Effects of salinity on juvenile Cyclopterus Lumpus (lumpfish) and their temporal and spatial distribution in the Great Bay Estuary, New Hampshire

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Effects of salinity on juvenile Cyclopterus Lumpus (lumpfish) and their temporal and spatial distribution in the Great Bay Estuary, New Hampshire

Abstract
Lumpfish (Cyclopterus lumpus) is a semi-pelagic species that is broadly distributed in the temperate portions of the North Atlantic. The lumpfish is also a commercially important species in Iceland and the Netherlands, where it is fished for roe that is used for caviar. Moreover, several recent studies have shown that lumpfish juveniles are useful ‘cleaner fish’ in the Atlantic salmon aquaculture industry. Despite the importance of the species, little is known about its physiology and ecology. The overall goal of this research was to investigate if, and how, salinity affects the physiology and ecology of juvenile lumpfish.

To determine the effect of salinity on oxygen consumption rates of juvenile lumpfish, juveniles were exposed to five salinity treatments (10, 15, 20, 25, 30 ppt) and oxygen consumption rates were measured. Standard metabolic rates (SMR) were calculated using SMR = (V*\Delta C_{\text{wo2}}) / (\Delta t*Mf), where V is the volume of the respirometry chamber, \Delta C_{\text{wo2}} is the slope of the decrease in dissolved oxygen, \Delta t is the change in time, and Mf is the mass of the individual fish. Results showed that juveniles had the lowest SMR at 10 ppt and the highest at 20 ppt. However, they were able to tolerate salinities down to 5 ppt for a week without visual signs of stress. This information will be helpful in informing the management of coastal and fishery resources, as well as those who wish to use lumpfish in aquaculture operations.

Little is known about the distribution of juveniles, therefore to determine the temporal and spatial distribution of juvenile lumpfish in the Great Bay Estuary, NH, sampling took place in 2015 and 2016 from June through September. Juveniles were caught, using dip nets, from macroalgae growing on floating docks at four different locations along the Piscataqua River and Great Bay. Lumpfish were found at all locations, except for the mouth of the Great Bay site (JEL) in all four months. Water temperatures ranged from 8.6 – 22.3 °C, and salinities ranged from 21.9 – 33.95 ppt where lumpfish were found. While juvenile lumpfish were never captured at the Great Bay sampling station, there is some anecdotal information they are occasionally found in the Bay, and thus that they are able to tolerate lower salinities and warmer temperatures than previously thought. With the changing climate, it is important to fully investigate the abiotic factors that influence the temporal and spatial distribution of juvenile lumpfish.

Keywords
Cyclopterus lumpus, distribution, lumpfish, salinity tolerance, Biology, Ecology

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EFFECTS OF SALINITY ON JUVENILE CYCLOPTERUS LUMPUS (LUMPFISH) AND THEIR TEMPORAL AND SPATIAL DISTRIBUTION IN THE GREAT BAY ESTUARY, NEW HAMPSHIRE

BY

Jenna Rackovan
B.S., Gettysburg College, 2007

THESIS

Submitted to the University of New Hampshire in Partial Fulfillment of the Requirements for the Degree of

Master of Science in Marine Biology

December, 2016
This thesis has been examined and approved in partial fulfillment of the requirements for the degree of Masters of Science in Marine Biology by:

Thesis Director, William H. Howell, Emeritus Professor of Biological Science

Elizabeth Fairchild, Research Associate Professor of Biological Sciences

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On December 2, 2016

Original approval signatures are on file with the University of New Hampshire graduate school.
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ABSTRACT

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By

Jenna Rackovan

University of New Hampshire, December, 2016

Lumpfish (Cyclopterus lumpus) is a semi-pelagic species that is broadly distributed in the temperate portions of the North Atlantic. The lumpfish is also a commercially important species in Iceland and the Netherlands, where it is fished for roe that is used for caviar. Moreover, several recent studies have shown that lumpfish juveniles are useful ‘cleaner fish’ in the Atlantic salmon aquaculture industry. Despite the importance of the species, little is known about its physiology and ecology. The overall goal of this research was to investigate if, and how, salinity affects the physiology and ecology of juvenile lumpfish.

To determine the effect of salinity on oxygen consumption rates of juvenile lumpfish, juveniles were exposed to five salinity treatments (10, 15, 20, 25, 30 ppt) and oxygen consumption rates were measured. Standard metabolic rates (SMR) were calculated using SMR = (V*ΔC_{wo2})/(Δt*M_f), where V is the volume of the respirometry chamber, ΔC_{wo2} is the slope of the decrease in dissolved oxygen, Δt is the change in time, and M_f is the mass of the individual fish. Results showed that juveniles had the lowest SMR at 10 ppt and the highest at 20 ppt. However, they were able to tolerate salinities down to 5 ppt for a week without visual signs of stress. This information will be helpful in informing the management of coastal and fishery resources, as well as those who wish to use lumpfish in aquaculture operations.
Little is known about the distribution of juveniles, therefore to determine the temporal and spatial distribution of juvenile lumpfish in the Great Bay Estuary, NH, sampling took place in 2015 and 2016 from June through September. Juveniles were caught, using dip nets, from macroalgae growing on floating docks at four different locations along the Piscataqua River and Great Bay. Lumpfish were found at all locations, except for the mouth of the Great Bay site (JEL) in all four months. Water temperatures ranged from 8.6 – 22.3 °C, and salinities ranged from 21.9 – 33.95 ppt where lumpfish were found. While juvenile lumpfish were never captured at the Great Bay sampling station, there is some anecdotal information they are occasionally found in the Bay, and thus that they are able to tolerate lower salinities and warmer temperatures than previously thought. With the changing climate, it is important to fully investigate the abiotic factors that influence the temporal and spatial distribution of juvenile lumpfish.
INTRODUCTION

Systematics and Life History

Lumpfish (*Cyclopterus lumpus*) are part of the order Scorpaeniformes and a member of the family Cyclopteridae (Davenport 1985), which is comprised of 28 species and six genera (Nelson 2006). This is subject to change as there has been some debate on whether or not some nominal species are actually just different sexes of the same species (Hatano et al. 2015). However, *Cylopterus lumpus* is the only species of the genus *Cyclopterus*. Lumpfish are a semi-pelagic species that spend most of their adult life in deep, cold offshore waters. They are generally found around 60 m in depth, but have been found to travel down to 300-400 m in depth (Blacker 1983; Kennedy et al. 2016). There are three distinct genetic groups of *Cyclopterus*: 1) Maine-Canada-Greenland; 2) Iceland-Norway; and 3) Baltic Sea (Pampoulie et al. 2014). In the western North Atlantic, lumpfish range from Greenland to New Jersey (Collins 1979; Bigelow and Schroeder 2002). *Cyclopterus* are mostly cartilaginous and are usually grayish in color. They have adapted a ventral suction disk, which is formed from their modified pelvic fins and use it to adhere to rocks, algae, and other marine structures (Bigelow and Schroeder 2002).

During the spring and early summer the adults move inshore to spawn along the rocky coasts (Cox and Anderson 1922a; Goulet et al. 1985a; Bigelow and Schroeder 2002a). Males and females are sexually dimorphic and the females are larger than the males (Cox and Anderson 1922a; Goulet 1988). The males, who are bright red during spawning season, locate a nest that is in either a crevice or depression in the substrate or in boulders (Goulet 1988). Females spawn 2-3 demersal egg masses and then move offshore, while the males stay with the eggs until hatching, approximately 6-8 weeks depending on water temperatures (Cox and Anderson 1922a;
Collins 1976a; Goulet et al. 1985a; Martin-Robichaud 1991). Throughout this time, the males guard the eggs from predators and continually fan the eggs for aeration (Goulet et al. 1985a). Once hatched, the larvae begin to feed after about one week (Benfey and Methven 1986).

The juveniles leave the nest area in the early summer and live among the macroalgae. Moring (1989) found that juveniles less than 26 mm TL live among Zostera (sea grass) beds or Laminaria (kelp), and then move to Ascophyllum nodosum (rockweed) in the inner bays. Juveniles also use tidepools during the summer as nursery habitats (Moring 1989a). In the warmer months (Jul-Sept), they are found primarily in the upper 0.5 m of the water column (Daborn and Gregory 1983). During this life history stage the juveniles feed on small invertebrates such as amphipods, copepods, isopods, cumaceans and even small fish larvae (Moring 1989; Tully and O’Ceidigh 1989; Davenport and Rees 1993). Lumpfish grow relatively quickly in their first year of life, reaching approximately 35 to 70 mm TL (Martin-Robichaud 1991). During this time, they are able to divert the majority of their energy towards growth since they cling to algae and wait for prey to pass by (Brown 1986a; Killen et al. 2007a). Juveniles have a very high association with seaweed. They depend on it for transportation as it passively drifts, protection from predators, and an increase in food sources (Vandendriessche et al. 2007).

At approximately one year of age, the juveniles become mostly pelagic and begin to move offshore. Lumpfish can live up to approximately 10 to 15 years and reach sexual maturity in two to three for males and three to four for females (Albert et al. 2002; Hedeholm et al. 2014). This age estimate is different than was previously reported by Thorsteinsson (1981), who found the spawning age to be five to ten for females and four to seven for males. Although they do not feed during the spawning periods, adults have been found to eat crustaceans, polychaetes, and jellyfish throughout the rest of the year (Cox and Anderson 1922). The average adult reaches a
maximal length of about 38-40 cm (15 to 16 in.), however the largest lumpfish recorded was a female, which was approximately 58 cm (23 in.) in length (Bigelow and Schroeder 2002). The only known predators for lumpfish are the harbor seal (de la Vega et al. 2016), hooded seal (Haug et al. 2007) and Greenland shark (Nielsen et al. 2014).

**Commercial Uses**

Lumpfish is a commercially important species in Iceland, Newfoundland and Norway where it is harvested for its roe. The commercial fishery began to flourish in the 1950’s and 60’s due to a caviar demand in Germany and France (Johannesson, 2006; Stevenson and Baird, 1988). Since then the fishery has become quite lucrative. In 2011 lumpfish roe sales amounted to approximately $33 million (Johannesson 2006a). According to the Food and Agriculture Organization of the United Nations, the total catch for lumpfish in 2013 and 2014 was 20,365 and 12,706 tonnes, respectively (FAO 2016). The fisheries in both Greenland and Iceland were deemed sustainable in 2015 by the Marine Stewardship Council (MSC 2016). In addition, there is a small recreational fishery for lumpfish meat, however this only targets the males and does not contribute much to the overall fishery. Despite being such a commercially important species, very little is known about the life history and ecology of lumpfish.

Recent studies have shown that juvenile lumpfish are useful in the Atlantic salmon aquaculture industry as ‘cleaner fish’ that remove sea lice from the salmon. Sea lice can cause multiple hemorrhages on the surface of the body, allowing access to pathogens that weaken the fish (Bravo 2009). Salmon farmers have been relying on cleaner fish, such as the Ballan wrasse (*Labrus bergylta*) to reduce sea lice since 1988 (Skiftesvik et al. 2014). In 1998, farmers began to use chemical pesticides (e.g. Emamectin benzoate (marketed as SLICE®)) to delouse the fish, but many of the sea lice populations have become resistant to this chemical treatment (Denholm
et al. 2002; Skiftesvik et al. 2014). Costello (2009) estimated that the cost of sea lice control globally was €305 million based on the 2006 salmonid production. As a result, cleaner fish have started to resurface as an alternative to these chemicals since their use is less costly. There is some debate on whether or not wrasse is being overfished for this purpose. There also have been studies that show that some species of wrasse cannot tolerate low temperatures (≤4°C), especially when caught in the summer months (Sayer and Davenport 1996; Sayer and Reader 1996); this can result in wrasse becoming inactive and, therefore, no longer effective at feeding on the lice.

Juvenile lumpfish provide an alternative to wrasse because they are easily reared in a hatchery (Brown et al. 1989). Juvenile lumpfish are opportunistic feeders, and when placed in aquaculture pens with salmon they feed on the sea lice (Imsland et al. 2014a, 2014c, 2015a). This provides an environmentally friendly, natural way to help rid aquaculture species of sea lice. Their use could be beneficial to other species, or to salmon farms located in lower salinity environments, depending on the salinity tolerances of juvenile lumpfish.

**Salinity Tolerance**

Many studies have investigated salinity tolerances and preference of different estuarine species. Serkov (2003) observed the salinity tolerance for seven different teleost fish in Japan, including multiple species of the distantly related sculpin, and found that most could tolerate low salinities, including fresh water for multiple days, but would then begin to show signs of stress and even death. Respiration has been used as an indicator of salinity induced stress in many studies. For example, a study on juvenile hogchokers (*Trinectes maculatus*), an estuarine species, showed that salinity affects metabolic rates (Peterson-Curtis 1997). Although the results
varied between lengths of time in each salinity (0, 7 and 15ppt), exposure to the freshwater produced consistently higher oxygen consumption rates.

Many marine teleost fish are very strong osmoregulators and can survive for short periods of time in undesirable environments. Moser and Miller (1994) tested juvenile spot (*Leiostomus xanthurus*) in eight different salinities between 0 and 34ppt, and found that their metabolism stabilized after approximately three hours after rapid salinity changes. They reported, however, that it was more difficult for the fish to adjust to low salinities than higher ones. The effect of salinity on the respiration rates of juvenile lumpfish has not been studied. The overall goal of this research was to investigate if, and how, salinity affects the physiology and ecology of juvenile lumpfish. The specific objectives were to: 1) determine the effect of salinity on oxygen consumption rates of juvenile lumpfish; and 2) determine the temporal and spatial distribution of juvenile lumpfish in the Great Bay Estuary, especially in relationship to environmental salinity.
Chapter 1: EFFECTS OF SALINITY ON RESPIRATION RATES OF JUVENILE LUMPFISH
CYCLOPTERUS LUMPUS

Abstract

The lumpfish (Cyclopterus lumpus) is a commercially important species in Iceland and the Netherlands, where it is fished for roe that is used for caviar. Moreover, several recent studies have shown that lumpfish juveniles are useful ‘cleaner fish’ in the Atlantic salmon aquaculture industry. Despite the importance of the species, little is known about its ecology. This study examined how one environmental variable (salinity) impacted the oxygen consumption of juvenile lumpfish. Juveniles were exposed to five salinity treatments (10, 15, 20, 25, 30 ppt) and oxygen consumption rates were measured. Standard metabolic rates (SMR) were calculated using $SMR = \frac{V \cdot \Delta C_{WO2}}{\Delta t \cdot M_f}$, where $V$ is the volume of the respirometry chamber, $\Delta C_{WO2}$ is the slope of the decrease in dissolved oxygen, $\Delta t$ is the change in time, and $M_f$ is the mass of the individual fish. Results showed that juveniles had the lowest SMR at 10 ppt and the highest at 20 ppt. However, lumpfish were able to tolerate salinities down to 5 ppt for a week without visual signs of stress. This information can be helpful in informing the management of coastal and fishery resources, as well as those who wish to use lumpfish in aquaculture operations. Having a better understanding of their tolerance also could lead to the use of lumpfish as cleaner fish for cultured fish species in lower salinity environments.

Introduction

Lumpfish (Cyclopterus lumpus) are a semi-pelagic, cold water species that spend the majority of their life offshore. In the western North Atlantic, they range from Greenland to New Jersey (Bigelow and Schroeder 2002). They are a commercially important species that have
been harvested since the 1950’s for their roe, which is used for caviar (Stevenson and Baird 1988; Johannesson 2006). Recently the juveniles of this species have been used as “cleaner fish” in the salmon aquaculture industry. Imsland et al. (2014a, 2014b, 2015b) found that not only did lumpfish reduce the number of sea lice in the pen, but also they ate more adult female lice, which reduced the potential of reinfection. Due to this discovery, the number of lumpfish hatcheries has been growing rapidly. In Norway alone, there were 16 lumpfish hatcheries that produced five million juveniles in 2014 and approximately 12-14 million in 2015 (Vargas 2015). Despite their commercial importance for roe and as “cleaner fish”, there is relatively little known about lumpfish physiology and ecology.

Juvenile lumpfish tend to spend their first year of life inshore in brackish water, before moving offshore in their second year (Daborn and Gregory 1983; Moring and Moring 1991). The structural habitat preference and diet of juvenile lumpfish is well known. Moring (1989) found that juveniles less than 26 mm TL live among Zostera (sea grass) beds or Laminaria (kelp), and then as they grow they move to Ascophyllum nodosum (rockweed) in the inner bays. Juveniles also have been found using tidepools during the summer as nursery habitats (Moring 1989). Young-of-the-year are primarily found in the upper 0.5 m of the water column (Daborn and Gregory 1983). During this life history stage the juveniles feed on small invertebrates such as amphipods, copepods, isopods, cumaceans and even small fish larvae (Moring 1989; Tully and O’Ceidigh 1989; Davenport and Rees 1993). Despite their apparent preference for inshore brackish habitats, their tolerance of low salinity, has not been studied. Salinity is considered to be one of the primary environmental factors affecting the growth and survival of marine organisms (Chen et al. 1996). It is also highly associated with species assemblage (Gunter 1956; Martino and Able 2003; Glover et al. 2012) and, thus, is an extremely important parameter.
influencing fish distribution. Therefore, studying the salinity tolerance of this species can help shed light on both lumpfish physiology and ecology.

There are many published reports concerning the salinity tolerances and preferences of nearshore and estuarine species. Hyndman and Evans (2009), for example, studied a distantly related species, the longhorn sculpin (*Myxocephalus octodecrimspinus*), and found that they could tolerate low salinities (10% seawater) for two days, but after day six, they began to show visual signs of stress. One way to determine salinity tolerance is to measure oxygen consumption, and therefore metabolic rate, of a species when exposed to different salinities. For example, metabolic rates of juvenile grey snapper (*Lutjanus griseus*) were compared when exposed to multiple combinations of temperature and salinity (Wuenschel et al., 2005). At increased salinities (5-45 psu) and higher temperatures (30-33°C), there was an effect on juvenile gray snapper oxygen consumption, which resulted in a higher energy cost for the juveniles. Similar studies have never been conducted on lumpfish, and are needed to clarify if lumpfish are suitable ‘cleaner fish’ in aquaculture operations located in low salinity environments. Therefore, the goal of this study was to determine the preference and salinity tolerance of juvenile lumpfish by examining the effects of salinity on oxygen consumption, and thus their metabolic rates.

Material and Methods

*Data Collection*

Adult broodstock were caught with 91 m long, 20.3 cm mesh, monofilament gillnets. Nets were set sub-tidally (3-5 m deep) along the rocky shore near New Castle, NH, USA (Fig. 1.1). Soak times were approximately 24 hours. Collections were made in April and May in both 2015 and 2016. Adults also were caught offshore by commercial trawlers (Fig. 1.2). All fish
were transported back to the University of New Hampshire’s (UNH) Coastal Marine Laboratory (CML), where they were placed as individuals or spawning pairs into 0.9-meter diameter tanks with flowing seawater, and held until the end of the spawning season. In 2015 only males were caught, and in 2016 none of the spawning pairs collected produced eggs. In 2015, young-of-the-year lumpfish, 15-35 mm in length, were caught off the UNH pier (New Castle, NH, USA) in July and August. Juveniles were kept at the CML in six separate, 10-liter tanks (15/tank) supplied with flow-through ambient seawater which ranged from 28-31ppt salinity and 7-20°C and fed frozen brine, *Mysis* shrimp, or white worms (*Enchytraeus albidus*) daily.

In April of 2016, a single lumpfish egg mass was harvested off the Maine (USA) coast (Fig. 1.2) by SCUBA divers, returned to the CML, and held in a 0.9-meter diameter tank with flow-through, ambient, filtered seawater, which ranged from 28-31ppt salinity and 8-11°C. Hatching occurred 24-37 days post-collection. Three days after hatch, larvae were fed enriched (Ori-Green, Skretting™) *Artemia* napulii 2-3 times/day (1000 nauplii/L) for two weeks before being weaned onto a dry formulated diet (Gemma Micro, Skretting).

*Standard metabolic rates*

To determine the salinity tolerance of juvenile lumpfish, salinity experiments were conducted in the CML when the juveniles were approximately two to three months old and 20 to 48 mm TL. Ten individuals were placed into five different 3-liter tanks (Fig. 1.3). Five individuals were acclimated to the desired test salinity by slowly adding de-ionized fresh water over a six-hour period to represent a tidal cycle. A second set of five fish was left in the test salinity for two extra hours (total of eight hours) before experiments were run so that all salinities could be tested twice in a single day. Temperature in the acclimation and oxygen consumption chambers was kept at approximately 17-19 °C by immersing the chambers in a heated water
bath. Salinity was monitored using a YSI (Yellow Springs Instrument) professional plus. Once the fish were acclimated, they were measured (total length) and weighed before being transferred individually into a small-enclosed glass, 550 ml chamber of the desired salinity. Their rate of oxygen consumption was monitored for 30 minutes to obtain a standard metabolic rate (SMR). The water for each experiment was aerated (with an air stone) to approximately 100% oxygen saturation for at least 24 hours prior to the experiment. Oxygen levels in the respiration chambers were measured using a Vernier system oxygen probe, and temperatures were monitored with a Vernier system temperature probe. Both probes were inserted into the glass chamber through a rubber stopper. Five different salinities were tested (10, 15, 20, 25, 30 ppt), with the control being 30 ppt. Each salinity treatment was tested 14 times with a different individual for a total of 70 trials throughout the experiment.

*Salinity tolerance*

After the 30 minute trials, all fish tested in the same salinity were pooled together and transferred into 10 liter aerated tanks, containing water of the same test salinity, and monitored for approximately one week. Long term exposure effects, such as swimming ability and appetite were monitored and any mortalities were recorded. Ten fish from the 10ppt tanks were transferred to 5ppt and observed for another week. Five fish from 5ppt were placed in 0ppt to observe freshwater tolerance.

*Salinity Gradient*

Salinity preference was determined using a plexiglass salinity gradient tank (118.75 long x 30.5 wide x 20.3cm deep) that was constructed such that freshwater and saltwater sources entered from opposite ends, with water drained from the saltwater end (Fig. 1.4). A light source was suspended above the gradient tank to insure equal light intensity throughout the tank.
Although the salinity gradients varied over the course of the trials due to tidal differences in ambient salinity, gradients were generally in the range of 13 – 23 ppt. Each trial began with eight juveniles being placed into a cylindrical tube that was acclimated to an ambient salinity (13 - 21 ppt) for five minutes at various locations in the tank depending on the trial. After the five-minute acclimation period, the fish were released and able to swim freely around the tank. The location of the eight fish in the gradient tank, and the salinity near each fish, were recorded hourly, for five hours. This was repeated eight times, for a total of 64 fish.

Data Analysis

Standard metabolic rates (SMR) were calculated using the metabolic rate calculation developed by (Clark et al. 2013):

\[
\text{SMR} = \frac{(V\Delta C_{\text{wo2}})}{(\Delta t M_f)}
\]

where \(V\) is the volume of the respirometry chamber, \(\Delta C_{\text{wo2}}\) is the slope of the decrease in dissolved oxygen, \(\Delta t\) is the change in time, and \(M_f\) is the mass of the individual fish. Metabolic rates between individuals at the different salinities were tested for normality, homogeneity, and non-additivity and 2015 data were then log transformed using R version 3.3.1. An ANOVA, followed by Tukey’s mean comparison test, was used to test the null hypothesis that there were no significant differences between the metabolic rates for fish tested at five different salinities.

The salinity gradient tank was separated for analysis into three areas, each associated with a range of salinities: area 1 (11 – 15 ppt), area 2 (15 – 20 ppt), and area 3 (20 – 25 ppt). The percent of juveniles present in each of these areas was recorded each hour. Data were analyzed using a Kruskall- Wallis nonparametric test. Fish distribution for each hour (all trials combined) and each trial (all hours combined) were plotted.
Results

*Standard metabolic rates*

In 2015, mean oxygen consumption also referred to as standard metabolic rates (SMR) were significantly different between salinities ($F = 4.09, p = 0.005$) (Fig. 1.5). One negative value, likely due to a malfunction of the DO probe, was removed from the data set. Fish in 10 ppt showed the lowest metabolic rates, and fish in 20 ppt had the highest. Fish showed no external signs of stress (no longer eating or swimming irregularly) in any of the tested salinities. There were no significant differences in SMRs of fish exposed to the same range of salinities in 2016 ($F = 1.67, p = 0.168$) (Fig. 1.6). However, fish at 10 ppt still had the lowest metabolic rates and fish at 20 ppt had the highest. When the two years were combined, there were significant differences between salinities ($F= 4.31, p = 0.003$). Fish exposed to 10 ppt had a significantly lower SMR than those exposed to 20, 25, and 30 ppt. There was no difference between fish exposed to 15ppt from those exposed to any other salinity (Fig. 1.7). There were no significant differences between temperatures or between the two sets of salinity experiments in any given day. There was no clear relationship between SMR and weight ($r = 0.01, p = 0.235$), including no significant difference by weights of the fish tested between salinities ($p >0.05$) (Fig. 1.8).

*Salinity tolerance*

The fish placed in each of the test salinities for one week in 2015, showed 90-100% survival in 5 ppt through 30 ppt (Fig. 1.9). All five fish that were exposed to 0 ppt died within 28 hours (Fig. 1.9). In 2016, there was 60-100% survival in all salinities tested, with fish held at 10 and 25 ppt showing the lowest survival (Fig. 1.9).
Salinity gradient

Juvenile lumpfish showed a preference for high salinity waters in the salinity gradient experiment (H = 19.88, p < 0.01) (Fig. 1.10). However, when the trials were analyzed by hour, there did not seem to be a difference in salinity preferences overtime (Fig. 1.11). Juvenile lumpfish appeared to prefer salinities > 18 ppt in all trials compared to lower salinities (Fig. 1.12).

Discussion

Standard metabolic rates

Lumpfish appear to have a high tolerance for a variety of salinities. For example, juveniles can tolerate salinities down to 5 ppt for at least a week. However, when given a choice between salinities, they preferred salinities of 20 ppt or higher. These results could indicate that juvenile lumpfish are euryhaline. Standard metabolic rates vary significantly among different species and can change based on many different factors. Juvenile lumpfish SMR ranged from 0.002 to 0.035 mg O$_2$L$^{-1}$g$^{-1}$min$^{-1}$ (0.098 to 1.035 mgO$_2$hr$^{-1}$) throughout all salinities. Like several other teleost fish, their SMR was lowest at 10 ppt. For example, juvenile winter flounder (Pseudopleuronectes americanus) had the lowest oxygen consumption rates at 10 and 20 ppt (Frame 1973) and flathead grey mullet (Mugil cephalus), when exposed to a variety of salinities, had the lowest metabolic rates at 5 ppt (Cardona 2000). Juvenile hogchokers had the highest oxygen consumption rates in freshwater, but the lowest in 7 ppt; salinity had no effect on growth rates (Peterson-Curtis 1997b). The similarities between these estuarine species and juvenile lumpfish could indicate that lumpfish are euryhaline, and able to extend their range into estuarine habitats.
Sampaio and Bianchini (2002) studied the blood osmolality of the euryhaline flounder, *Paralichthys orbignyanus*, and estimated their isosmotic point to be at 328.6 mOsm kg\(^{-1}\) H\(_2\)O, which corresponded to 10.9 ppt. Similar values for isosmotic points were seen for other estuarine, euryhaline species, such as *Cyprinodon variegatus*, *Scopthalmus maximus* and *Sparus aurata* (as reviewed in Sampaio and Bianchini 2002). Although the osmolality of the blood of the lumpfish was not measured in this study, it is possible that it was similar to other euryhaline species (i.e. ~325 mOsm kg\(^{-1}\) H\(_2\)O). If so, the lumpfish were nearly isosmotic at 10 ppt, which would explain why the SMR was the lowest in fish exposed to 10 ppt salinity. Yet, despite this, when given a choice, they preferred 20 ppt seawater.

Metabolic rates have been reported for lumpfish, but not in relation to salinity. Killen et al. (2007b) found the standard metabolic rate, in both wild caught and lab reared lumpfish, was 0.22 to 0.27 mgO\(_2\)g\(^{-1}\)hr\(^{-1}\) at 11 °C in seawater. There was no difference between standard metabolic rates for lumpfish across size classes. Results from the current study showed an average SMR of 0.52 mgO\(_2\)g\(^{-1}\)hr\(^{-1}\) in 30 ppt at an average temperature of 18.4 °C. These differences in SMR suggest that there are many different factors that can affect metabolism, including temperature and size. The effect of temperature on metabolic rates was studied in a meta-analysis of 69 different species and found that as temperature increased, the metabolic rate increased too (Clark and Johnston 1999). However, this link between temperature and metabolic rate may not always be positive, as there also may be evolutionary temperature adaptations in different species that show a different relationship (Clarke and Fraser 2004). It also has been determined that resting metabolic rate increases with weight in most teleost fishes (Clark and Johnston 1999). In this study, however, there was no relationship found between weight and standard metabolic rate for juvenile lumpfish (Fig. 1.8). This was likely due to the small range
of weights (0.23 – 4.16 g) used in this experiment. If the weight range had been expanded, a pattern similar to that proposed by Clark and Johnston (1999) might have been observed.

*Salinity tolerance*

Juvenile lumpfish have a high tolerance for salinities as low as 5 ppt for one week, with no external signs of stress. In the week-long exposure experiments, the fish continued to eat and swim normally. This suggests that lumpfish may be more euryhaline than previously thought. There have been many studies on salinity tolerance of marine teleost fishes. Moser and Miller (1994) studied the salinity tolerance of spot (*Leiostomus xanthurus*) by exposing them to changes in salinity from 0-34 ppt. They found that spot tended to acclimate after three hours, and that they were better at adapting to rapid increasing versus, decreasing salinities. When salinity tolerance of the juvenile goliath grouper (*Epinephalus itajara*) was tested, it was found that the juveniles could tolerate freshwater, but it would take about 72 hours for the blood osmolality to stabilize. In another study (Wu and Woo 1983), the salinity tolerance of 13 different marine species exposed to a lower salinity over the course of two weeks was examined. At the end of this experiment, all species survived in salinities as low as 10 ppt but only three species survived in 3 ppt. Finally, Schultz and McCormick (2012) compiled the results from 108 different salinity tolerance studies on 141 different species and found that most euryhaline species on average could survive as low as 4.5 ppt when placed directly into different salinities and 7 ppt when gradually brought to different salinities. These many different examples of salinity tolerance in marine fishes suggest that multiple species have salinity plasticity in which they are capable of tolerating a range of salinities, even when they are not usually exposed to them in their natural habitats. The findings from the previously mentioned studies, along with the results from this
current study, indicate that many marine fish, including the lumpfish, can survive in these low salinity environments for multiple days or longer.

Survival rates for juvenile lumpfish, in salinities ranging from 5 to 30 ppt over a one-week period, were between 60 - 100% (Fig. 1.9). The lowest survival rates for both years were at 25 ppt (64 – 88%). However, the lower rates of survival across salinities in 2016, were not necessarily due to salinity effects. In 2016, the fish were fed pellets instead of frozen mysids, and these fouled the tanks faster, causing a decrease in oxygen and water quality. Also in that year, there were three days when the water temperature in the experimental tanks was around 20°C, exposing the lumpfish to warmer than average temperatures. Further, the fish used in 2015 were wild caught juveniles, while those used in 2016 were cultured in the laboratory. These three factors could have caused the higher mortalities seen in the second year. Kefford (2004) found that fish usually die within 48 to 72 hours of exposure to lethally high or low salinities. The relatively high survival rates over an extended time at all salinities, apart from freshwater, indicate a wide salinity tolerance for juvenile lumpfish.

Salinity gradient

Studying salinity preference in the lab can help determine habitat preference for a species. Salinity preference studies have been performed for many different euryhaline species, such as the African jewelfish (Rehage et al. 2015), grey snapper (Serrano et al. 2010) and European flounder (Bos and Thiel 2006). In this study, it was determined that although juvenile lumpfish could tolerate lower salinities, when given a choice, they preferred salinities of 20 ppt or higher. The gradient tank used for these experiments was small and variable, not always having consistent salinity ranges. However, it was still apparent that lumpfish showed a stronger preference for the higher salinities available (Fig. 1.10). A larger experimental gradient tank,
able to produce a larger range in salinities (0 – 30 ppt), would perhaps produce more accurate results. Lumpfish in the wild prefer to cling to substrates and remain stationary rather than actively swim (Brown 1986b). Since the substrates often include drifting macroalgae, the juveniles are likely carried by the tide, at least for brief periods, into lower salinities. Thus, while they behaviorally prefer higher salinities, they may have evolved to tolerate lower salinities.

Conclusions

These studies show that juvenile lumpfish can tolerate low salinity environments, although the physiological mechanisms remain unclear. Future studies should document the blood osmolality of juvenile lumpfish held at different salinities to determine their isosmotic point, as well as determine if they are strong osmoregulators. While we now know that juveniles between the sizes of 0.2 and 4.16 g can tolerate lower salinities, it remains unclear if they can continue to tolerate these lower salinities as they get older. Do they become less tolerant as adults, when they are living offshore? Clearly further investigations are needed to determine metabolic rates and salinity tolerance of different size classes. The growing need for cleaner fish in the aquaculture industry makes it important to understand the physiology of juvenile lumpfish. Having a better understanding of their tolerance also could lead to the use of lumpfish as cleaner fish for cultured fish species in lower salinity environments, such as steelhead trout.
Figure 1.1. Collection sites of adults and juvenile lumpfish in 2015.
Figure 1.2. Adult lumpfish and egg mass capture locations in 2016.
Figure 1.3. Diagram of experimental design. The top five small boxes represent the 3-liter tanks where fish were acclimated to desired salinities (represented by the numbers). The bottom five tanks were kept at desired salinities with 100% oxygen. The small cylinder represents the experimental chamber with oxygen and temperature probes.
Figure 1.4. Salinity gradient tank setup design. The tank was separated into three areas, each associated with a range of salinities: area 1 (11 - 15 ppt), area 2 (15 - 20 ppt), and area 3 (20 - 25 ppt).
Figure 1.5. Box plot showing salinity effects on oxygen consumption rates of juvenile lumpfish in 2015. Different letters above plots indicate significant differences in metabolic rates.

Figure 1.6. Box plot showing salinity effects on oxygen consumption rates of juvenile lumpfish in 2016. Different letters above indicate differences in metabolic rates.
Figure 1.7. Box plot showing salinity effects on oxygen consumption rates of juvenile lumpfish for both years combined. Different letters above plots indicate significant differences in metabolic rates.
Figure 1.8. Standard metabolic rates in relation to weight of juvenile lumpfish.
Figure 1.9. Proportion of juvenile lumpfish that survived one week in each salinity in both 2015 (top) and 2016 (bottom).
Figure 1.10. Mean percent of juvenile lumpfish present in the salinity gradient tank areas (Area 1: 11 – 15 ppt, Area 2: 15 – 20 ppt, Area 3: 20 – 25 ppt).
Figure 1.11. Number of juvenile lumpfish present at each salinity in each hour in the trial, all trials combined.
Figure 1.12. Number of juvenile lumpfish present at each salinity during a specific trial, all hours combined. The dotted vertical line represents the highest salinity available during that trial.
Chapter 2: SPATIAL AND TEMPORAL DISTRIBUTION OF JUVENILE CYCLOPTERUS LUMPUS IN THE GREAT BAY ESTUARY, NEW HAMPSHIRE

Abstract

Lumpfish (*Cyclopterus lumpus*) is a semi-pelagic species that is broadly distributed in the temperate portions of the North Atlantic. Little is known about the distribution of juveniles, therefore this study was the first attempt to determine juvenile lumpfish distribution in near-coastal and estuarine locations, as represented by the Great Bay Estuary, NH. Sampling took place in 2015 and 2016 from June through October at four different locations along the Piscataqua River and Great Bay. Using dip nets, juveniles were caught, from macroalgae growing on floating docks in all four months and all locations, except for the Great Bay site (JEL). Water temperatures ranged from 8.6 – 22.3 °C and salinities ranged from 21.9 – 33.95 ppt where lumpfish were found. Juvenile lumpfish were never captured at the Great Bay sampling station, therefore it is still unclear whether or not they are using the Bay as a nursery ground. With the changing climate, it is important to fully investigate the abiotic factors that influence the temporal and spatial distribution of juvenile lumpfish.

Introduction

Lumpfish (*Cyclopterus lumpus*) is a semi-pelagic species that is broadly distributed in the temperate portions of the North Atlantic. During the spring the adults move inshore along the rocky coast to spawn (Cox and Anderson 1922; Goulet et al. 1985; Bigelow and Schroeder 2002). Once they spawn, females move offshore and the males stay with the eggs, aerating them until hatching (Cox and Anderson 1922; Collins 1976; Goulet et al. 1985). In the months following hatching, the emergent juveniles live among the algae floating at the surface, in sea
grass beds, or in tidepools. After the first year, juveniles are thought to become more pelagic and move offshore (Daborn and Gregory 1983; Moring 1989; Moring and Moring 1991). However, Davenport and Rees (1993) found juveniles older than one year attached to Ascophyllum floating near the surface in the Irish Sea, indicating that juveniles stay neustonic for longer than their first year.

There are many different euryhaline species that spend the first year or two of life in estuarine nursery grounds before moving offshore as juveniles or young adults. Examples include winter flounder (Pseudopleuronectes americanus) that spend their first two years of life in estuaries (Pereira et al., 1999), and weakfish (Cynoscion regalis) that spend approximately six months in the estuary (Paperno et al. 2000) before moving offshore as young adults. The juveniles of a closely related species of snailfish (Liparis coheni) also are found in the Gulf of Maine and tend to be found in estuaries in the early spring/late summer in salinities ranging from 12-34 ppt (Able 1976). The Great Bay Estuary in New Hampshire, where salinities average 15-25 ppt in the spring through the summer (Watson et al. 1999), functions as a nursery ground for many different Gulf of Maine species, such as rainbow smelt (Osmerus mordax) (Ganger 1999) and winter flounder, (Armstrong 1997; Bailey 2013). Great Bay Estuary, therefore, could serve as potential habitat for young-of-the-year lumpfish too.

There have been multiple distribution studies on adult lumpfish, and except during their breeding season when the adults move inshore, lumpfish are widely distributed throughout their geographical range, including in the Norwegian Sea (Holst 1993) and Barents Sea (Eriksen et al. 2014). However, there is less information about the distribution of juveniles. Juveniles tend to be found in 5-7 °C (Eriksen et al. 2014) and Daborn and Gregory (1983) found that juveniles are present in the Bay of Fundy from July through September. During those months they tend to be
in the upper 0.5 m of the water column. Aside from these studies, there has been very little focus on juvenile distribution in the first year of life, particularly in relation to salinity, and to date there have been no studies on the distribution of lumpfish in New Hampshire waters.

Climate change is affecting the productivity, habitat, and relative abundance of fish in many marine communities (Hollowed et al. 2013). Species distributions are shifting, and species like the lumpfish, which are typically found in cold water, are being forced to adapt. Understanding the physiology and ecology of lumpfish will allow us to predict what changes may occur as seawater temperatures rise. Moreover, since juvenile lumpfish are used as ‘cleaner fish’ in the Atlantic salmon aquaculture industry, knowledge of their physiology and ecology will facilitate and expand their use. Therefore, the goal of this study was to determine the temporal and spatial distribution of juvenile lumpfish in the estuarine and coastal waters of New Hampshire and use these data, in part, to determine their thermal and salinity tolerance.

Material and Methods

Study Area

The Great Bay Estuary, located in southeastern New Hampshire, is the largest estuarine system in New Hampshire comprising approximately 1,645 square kilometers. Large parts are shallow mudflats, but deep channels are found throughout. It is fed by seven rivers (Fig. 2.1), including the Piscataqua River, which forms the border between Maine and New Hampshire. A total of four sites, spanning the natural salinity gradient, in the lower-, mid-, and upper-estuary (Fig. 2.1) were sampled weekly from June through September in 2015. The upper most site, Jackson Estuary Laboratory (JEL), located at the mouth of Great Bay had an average depth of 2.4 m and was primarily in an eelgrass bed for otter trawling and plankton netting. The upper-middle site, Great Bay Marina (GBM) had an average depth of 2.3 m and was part sand and part
eelgrass. The lower-middle site, Great Cove Marina (GCM) had an average depth of 2.0 m and was a mixture of mud and sand. The lower site, Wentworth Marina (WM), had an average depth of 2.6 m and was primarily sand (Fig. 2.1).

Data Collection

To monitor the distribution and abundance of juvenile lumpfish that could occur in the estuary, multiple gear types were employed at each site in 2015. Using a 5 m (spread) otter trawl with a 1.25 cm codend liner, three 125 m tows were conducted to estimate benthic juvenile lumpfish distribution. All fish and piscivorous crabs were identified, measured to the nearest mm, and retained in buckets until all three tows were completed, after which all organisms were returned to the water alive. Otter trawl sampling was discontinued in September since no lumpfish were found in this gear.

Three replicate, 125 m surface tows were made with a 0.5 m diameter, 500 µ plankton net parallel to the otter trawls to collect juveniles attached to floating macroalgae on the surface. Contents of each net haul were examined for lumpfish, and then discarded. Plankton sampling sites were moved into the channel at each site in September due to floating debris at the original sites. Finally, dip nets were used along the sides of docks and piers by scraping approximately 1m through the macroalgae growing on these structures to collect juveniles attached to the macroalgae. This was done a total of 10 times on each sampling occasion at all study site locations.

In 2016, only the dip net sampling continued because no lumpfish were caught in trawls and only five were ever caught in plankton tows in 2015. In addition, lines of kelp (*Laminaria saccharina*) and artificial kelp (burgundy duck fabric) also were strung off of each dock and
checked weekly. All organisms caught were identified, measured, and placed into buckets until all sampling had finished, then returned to the water.

A YSI temperature/oxygen probe and refractometer were used to measure temperature, dissolved oxygen, and salinity at each site on every sampling occasion (taken at high tide in 2015). In addition, daily average salinity data for the Coastal Marine Laboratory (CML) and the Great Bay Estuary were obtained from the Northeastern Regional Association of Coastal Ocean Observing Systems (NERACOOS) and the Great Bay National Estuarine Research Reserve (GBNERR).

**Data Analysis**

Spatial and temporal distributions were plotted in GIS and analyzed in R version 3.3.1. To reduce over dispersion and account for the excessive zeros in the data, a zero inflated negative binomial model (ZINB) of the catch per unit effort (CPUE) by sampling location for dip net samples, followed by a type three analysis of variance, was used to analyze differences between sites. The sampling site JEL was excluded from analyses since there were no fish caught at this site.

**Results**

During the distribution survey for juvenile lumpfish, many other non-target fish and piscivorous crab species were collected. The green crab (*Carcinus maenas*) was the most abundant species found with the otter trawl at all sites, with the exception of JEL, where fourspine sticklebacks (*Apeltes quadracus*) was the most abundant species (Table 2.1). Silversides (*Menidia menidia*), winter flounder (*Pseudopleuronectes americanus*), and fourspine sticklebacks were the next most abundant species, especially at the JEL site (Table 2.1). Green
crab also was the most abundant species caught with dip net sampling, and cunner
\textit{(Tautogolabrus adpersus)} was the most abundant species found in the artificial kelp lines
(Table 2.2).

In 2015, temperature ranged from 9.7 to 23.7 °C across all sites, with the lowest
temperatures occurring at the coast (WM) and increasing further up the estuary. The highest
temperatures were in the Bay (JEL) (Fig. 2.2). In 2016, the temperature ranged from 12.1 to
24.8 °C with similar patterns to 2015 (Fig. 2.3). The salinity ranged from 20.8 to 33.6 ppt across
all sites in 2015 with the lowest and highest being at GCM (Fig. 2.2). Salinities were relatively
consistent throughout all sites over the study period in 2016, however, they decreased slightly up
estuary. In this year, the salinity ranged from 21.5 to 33.95 ppt, with one outliner from JEL at
12.1 ppt (Fig. 2.3). Weekly average salinities at JEL showed the lowest salinity of 16 ppt in
June. Dissolved oxygen was consistent across all sites and ranged from 6.0 to 10.13 mg/L in
both years (Figs. 2.2, 2.3).

Juvenile lumpfish were caught using plankton nets, dip nets, and with both artificial and
real kelps lines. Only five lumpfish were caught using a plankton net throughout the whole
study. Three lumpfish were caught using artificial kelp lines at WM and GBM, and 11 were
captured using real kelp lines at WM, GCM, and GBM (Table 2.3). There was a total of 33
juvenile lumpfish caught with dip nets throughout all sites in 2015, and 79 in 2016 (Fig. 2.4).
The majority of these fish ranged from 10 to 25 mm in length in all four sampling months (Figs.
2.4, 2.5). In 2015, more lumpfish were found in September than in any other month, at all sites
except for JEL, where none were ever caught (Fig. 2.6). This distribution shifted in 2016, when
the largest number of lumpfish was found in August (Fig. 2.7). The highest number of lumpfish
was found at the WM site in 2015 and at the GBM site in 2016.
Catch per unit effort (CPUE) was the highest at GCM in 2015 and there were no lumpfish caught at JEL throughout the sampling season (Fig. 2.8). There was no significant difference in CPUE between the three sites where lumpfish were found (p = 0.51). There was no significant difference between salinities at all four sites during collection (p = 0.31). In 2016, both WM and GBM had the highest CPUE, and again there were never any lumpfish caught at JEL (Fig. 2.9). There was no significant difference in CPUE between the three sites where lumpfish were found (p = 0.39). There was a significant difference between JEL salinity compared to the other three sites during collection (p < 0.0001). When both years were combined, all three sites where lumpfish were found had very similar CPUE (Fig. 2.10).

Discussion

Juvenile lumpfish were found at three of the four locations throughout all four sampling months, in both years, in salinities ranging from 21.9 – 33.95 ppt. The most efficient collection method employed during this study was dip netting through macroalgae growing off the sides of piers where the juveniles were attached to the kelp within one meter of the surface. No lumpfish were ever collected at the JEL site in Great Bay. The prevailing theory was that their absence was due more to a lack of preferred macroalgae (*Laminaria spp.*) rather than unfavorable temperatures and salinity. Therefore, to increase capture rates and to create potential habitat for juvenile lumpfish, both artificial and real kelp lines were hung at each of the four sampling locations in 2016. While there were some lumpfish found on the artificial kelp, the real kelp lines appeared to be the preferred habitat; still no juveniles were collected in Great Bay (JEL).
While there have been some studies of lumpfish distribution, most of these did not focus on juveniles. Cox and Anderson (1922) studied lumpfish in Canadian waters and found that lumpfish of all sizes ranged throughout the main portion of the Bay of Fundy and into Passamaquoddy Bay, in low temperatures and high salinities, but did not venture into the upper parts of Passamaquoddy Bay. The upper parts of Passamaquoddy Bay, at the mouth of St. Croix River, averages ~12 ppt (Ketchum and Keen 1953). While Cox and Anderson (1922) seem to suggest that lumpfish avoid lower salinities, there have been some studies that may indicate that lumpfish are venturing into lower salinity environments. Lumpfish are found throughout the Baltic Sea, including areas with lower salinities such as the Gulf of Gdańsk. Here salinities range from 7-8 psu in the upper 60-70 m and are greater (>12 psu) in the deeper areas (Tomczak et al. 2016). Juvenile lumpfish also were found in trawl surveys in other areas of the Baltic Sea where the average salinity is 10 psu (ICES trawl surveys). These previous studies suggest that while juveniles may prefer high salinity environments, they do appear in lower salinities as well. Similar results were seen in laboratory studies, where juveniles were given a choice between salinities; while some juveniles would venture into lower salinities the majority preferred salinities > 20 ppt (refer to chapter 1).

The observed spatial distribution of juvenile lumpfish in New Hampshire ranged from the coast (WM) to Little Bay (GBM). While no juveniles were ever caught during this survey at the mouth or in Great Bay (JEL), one juvenile lumpfish was caught in a plankton net by another University of New Hampshire (UNH) researcher in June of 2015 (Elizabeth Morrissey, pers. comm.). In October and November of 2016, four juvenile lumpfish were found clinging to a rope tied to a tracking device by UNH students in Great Bay (Meghan Owings, pers. comm.).
These observations indicate that juvenile lumpfish are present in Great Bay, although they were never detected in the current study.

Juvenile lumpfish began to appear around mid-June, peaked in August, and then began to decline in September (Figs. 2.5, 2.6). Average seawater temperatures when they first appeared, at peak, and began to decline were 14.6, 19.2, and 17.4 °C, respectively. A similar timeframe was found for juveniles in the Bay of Fundy by Daborn and Gregory (1983). Juvenile lumpfish also have been found in the Barents Sea in August and September in temperatures ranging from 5-7 °C (Eriksen et al. 2014). During that study, which included a 30-year dataset, juveniles were found in the Barents Sea in higher densities in warmer years. Similar temperature preferences have been seen for the pacific spiny lumpfish (*Eumicrotremus asperrimus*) as well. *E. asperrimus* juveniles prefer temperatures ranging from 4-6 °C in the Sea of Japan (Antonenko et al. 2009). These previously mentioned studies have all taken place in northern latitudes where water temperatures remain relatively cool throughout the summer months. The current study is the first to take place near the southern ranges of this species and may indicate a broader temperature range, at least for juvenile lumpfish.

Previous studies have indicated an association between juvenile presence and algae (Cox and Anderson 1922; Daborn and Gregory 1983; Moring 1989). Moring (1989) found a high association with juveniles in tidepools with *Zostera* and *Laminaria*, while Daborn and Gregory (1983) found a high association with floating seaweed in the Bay of Fundy. In the current study, only five juveniles were caught on the surface in floating algae (*Ascophyllum*), while the majority were found in association with *Laminaria* attached to piers. Results from the current study support the fact that juvenile lumpfish have an association with certain algal species that create specific, preferred habitats. Juvenile lumpfish were only caught in association with a few other
species: *Carcinus maenas*, *Tautogalabrus adspersus*, and *Pholis gunnellus*. Co-occurrence is an indication of overlap in habitat preferences, which also could lead to competition. Increased populations of any of these species, specifically of the invasive *C. maenas*, could affect juvenile lumpfish populations.

Conclusions

Juvenile lumpfish were found at locations in the estuary with salinities as low as 22 ppt. While there were no juveniles caught during this survey in Great Bay, there have been individuals caught in other surveys, leading us to believe that there are juvenile lumpfish present far up into the estuary. Our inability to capture juveniles at the uppermost sampling site (JEL) may have been associated with the lack of macroalgae at this location. Despite adding kelp lines in the second year to help create preferred habitat, still no lumpfish were caught. This may indicate that the numbers in the upper bay were small or that the conditions at the sampling location (e.g. current speed, prey availability) were unfavorable. If, indeed, the numbers occupying the upper bay are small, it may indicate that a combination of higher temperatures and lower salinities may be deterring lumpfish from entering the bay. Increased temperatures may cause mortalities in juveniles if there are extended periods of time above their critical thermal maximum, which is between 21.9 – 22.3 °C (Ern et al. 2016). Temperatures in Great Bay averaged around 22.5 °C in July and August in both years which may be reaching the upper thermal limits of juvenile lumpfish. Higher temperatures also may be attributing to habitat degradation since kelp, which also is a cold-water species, cannot tolerate extended periods of warmer temperatures.
In 2016, 79 juvenile lumpfish were caught using dip nets, while in 2015 only 33 were collected. This difference in numbers between years could be a normal fluctuation in year class strength or could be an indication of other factors affecting the population (i.e. temperature, salinity, prey availability, etc.). There were only four study locations along the salinity gradient of the river into the bay. To further resolve the small number of sampling locations, future studies should include more sampling locations throughout the estuary in order to fully understand the distribution of juvenile lumpfish. With the changing climate, it is important to completely investigate what factors are truly driving juvenile lumpfish distribution and habitat preferences so that we are able to understand this important commercial fishing and aquaculture species better.
Table 2.1. Species composition and abundance from 2015 sampling with Dip netting (DN), Otter trawl (OT), and Plankton nets (PN) at all four sampling locations from June through September.

<table>
<thead>
<tr>
<th>Genus</th>
<th>Species</th>
<th>JEL</th>
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<th>GCM</th>
<th>WM</th>
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</tr>
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<td>Apeltes</td>
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Table 2.2. Species composition and abundance from sampling with dip netting (DN), artificial kelp (AK), and real kelp (RK) at all four sampling locations in 2016 from June through September.

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<th>AK</th>
<th>RK</th>
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<th>RK</th>
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<th>AK</th>
<th>RK</th>
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Table 2.3. Lumpfish counts from sampling on artificial kelp (AK) and real kelp (RK) by month at all four sampling locations in 2016.

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Figure 2.1. Study locations for all gear types in 2015 and 2016. Locations were the Jackson Estuarine Laboratory (JEL), Great Bay Marina (GBM), Great Cove Marina (GCM), and Wentworth Marina (WM).
Figure 2.2. Environmental data from all sampling locations in 2015. Daily Coastal Lab averages come from the Northeastern Regional Association of Coastal Ocean Observing Systems (NERACOOS) and daily Great Bay averages come from the Great Bay National Estuarine Research Reserve (GBNERR).
Figure 2.3. Environmental data from all sampling locations in 2016. Daily Coastal Lab averages come from the Northeastern Regional Association of Coastal Ocean Observing Systems (NERACOOS) and daily Great Bay averages come from the Great Bay National Estuarine Research Reserve (GBNERR).
Figure 2.4. Length frequency distribution of juvenile lumpfish from dipnet sampling in 2015 and 2016 at all sites combined.
Figure 2.5. Length frequency distribution by month of juvenile lumpfish from dipnet sampling in both years at all sites combined.
Figure 2.6. Monthly distribution of juvenile lumpfish from dipnet sampling in 2015.
Figure 2.7. Monthly distribution of juvenile lumpfish from dipnet sampling in 2016.
Figure 2.8. Catch per unit effort for juvenile lumpfish caught by dipnet by location in 2015. There were no significant difference between sites where lumpfish were found.

Figure 2.9. Catch per unit effort for juvenile lumpfish caught by dipnet by location in 2016. There were no significant difference between sites where lumpfish were found.
Figure 2.10. Catch per unit effort for juvenile lumpfish caught by dipnet by location with both years combined. There were no significant difference between sites where lumpfish were found.
GENERAL CONCLUSIONS

The results from this research have provided more insight into the physiology and ecology of the juvenile lumpfish. Juveniles were found to survive for several days in salinities as low as 5 ppt with no visible signs of stress. Lowest standard metabolic rate was found at 10 ppt and the highest at 20 ppt. Although blood osmolality was not measured, it likely matched the osmolality of seawater at 10 ppt. When given a choice, however, juveniles tended to prefer salinities of 20 ppt or higher. These studies show that juvenile lumpfish can tolerate low salinity environments, and are more euryhaline than previously thought, although the physiological mechanisms remain unclear. Clearly further investigations are needed to fully understand the salinity tolerance of juvenile lumpfish, their osmoregulatory capabilities, and if ontogenetic changes occur.

In the wild, juveniles were found in salinities as low as 22 ppt and temperatures as high as 22.5 °C, but were never found in Great Bay, NH during this study. Temperatures in Great Bay exceed the thermal threshold of lumpfish, and may be a contributing factor to the paucity of lumpfish in this location. Juvenile lumpfish have, however, been seen in the Great Bay by other researchers, suggesting that they may be using the upper estuary as a potential habitat, at least for certain parts of the year depending on temperature and salinity. Since it is likely that spawning occurs only in coastal locations, the mechanisms whereby juveniles arrive at estuarine locations should be investigated.

With the changing climate and the growing need for cleaner fish in the aquaculture industry, it is important to completely understand the physiology of lumpfish and investigate what environmental factors are determining juvenile lumpfish distribution and habitat preferences. Although much remains to be learned, results from both laboratory experiments and
the distribution studies indicate that lumpfish may have a higher tolerance for lower salinities than previously thought, and may thus be suitable for use as a cleaner fish in brackish water aquaculture operations. Since these areas typically reach higher temperatures than coastal waters, it will be important to investigate the temperature tolerance of juvenile lumpfish as well.
Literature Cited


APPENDIX

University of New Hampshire

Research Integrity Services, Service Building
51 College Road, Durham, NH 03824-3585
Fax: 603-862-3554

19-Dec-2014

Howell, William H
Biological Sciences, Rudman Hall
Durham, NH 03824

IACUC #: 141106
Project: Effect of Salinity on the Oxygen Consumption Rate of Juvenile Lumpfish (Cyclopterus lumpus) in the Laboratory, and their Temporal and Spatial Distribution in the Great Bay Estuary-Piscataqua River, New Hampshire
Category: D
Approval Date: 11-Dec-2014

The Institutional Animal Care and Use Committee (IACUC) reviewed and approved the protocol submitted for this study under Category D on Page 5 of the Application for Review of Vertebrate Animal Use in Research or Instruction - Animal use activities that involve accompanying pain or distress to the animals for which appropriate anesthetic, analgesic, tranquilizing drugs or other methods for relieving pain or distress are used.

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. Information about the program, including forms, is available at http://unh.edu/research/occupational-health-program-animal-handlers.

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

For the IACUC,

[Signature]
Dean Eder, D.V.M.
Vice Chair

cc: File