Reducing cannibalism among larval striped bass (*Morone saxatilis*): effect of rearing density and *Artemia* prey abundance

By

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Abstract

Striped bass larvae (range 16 to 38 days post-hatch, dph), in a series of trials (Trial 1 to 3) of 14 days duration in recirculation systems (tank volume 100 to 200L), exhibited survival and growth that was positively related to ration (70 to 350 stage II enriched *Artemia*/larva/meal, five meals daily) but independent of stocking density (range 1 to 9 larvae/L). Similarly, in smaller static water tanks (15L volume), 2x2 factorial trials (Trial 4 and 5) comparing stocking density (3 and 15 larvae/L, 12 to 28dph) and ration (50, and either 10 or 200 *Artemia*/larva/meal, five meals daily), survival, growth were significantly dependent on ration ($p<0.001$) but independent of stocking density, with no interaction effects. The incidence of cannibalism was inversely related to ration size but independent of stocking density. For efficient production of juveniles in recirculation systems, survival of >90% and cannibalism of <7% can be achieved by offering 250 enriched *Artemia*/larva/meal, five times per day (09:00-21:00h) at a density of up to 15 Larvae/L from 24 to 38dph.

List of Abbreviations Used

ANOVA	Analysis of variance
BL/S	Body length per second
BW	Body weight
COSEWIC	Committee on the Status of Endangered Wildlife in Canada
CV	Coefficient of variation
Dal-AC	Dalhousie University Agricultural Campus
DFA	Department of Fisheries and Aquaculture-Government of Nova Scotia
DFO	Department of Fisheries and Ocean
dph	Days post-hatch
EMS	Electromagnetic stirring
FW	Fresh water
g/L	Gram per liter
GIL	Growth in length
GLM	General linear model
ISB	Inflated swim bladder
LD	Light : dark cycle
LED	Light-emitting diode
m^2	Square meter
ml	Milliliter
MS222	Tricaine methanesulfonate
NS	Nova Scotia
NSB	Non-inflated swim bladder
NTU	Nephelometric turbidity unit
ppm	Parts per million
ppt	Parts per thousand
PVC	Polyvinyl Chloride
R	Ration
RAS	Recirculation aquaculture system
S	Stocking density
SGR	Specific growth rate
SE	Standard error
SW	Sea water
TAN	Total ammonia nitrogen
TL	Total body length
µg	Microgram
µm	Micrometer

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Chapter 1: Introduction

Globally, the impetus to develop marine aquaculture is strong due to over-exploitation of the wild fishery. After wild capture fishery production plateaued in the late 1980s at around 90 million tonnes (MT) per annum, aquaculture has been responsible for supplying the continued growth in human consumption of fish. Consequently, fish production contributed by aquaculture increased from 17.2MT in 1990 to 114.5MT in 2018 (FAO 2020).

Among marine teleosts in the wild, recruitment success is linked to nutritional, physical and climate-related variables. Among striped bass (*Morone, saxatilis*), inter-annual variability in abundance of under-yearling juveniles can differ more than 30-fold depending on the survival rate through the larval stage (Shideler and Houde 2014). The larval stage is often a bottleneck to recruitment due to high mortality rates, a consequence of their complex ontogeny and delicate nature. To compensate for the high mortality rate, many marine fish species allocate a large portion of their resources to reproductive activities, producing a large number of eggs, and are known as R-Strategists (Adams 1980**).** The two most famous hypotheses that explained the recruitment variability among marine fish are the critical period (Hjort 1914), and its extension, the match-mismatch (Cushing 1990). Both emphasized the importance of suitable abundance and type of planktonic prey during the critical first-feeding period. Oiestad (1985) proposed a new hypothesis to

emphasize the importance of predation and cannibalism on survival of larval fish. More recently, it was proposed that recruitment success is influenced by multiple interacting factors including temperature, hydrodynamics, predation, prey availability, and larvae growth rate (Houde 2008). For striped bass larvae in Chesapeake Bay for example, the recruitment success was influenced by the spring freshwater flow and air temperature, which control the environmental and hydrographic conditions that influence spatio-temporal overlap of larvae and zooplankton (Martino and Houde 2010). When suitable prey are available, the rapid growth exhibited by fish larvae can quickly reduce their vulnerability to predation and increase their chances of survival and attaining recruitment. The feeding success of larval fish is dependent on the development of sensory organs such as the eye, lateral line, olfactory and systems related to locomotion such as swim bladder, gills and myotomes and environmental conditions such as light intensity and hydrodynamics (Hubbs and Blaxter 1986).

During intensive culture of larval marine fish, despite the ability to control environmental conditions, food availability and stocking density, the correct protocols for good survival and growth remains uncertain for many species. Cannibalism can be particularly problematic. It is a feeding strategy that involves killing and consuming the whole or part of an individual of the same species and has been recorded for 390 teleost species from 104 families (Pereira et al. 2017). During intensive fish culture, cannibalism can have a great impact on the survival of larval teleosts. Among sharptooth catfish (*Clarias gariepinus*) for example, losses due to cannibalism ranged from 10 to 97.5%

(Hecht and Appelbaum 1988). Among striped bass larvae, by comparison, a cannibalism rate of 12 to 44% occurred during the first month larval stage (Braid and Shell 1981). In oligotrophic oceanic waters, cannibalism has been considered a population regulatory strategy by decreasing starvation induced mortality among, for example, larval bluefin tuna (*Thunnus thynnus*; Uriarte et al. 2019). The incidence of cannibalism among larval fish is regulated by many factors, such as prey density, stocking density, light intensity, size variation, prey type (Hecht and Pienaar 1993, Pereira et al. 2017). The encounter rate between predator and prey are critical to survival of larval fish (Rothschild and Osborn 1988). In this thesis, encounter rate was manipulated by utilizing prey density and fish stocking density as experimental factors.

Striped bass are an anadromous teleost fish of the family *Moronidae*. The natural range of striped bass is along the Atlantic coast of North America from the St. Lawrence River in Canada to the St. Johns River in Northeastern Florida (Hardy 1978). In the Shubenacadie River estuary in Canada, spawning mostly occurs in May and June after the accumulation of 11 to 20 degree-days above 12℃ (Duston et al. 2018). The body size of striped bass larvae is similar to many other marine pelagic fish, ranging from 3.5mm at the yolk sac stage to 5 to 7mm at the first feeding stage (Duston et al. 2018). In the Shubenacadie River estuary, small adult harpacticoid copepods are the initial prey for first feeding larvae and remain the dominant food until larvae reach 15mm total length (TL; Findlay 2019). Mysids become the main prey when larvae are 16 to 20mm TL and early juveniles (21 to 40mm TL) mostly feed on amphipods (Duston et al. 2018, Findlay 2019).

Juveniles then migrate from the estuary into Cobequid Bay and remain for the growing season during the summer. Striped bass typically reach maturation in 4 to 6 years for females and 3 to 4 years for males (Berlinsky et al. 1995). In Chesapeake Bay, the recruitment of striped bass larvae was strongly associated with the spatio-temporal match between high abundance of zooplankton prey and larval striped bass production (Martino and Houde 2010). The growth and survival of the young of the year striped bass was density dependent in Chesapeake Bay except for the Potomac River (Martino and Houde 2012).

Striped bass culture at Dalhousie University Agricultural campus (Dal-AC) was initiated in 1996 by J. Duston. Research efforts have included effects of light intensity on larval feeding (Duston and Astatkie 2012; MacIntosh and Duston 2007; Sampson et al. 2013), and juvenile grow out trials (Duston et al. 2004, Tonning 2019). Striped bass is a potential species to diversify a Nova Scotia aquaculture sector that is currently dominated by salmonids (DFA 2019). Cannibalism during the larval stage beginning around 12 dph has been clearly evident among striped bass reared at Dal-AC, hence the motivation for this thesis. Similarly, cannibalism has been reported among US striped bass larvae in culture associated with insufficient live prey (Braid and Shell 1981; Paller and Lewis 1987). Live *Artemia* prey are essential for cultured striped bass larvae, resulting in better growth and less cannibalism than larvae fed a formulated diet (Paller and Lewis 1987). The effect of live prey density on larval striped bass growth and survival has been reported in only four papers, the most recent being almost 30 years ago (Chesney 1989; Eldridge et al. 1981; Houde and Lubbers 1986; Tsai 1991). However, among the primary literature on striped

bass there are no empirical data on the daily ration and stocking density requirements through larval development. Solving the cannibalism problem would be a significant step forward toward the goal of commercializing striped bass aquaculture in Nova Scotia. Hence, the objective of this thesis was to gain a better understanding of the relationship between stocking density, ration and striped bass survival, growth and cannibalism. Chapter 2 reviews the state of knowledge of the life history and factors affecting the survival-growth-cannibalism dynamic of larval striped bass, including recent unpublished experiments preceding this thesis. Chapter 3 describes the general materials and methods. Chapters 4 and 5 present and discuss the results of a series of trials that quantified effect of a wide range ration levels and stocking densities on larvae survival and growth. Chapter 6 presents and discusses the main and interaction effect of stocking densities and ration sizes on larvae survival, cannibalism and growth. Chapter 7 is the conclusion of this thesis.

Chapter 2: Literature review

This chapter reviews the state of knowledge and establishes the rationale for the research and the experimental approach. Section 2.1 presents the striped bass life cycle; section 2.2 considers the history and the future prospects of striped bass aquaculture in North America. Section 2.3.1-2.3.3 reviews the relationship between prey density, stocking density and cannibalism on survival and growth of pelagic larval fish.

2.1 Striped bass life history

Striped bass (*Morone saxatilis*) is an anadromous teleost fish of the family *Moronidae*, the temperate basses. The natural range of striped bass is along the Atlantic coast of North America from the Saint Lawrence River in Canada to the Saint Johns River in Northeastern Florida. They are also found in the Gulf of Mexico from western Florida to Louisiana (Hardy 1978). In Atlantic Canada, there are two genetically discrete populations: the Bay of Fundy population spawns in the Shubenacadie-Stewiacke River and the Miramichi population (Wirgin et al. 1993). Historically they spawned in five rivers: the Saint John and Miramichi Rivers in New Brunswick, the Annapolis and Shubenacadie-Stewiacke Rivers in Nova Scotia and the St. Lawrence River in Quebec. The Saint John River population was confirmed only spawning once in 1975 in Belleisle Creek (Andrews et al. 2017). In a recent study, significant differences in the DNA sequence was found among juvenile striped bass sampled from Saint John River, Shubenacadie River, Hudson River

and Chesapeake Bay indicating the genetically distinct population of striped bass within the Saint John River still exists (Leblanc et al. 2018). The Annapolis River population is believed to have been extirpated in the last 40 to 50 years (Douglas et al. 2003). In the Bay of Fundy, the Shubenacadie-Stewiacke River is the only productive spawning area (COSEWIC 2012). In Canada, striped bass are exclusively anadromous, migrating between freshwater spawning areas and brackish or saltwater feeding areas in coastal areas. Age at maturity among populations in Atlantic Canada is about 3 to 4 years for males and 4 to 6 years for females (Berlinsky et al. 1995). Striped bass are iteroparous and highly fecund; females with mean fork length 81.5cm (range 77 to 86cm) and 6.68kg (range 5.72 to 8.23kg) and age 9.5 years (7 to 13 years old) sampled from the Shubenacadie River, had a mean fecundity of 905,254 eggs (range 0.5 to 1.4×10^6 eggs; MacInnis 2013). The spawning season in the Shubenacadie-Stewiacke River system typically begins in late May and lasts for 38 days, on average (Duston et al. 2018). Eggs are mostly distributed between 1 and 5ppt salinity (Duston et al. 2018). At 17°C, eggs hatch in about 48 hours (Hardy 1978). The larval development of striped bass can be divided into three stages, the yolk sac larvae TL ranges from 3-8 mm (Doroshev 1970); the fin-fold larvae stage lasts for 10-13 days (TL range 8 to 12mm; Hill et al. 1989); post fin-fold larvae reach about 30 mm length in 20-30 days (Hill et al. 1989). In culture, an upwelling water current is required to keep the eggs and larvae from settling on the bottom and suffocating, especially in the first 24 hours post-hatch (Rees and Harrell 1990). Total yolk absorption coincides with the onset of feeding at 5-7 days post-hatch (dph, Eldridge et al. 1981), at which time larvae inflate their swim

bladder and have a functional mouth. An oil globule provides additional endogenous nutrition enabling striped bass larvae to survive starvation up to 31 dph (Eldridge et al. 1981). In the Shubenacadie River, prey items among wild striped bass larvae start with small (<0.5 mm) copepods when the TL is around 6mm. Lack of suitable type and size of copepod prey in June is proposed to be an important lethal factor in the Shubenacadie River (Duston et al. 2018). In Chesapeake Bay, there is better evidence the spatial and temporal abundance of zooplankton matches the peak production of striped bass larvae, a factor critical to larvae survival and recruitment success (Martino and Houde 2010). The estuarine turbidity maximum (ETM) there provides a favorable nursery habitat for striped bass larvae, because of the high concentration of prey, mostly copepods (*Eurytemora affinis*) and a cladoceran (*Bosmina longirostris*, Martino and Houde 2010). Years with high freshwater flow are associated with better recruitment, enhancing the encounter rate and feeding opportunities near the ETM (Martino and Houde 2010, Shideler and Houde 2014).

The survival and growth of striped bass larvae changes throughout development and is dependent on an interaction between temperature and salinity. Among 10-17 dph larvae, 26 ℃ at 10ppt salinity resulted in best growth and survival, whereas at 30ppt, 21℃ resulted in best survival (Cook et al. 2010). Among larvae 23-29 dph, highest survival and growth was at 26℃, independent of salinity (1-30ppt, Cook et al. 2010). The minimum oxygen requirement for egg development is 3-5 mg/L, and larvae require 5-6 mg/L (Harrell and Bayless 1981). During the larval stage, low salinities (3-5 ppt) can enhance survival and growth and is essential for the Shubenacadie stock (Duston et al. 2018). In freshwater,

survival of larval striped bass is very poor (Bayless 1972). The highest density of larvae in the Shubenacadie River was between 1 and 10ppt and decreased significantly out of this salinity range (Duston et al. 2018). Swimming ability improves as the fish grow, enabling young striped bass in the Shubenacadie River to control their positioning better, and is associated with their distribution broadening throughout the summer (MacInnis 2013). By August and September, young-of-the-year (YOY) juveniles are widely distributed between the Shubenacadie and Stewiacke Rivers, and Cobequid Bay and the inner portion of Minas Basin (Bradford et al. 2015). The severity of winter following the first growing season can affect the recruitment variability of YOY striped bass (Hurst and Conover 1998). YOY striped bass need to achieve a body length of around 10cm at the end of first growing season to survive over winter (Bradford et al. 2012, Bradford and Chaput 1997, Hurst and Conover 1998).

2.2 Striped bass aquaculture

Since the earliest European settlements, striped bass has provided important commercial and recreational fisheries in Atlantic Canada (Rulifson and Dadswell 1995). The commercial harvest of striped bass in Atlantic Canada was mostly reported in Nova Scotia and New Brunswick and primarily from bycatch of other species such as American shad (*Alosa sapidissima*) and Atlantic salmon (*Salmo salar*, Rulifson and Dadswell 1995). Striped bass commercial landings in Nova Scotia was prohibited in 1970 and New Brunswick in 1986 due to low spawning success and stock abundance (Rulifson and

Dadswell 1995). In the late 1980's, following the success of salmon farming and the hybrid striped bass aquaculture in the US, the Canadian federal government invested in researching alternative aquaculture species, and the striped bass was considered a candidate in the Maritimes (Martin-Robichaud et al. 1991). A striped bass aquaculture program was initiated at St. Andrews Biological Station in 1988, and in 1989 expanded to the Huntsman Marine Science Centre (Cairns et al. 1999). A total of 19 facilities in eastern Canada engaged in striped bass research and development, including Nova Scotia Agricultural College (now Dal-AC; Cairns et al. 1999). Regrettably, a sustainable industry failed to evolve due to difficulties in capital investment, low seedstock production, lack of appropriate sites, inadequate equipment, and uncertain husbandry practices (Cairns et al. 1999, DFO 1999). However, the market demand remained, and striped bass aquaculture in ponds or tanks was believed to have the most potential (DFO 1999). The Two Rivers Bass Hatchery in Stewiacke, Nova Scotia was a privately operated facility at the confluence of the Stewiacke and Shubenacadie Rivers. The hatchery-produced juveniles for grow-out and provided seedstock to other facilities in PEI and Massachusetts (Cairns et al. 1999). However, the hatchery was unsuccessful as a business because of low production of juveniles associated with larval stage cannibalism linked to underfeeding of *Artemia* live prey (personal communication, B. Stone, farm manager, to J. Duston). Dr. J. Duston from Dal-AC remains interested in commercializing striped bass aquaculture as the market demand remains strong. Dal-AC is an ideal location for this research due to its proximity to the Shubenacadie River which facilitates studying striped bass eco-physiology and the

campus has the infrastructure to rear this species. Recent research on the striped bass larval stage led to refinement of rearing protocols in terms of lighting, salinity and temperature. Younger larvae (9-11 dph) capture prey more efficiently in dim light (4-34 lux) while older larvae (>22 dph) caught more prey in higher light intensities (34-461 lux), light threshold for visual feeding was between 0.0084 and 0.03 $\mu mol\ s^{-1}m^{-2}$ (MacIntosh and Duston 2007). Among juveniles, the optimal temperature for growth decreased as body size increased; 40g fish grew best at 24 to 28℃, fish over 500g grew better at a lower temperature of 16 to 20℃ (Duston et al. 2004).

In North America, the largest striped bass producer is Pacifico Aquaculture. Fish are grown-out in sea-cages in Baja California, Mexico, producing about 545 metric tons in 2018 and the predicted harvest for 2019 was 1200 tonnes (Monterey Bay Aquarium Seafood Watch Striped Bass Mexico 2020). In the US, hybrid striped bass, a cross between white bass *Morone chrysops* and striped bass, are the most popular cultured *Morone sp.* with an annual production in 2018 of 3941 tonnes (USDA 2019). The production strategy is semi-intensive in four stages: 1) egg incubation and hatching; 2) fertilized ponds with a high stocking density at 75,000 to 150,000 larvae per hectare; 3) juveniles at lower stocking density 25,000 to 60,000 per hectare; and 4) grow out at lower density of 8,000-10,000 per hectare (Harrell 1997). Culture of striped bass in Nova Scotia, has not advanced past small research trials on juvenile grow-out at North River Fish Farms Limited (NRFF). Nevertheless, the good performance of these fish motivated seven other farms in Nova Scotia to become licensed to grow striped bass. Striped bass culture can adopt a less

controversial method than open-net cage culture in coastal waters by growing them in constructed freshwater ponds, which meets the theme 'Low-impact and High-value Aquaculture' embraced by the Nova Scotia Government (Doelle and Lahey 2014). Towards the goal of sustainable striped bass aquaculture in Nova Scotia, the next challenge is to increase the production of juveniles by optimizing survival through the larval stage by addressing the cannibalism problem. The following section reviews the factors that contribute to the variability in survival and growth among larval fish including striped bass.

2.3 Factors affecting the survival of larval fish

Among intensively cultured teleosts, both biotic and abiotic factors affect the survival and growth of early life stages. The former include variation in genetic expression, sex, and body size (Hossain et al. 1998). Abiotic factors are usually those that can be controlled by culturists, including stocking and prey densities, temperature, turbidity, light intensity, photoperiod (Baras 2003). This thesis focuses on prey availability, stocking density as factors influencing cannibalism, survival, and growth among striped bass larvae.

2.3.1 Cannibalism

Cannibalism is a feeding strategy that involves killing and consuming the whole or part of an individual of the same species, irrespective of its stage of development. Cannibalism is more severe in larval and juvenile stages as young fish have a higher growth capacity than adults, they consume relatively large rations as a percentage of their body

mass, and their mouth gape is relatively big compared to the body (Hecht and Pienaar 1993). The number of families and species reported to exhibit cannibalism increased respectively from 36 and 106 in 1991 to 104 and 390 in 2017; of these, 150 species are within the captive fish category (Table 2.1; Pereira et al. 2017, Smith and Reay 1991). These direct observations of cannibalism were usually from aquaculture research as evidence of cannibalism in nature is sparse. However, among the primary literature on striped bass, only three studies reported cannibalism (Braid and Shell 1981, Paller and Lewis 1987 Rhodes and Merriner 1973). Among marine pelagic fish species, cannibalism was considered an evolutionary stable strategy in environments in which productivity is low (Nishimura and Hoshino 1999).

Table 2. 1 Species exhibiting cannibalism cited in this thesis and the sources.

Species	References
African sharptooth catfish *Clarias gariepinus*	Hecht and Appelbaum 1988
Walleye *Stizostedion vitreum*	Loadman et al. 1986
Striped bass *Morone saxatilis*	Braid and Shell 1981
European bass *Dicentrarchus labrax*	Katavić et al. 1989
Spotted seatrout *Cynoscion nebulosus*	Manley et al. 2014
Northern pike *Esox Lucius*	Kucharczyk et al. 1998
Dorada *Brycon moorei*	Baras et al. 2000
Perch *Perca fluviatilis*	Byström et al. 2012
Atlantic cod *Gadus morhua*	Folkvord 1997
Atlantic bluefin tuna *Thunnus thynnus*	Uriarte et al. 2019
Pikeperch *Sander lucioperca*	Zakęś 2012
African catfish *Clarias gariepinus*	Mukai et al. 2013
Giant grouper *Epinephelus lanceolatus*	Hseu et al. 2004
North American burbot *Lota lota maculosa*	Barron et al. 2013

Cannibalism causes two opposite effects on larval fish, on one hand, it increases the mortalities due to predation, on the other hand, it reduces the mortalities caused by

starvation (Nishimura and Hoshino 1999). Recently, DNA analysis of gut contents of wild bluefin tuna revealed the evidence of cannibalism in the natural habitats (Uriarte et al. 2019). Cannibalism was first reported in wild Atlantic bluefin tuna larvae (*Thunnus thynnus*) in the relatively oligotrophic environment of the Balearic Sea, where cannibalism decreased the starvation induced mortality and potentially improved larval tuna survival (Uriarte et al. 2019). In the wild, higher cannibalism rates would be beneficial for the survival of the population; however, in aquaculture, the primary goal is to rear larvae quickly and efficiently with minimum losses, so cannibalism needs to be controlled (Hecht and Pienaar 1993). Cannibalism is the bottleneck in the commercial rearing of many fish species due to massive mortalities during production. Among sharptooth catfish for example, cannibalism accounted for 97.5% of the mortalities when 14 dph larvae were stocked at 5 larvae/L and fed 5% BW of dry pellets per day for 26 days (Hecht and Appelbaum 1988). Two types of cannibalism occur: 1) tail-first and 2) head-first. Tail-first cannibalism, which occurs in the early larval phase, is independent of body size differences between prey and predator as the victim is partially ingested from the tail (Hecht and Appelbaum 1988). Growth heterogeneity occurs within a population practicing tail-first cannibalism as the nutrients that cannibals derive from consuming conspecifics are greater than those obtained through consumption of plankton prey. Tail-first cannibalism is eventually replaced by head-first cannibalism due to growth advantages achieved by tail-first cannibals in the early larval stage. Sharptooth catfish larger than the cohort average due to tail-first cannibalism then engaged in head-first cannibalism, where prey were

consumed beginning with the head to avoid the spiny fin rays of the dorsal or pectoral fins (Hecht and Appelbaum 1988). Head-first cannibalism requires body size difference between cannibals and prey and occurs in the later larval stages, it can be mitigated by size sorting to maintain similar sized fish per holding tank when fish are large enough to be size graded effectively in some species (Baras and Jobling 2002). In contrast, tail-first cannibalism can be only mitigated by adjusting the rearing conditions such as stocking density, light intensity, temperature, food regime (Baras and Jobling 2002). Among striped bass larvae reared at Dal-AC, tail-first cannibalism is very common when fish are 12 dph and about 10mm TL, underfeeding is one of the most common factors promoting tail-first cannibalism (J. Duston, *pers. comm*.).

Consistent with our experiences at Dal-AC, striped bass cannibalism reported in the literature starts among larvae ranging in age from 6 to 18 dph (Braid and Shell 1981, Rhodes and Merriner 1973). In tail-first cannibalism, larvae can cannibalize similar-size siblings, with trunk and tail attacks most common (Loadman et al. 1986). The percent mortalities caused by tail-first cannibalism is species specific, ranging from 1.5 to 12% of the initial population in European perch (*Perca fluviatilis*, Baras et al. 2003, Babiak et al. 2004, Król and Zieliński 2015) and 10 to 17% in pikeperch larvae (*Sander lucioperca* , Zakęś 2012) and up to 39.1% in dorada (*Brycon moorei*, Baras et al. 2000). The ratio of TL between cannibals and prey for successful head-first cannibalism varied by species: in Atlantic cod (*Gadus morhua*), a 25 to 75% difference in TL is required for successful ingestion (Folkvord 1997, Folkvord and Otterå 1993); 30% in giant grouper (*Epinephelus*

lanceolatus, Hseu et al. 2004); 50% in barramundi (*Lates calcarifer*, Ribeiro and Qin 2013); and 88% for pike (Szczepkowski 2009). In the primary literature on striped bass there are no studies examining the ratio of TL between cannibals and prey for a successful head-first cannibalism.

The intensity and rate of head-first cannibalism were positively affected by size heterogeneity within a cohort. Among Atlantic cod larvae the cannibalism rate was significantly affected by initial BW coefficient of variation (CV), ranging from 0.03%/day to 5%/day in the smallest (28%) and largest (68%) initial CV treatments respectively (Folkvord and Otterå 1993). Cannibalism first occurred when the max: min weight ratio exceeded 6.5:1 and was the primary mortality factor when this ratio exceeded 8.5:1 (Folkvord and Otterå 1993). The risk of head-first cannibalism might be better measured by the relative size between the largest and smallest individuals in the population rather than the coefficient of variation of TL or BW in some species (Folkvord 1997). Among North American burbot (*Lota lota maculosa*) for example, up to 86% of the larvae were lost due to cannibalism in a 15-day trial in one replicate tank (6.5L) stocked with 100 larvae which contained two larger fish, for tanks without any larger fish, the cannibalism was minimal to non-existent (Barron et al. 2013).

Head-first cannibalism can be significantly mitigated by size sorting. North American burbot (80 dph) were divided into three groups upon first exhibiting cannibalistic behavior: 1) the control, 2) passed and 3) retained grader groups with a mean TL of 11.8, 10, and 13.8mm respectively (Barron et al. 2013). Grading significantly reduced size heterogeneity

in the control relative to the retained grader group with 11.7 and 18.1% TL CV respectively (Barron et al. 2013). In turn, grading reduced the cannibalism rate significantly in passed and retained grader groups at 14.3 and 1.0% compared to 29% in the control group (Barron et al. 2013). Similarly, pikeperch were graded into three groups: small, large and unsorted (control) with the mean BW of 0.04, 0.08 and 0.06g and a CV of 27.9, 33.8 and 34.2% respectively. Cannibalism rates were lowest in the small body size group followed by the large body size group, and greatest in the unsorted group with cannibalism rates of 11.3, 21.3 and 29% respectively (Szczepkowski et al. 2011). However, the body size of fish larvae used in these two studies were relatively larger than other studies. Exploring the appropriate ration level and stocking density is key to reduce cannibalism among those exhibited cannibalistic in the very early age are too delicate to be graded, hence, the motivation for this thesis.

2.3.2 Larval fish feeding, growth and survival in relation to feeding regime

In aquaculture, selecting appropriate prey and their size is critical for the survival and growth of first feeding pelagic fish larvae (Van der Meeren and Næss 1993). Live feed is needed to stimulate first feeding among marine fish larvae of the altricial type which remain relatively underdeveloped state until the yolk sac is exhausted. Natural zooplankton are both difficult to culture on a large scale and difficult to collect from the wild environment. Due to these limitations, marine rotifers (*Brachionus plicatilis)* and brine

shrimp nauplii (*Artemia* spp.) have been introduced to the larval stages of most marine species as live feeds (Callan et al. 2003). In addition to prey types and sizes, prey density is also critical as most larval stages of marine fish have limited swimming ability, so encounter probability and feeding success increases with prey density (Slembrouck et al. 2009). Therefore, accurate estimates the optimal prey density for larval fish are essential for successful aquaculture production.

The feeding success of larvae improves rapidly with morphological development, age and experience, while first feeding larvae are the most vulnerable to starvation. Feeding success rate, defined as the percentage of larvae with prey captured, can be quantified by examining gut contents. Feeding success of larval anchovy (*Archosargus rhomboidalis*), offered the rotifer *Brachionus plicatilis* at a concentration of 10000/liter was 32% at 5 dph, increasing to 88% at 21 dph (Hunter 1972). This increase was associated with improved swimming speed, which was proportional to body length; the volume of water searched also increased exponentially with size (Hunter 1972). Likewise, among larval Atlantic cod, swimming speed, perception distance, search volumes increased with body size, all associated with improved prey capture (Von Herbing and Gallager 2000). Prey density also affects capture success. The feeding success of Atlantic cod larvae was significantly higher in 4000 *Artemia*/L treatments than lower prey densities (0, 500, 1000 and 2000 *Artemia*/L), increasing steadily from approximately 60% at 23 dph to 100% at 27 dph when offered 4000 *Artemia*/L (Puvanendran et al. 2002). Since encounter rate is proportional to prey density, larvae in lower prey density environments spend more energy locating and

capturing live *Artemia* (Puvanendran et al. 2002). Among striped bass larvae, prey capture rate increased with respect to both age and prey density under both dark and light conditions. In 50 and 800 *Artemia*/L treatments, the capture rate in the dark at 9 dph was 4 and 20 *Artemia*/larva/hour respectively, which increased to 26 and 220 *Artemia*/larva/hour at 22 dph (Duston and Astatkie 2012). Under light (20 lux) conditions, the capture rate among 8 and 11 dph larvae was about 15 *Artemia*/larva/hour; at 13 dph, increasing to 65 *Artemia*/larva/hour in 50 *Artemia*/L and over 80 *Artemia*/larva/hour in both 200 and 800 *Artemia*/L densities (Duston and Astatkie 2012). The sustainable swimming speed increased from 2.8 body lengths per second (BL/s) to 3.8 BL/s when striped bass larvae grew from 6.0-6.9 to 8.0-8.9mm TL (Meng 1993). The improvement of swimming speed contributes to the improving feeding success by increasing the volume of water larvae are capable of searching for food (Meng 1993).

Prey abundance is highly correlated with larval feeding success, growth and survival. Striped bass larvae were offered 0, 10, 100, 500, 1000 and 5000 *Artemia*/L from 7 to 30 dph with food and water changed daily. Larvae lost weight at <100 *Artemia*/L and grew at prey densities ≥100/L (Eldridge et al. 1981). The average daily instantaneous mortality rate was inversely proportional to food concentration; final survival rate was over 80% in 1000 and 5000/L treatments but decreased significantly to ca. 50% and 30% in 100 and 10 prey/L treatments (Eldridge et al. 1981). In another study on striped bass larvae, a lower prey density range was evaluated, from 0 to 1000/L depending on the prey types (Tsai 1991). The survival rate was also proportional to prey density irrespective of stages of *Eurytemora*

affinis, adult (0, 5, 10, 25, 50, 100/L), copepodite (10, 20, 50, 100, 200/L) and nauplius (50, 100, 250, 500, 1000/L) starting from 9 dph for 16 days (Tsai 1991). The relative nutritional value was similar among all three stages of prey, but due to the weight difference between each stage, one adult prey was equal to 1.45 copepodites or 11.12 nauplii; to achieve 60% survival from 9 to 24 dph, 100 adult/L or 145 copepodites/L or 1112 nauplii/L were needed (Tsai 1991). The minimum satisfactory prey density was not determined in this study because both survival and growth of larvae increased as the prey density increased (Tsai 1991). In contrast, over 90% survival was achieved when 5 dph larvae were offered 100 *Artemia*/L for 20 days (Chesney 1989). When offered *Eurytemora affinis* (70% of nauplii, 30% of copepodites and adults), by comparison, the survival rate increased from 61 to 87% at a density of 50 and 250/L respectively (Chesney 1989). The higher survival rate reported by Chesney (1989) relative to Tsai (1991) despite similar densities, might be because the prey density being checked and adjusted several times daily in the former study. Atlantic cod larvae, by comparison, were offered a mixed diet of rotifers and *Artemia* (1:1) at densities of 250, 500, 1000, 2000, 4000, 8000 and 16000/L for six weeks starting at 1 dph. Their growth was significantly higher at prey densities ≥4000/L as larvae swam significantly less prior to encounter prey, and had a significantly higher capture success, therefore, more energy was directed into somatic growth (Puvanendran and Brown 1999). The survival rate increased as the density increased from 250 to 4000 prey/L but decreased when increased further to 8000 and 16000 prey/L associated with possible water quality problems (Puvanendran and Brown 1999). Larvae offered 250 and 500 prey/L treatments

did not survive beyond days 11 and 24, indicating these prey densities were too low (Puvanendran and Brown 1999). Redfish (*Sebastes mentella*) larvae, in contrast to Atlantic cod, when offered a high prey density exhibited relatively poor survival and growth due to a 'confusion effect' (Laurel et al. 2001). Highest overall survival (26%) and growth (0.074mm/day) was achieved at 1500 prey/L compared to 7% and 0.055mm/day at both 500 and 4500 prey/L (Laurel et al. 2001). The high growth rate and survival in the intermediate prey density was attributed to higher capture efficiency. At 4500 prey/L, larvae were 'confused' needing to orient 10 times to capture a single prey compare to 2 to 2.5 orientations to catch a prey at densities of 500 and 1500 prey/L treatments (Laurel et al. 2001). Continuous feeding at high prey density may not benefit fish larvae because it may over-stimulate food ingestion, increase the time of gut evacuation and reduce digestive efficiency (Ma et al. 2015). Excess prey may also deteriorate water quality and affect the survival and growth of larval fish. The survival and growth of Pintado (*Pseudoplatystoma corruscans*) larvae were tested under *Artemia* rations of 50, 200, 350, 500, 650, 800, and 950 *Artemia*/L/day in 7L static water tanks with 15 larvae/L density. Larvae survival and growth increased between 200 and 350 *Artemia*/L/day and stabilized among the medium densities 500 and 650 *Artemia*/L/day and decreased above 800 *Artemia*/L/day associated with the highest level of ammonia, unionized ammonia and nitrite (Fernando Beux and Zanboni-Filho 2008). In this study, very small tanks (7L) were used and 50% of the water was changed daily, 100% of water was changed every three days. The deterioration in water quality indicates that larger rearing tanks or a higher water exchange rate are needed to

improve the water quality. The small static rearing tanks allow for replications in a small floor space; prey and larvae are secure and not lost down to the drain/outlet screen, and rearing larvae in relatively small numbers is more manageable. However, small static tanks need water changes regularly to maintain the water quality; further, the results from this small system may not extrapolate to a larger scale commercial system. Consequently, for this thesis, experiments were conducted in both large and small tanks. A recirculation system (40min exchange rate, ca.200L/tank) was used for Trial 1 (Chapter 4) and Trial 3 (Chapter 5); another recirculation system (29min exchange rate, ca.100L/tank) was used for Trial 2 (Chapter 4); the third system, static water tanks (15L/tank, 50% water exchange every day and 100% change every week) was used for Trial 4 and 5 (Chapter 6). Water quality in all systems was monitored twice per day to ensure it remained within a suitable range. Together, these trials provided valuable information on ration size and stocking density effect on cannibalism, survival and growth.

Food availability and quality had an impact on growth rates of striped bass, and by extension, on the variation in size of individuals of the same age define as growth depensation (Paller and Lewis 1987). Striped bass larvae exhibited growth depensation when inadequate food was supplied and resulted in losses due to cannibalism. Striped bass (8.6mm TL) offered a large amount of formulated feed (0.05-0.3g/fish/day) but few *Artemia* (100-600 *Artemia*/fish/day) exhibited both growth depensation and cannibalism, body length coefficient of variation (CV) increased from 12.8 to 23.3% after 21 days, and consumption of the inert diet was poor (Paller and Lewis 1987). By contrast, larvae fed a

large amount of *Artemia* (100-500 *Artemia*/fish/day) for 11 days and then converted to a combination of *Artemia* and formulated feed for 3 days did not exhibit growth depensation and cannibalism (Paller and Lewis 1987). Likewise, the BW CV of gold fish (*Carassius auratus*) was inversely related to daily ration, increasing from ca. 18% among larvae offered 50% BW/day of mixed (1:1) *Artemia* and dry feed to 75% among larvae offered 2.5% BW/day for 35 days (Kestemont 1995). Cannibalism was observed only among larvae offered the lowest ration, 2.5% BW/day, together with highest CV of BW (Kestemont 1995). Heterogeneity in body size facilitates the initiation of cannibalism, which then increases further because the smallest fish are usually consumed by the largest ones. The largest striped bass (42mm) was exclusively cannibalistic judging from gut contents, this fish was never observed consuming the supplied food, a mix of fish eggs and formulated feed (Paller and Lewis 1987). The cannibal larva may have consumed 40-60 conspecifics over a 21-day trial (Paller and Lewis 1987). At Dal-AC, cannibalism is consistently evident each year in the 1.5m diameter production tanks (700L) each holding a large, but unknown, number larvae and offered *Artemia* spp (J. Duston. *pers. comm*.). Cannibals are first evident around 12 to 14 dph with their larger size, predatory swimming behavior, sometimes with a fish protruding from their mouths. Despite the apparent incidence of fish with a large body size is rather low (<10% of the population), the decline in population size through to 30 dph can be very large. To reach the goal of establishing commercial aquaculture of striped bass, it is essential to develop rearing protocols to maximize seedstock production by minimizing losses due to cannibalism at the larval stage.

The main objective of this thesis was to devise feeding protocols to maximize growth and development of each individual larva and minimize cannibalism, balancing the economics underlying the relatively high cost of *Artemia* production.

2.3.3 Effect of stocking density on larval fish growth and survival

In intensive fish culture, improved efficiency in the early stages of production is needed to offset the high mortality rate, which usually includes increasing the stocking density (Hatziathanasiou et al. 2002). However, an overcrowded rearing environment may cause higher aggression and competition for food and space and elevated incidence of disease, which eventually leads to lower growth and survival. A stocking density below optimal increases the operating costs of a commercial hatchery. Optimal stocking density should result in the highest production that does not compromise survival and growth (Baras and Jobling 2002). Defining the optimal stocking density includes minimizing fish aggression and cannibalism (Katavić et al. 1989; Kaiser et al. 1995).

The effect of stocking density on survival and growth of larval fish is species specific. A negative relationship between stocking density and growth was exhibited by both larval giant gourami (*Osphronemus goramy*; range 0.6 to 19.2 larvae/L; Arifin et al. 2019) and walking catfish (*Clarias batrachus*; range 7 to 35 larvae/L; Sahoo et al. 2004). In contrast, growth was independent of stocking density among Atlantic cod (range 20 to 300 larvae/L; Baskerville-Bridges and Kling 2000).

An ontogenetic change in the effect of stocking density on growth and survival was

reported among European sea bass (*Dicentrarchus labrax*) between the larvae and post-larvae stage. Independence between stocking density and growth and survival over 30 days was observed among first feeding larvae (5mm TL) at stocking densities of 50, 100, 150, and 200 larvae/L when offered an excess of live prey (Hatziathanasiou et al. 2002). However, at the post-larval stage (35 dph, 17mm TL), an inverse relationship between stocking density and growth and survival occurred among fish stocked at 5, 10, 15 and 20 fish/L offered an excess of live prey for 11 days then weaned on a formulated diet for another 11 days (Hatziathanasiou et al. 2002).

By comparison, a positive relationship between stocking density and growth/survival was exhibited among perch larvae (0.8mg, 100L volume) stocked at 10, 32 and 100 fish/L densities and offered excess live *Artemia* (Baras et al. 2003). High stocking density resulted a more uniform growth between individuals (Baras et al. 2003). Tail-first cannibalism was evident earliest among perch larvae stocked at low density; as body size of cannibals increased, they ingested prey head-first completely (Baras et al. 2003). An earlier study on *P. fluviatilis*, also reported more homogeneous growth and reduced cannibalism at higher stocking densities (1 to 9 larvae/L, Mélard et al. 1996). High stocking density resulted in a perceptual confusion resulting in cannibals making unsuccessful attacks (Baras et al. 2003). Similarly, among northern pike larvae (*Esox lucius*) the success rate of cannibalistic attacks was inversely related to stocking density because a large number of targets in a tank was interpreted as causing perceptual confusion (Kucharczyk et al. 1998).

In other species, by contrast, higher stocking density resulted in lower survival rates.

Density effects on larval fish may trigger behavioral interaction between siblings, including competition for food and space. For example, among reba carp (*Cirrhinus reba*) fry, a lower survival rate at high stocking density was attributed to stronger competition for food and space as well as increased stress (Keer et al. 2018). Higher stocking density results in a higher encounter rate between potential cannibals and conspecifics, especially in species demonstrating "meet-and-eat" behavior, elevating the risk of cannibalism (Baras and Jobling 2002, Hecht and Pienaar 1993). The survival of pikeperch larvae (*Sander lucioperca L.*, 18 dph, 15.6mm and 35mg) fed excess artificial feed for 21 days was significantly higher at the lowest stocking density (6 larvae/L) compared to the highest density treatment (15 larvae/L). The incidence of cannibalism was significantly different between the lowest and highest stocking density treatments at 27 and 35% in respectively (Szkudlarek and Zakęś 2007).

Among the striped bass literature, the effect of stocking density on larvae growth, survival and cannibalism has not been investigated in a designed experiment, but inferences can be cautiously made by comparing data between studies. Relatively low survival was recorded in higher stocking densities in a smaller rearing volume. Only ca. 15% of striped bass larvae survived when stocked in 7.6L McDonald hatching jars in a recirculating system at a density of 79 larvae/L for two weeks starting from 5 or 7 dph, where prey *Artemia* was not a limiting factor (Braid and Shell 1981). Cannibalism contributed to the mortality, which ranged from 19 to 36% (Braid and Shell 1981). In other studies where striped bass were stocked in lower densities and a larger rearing volume, the survival rate

was relatively high. Over 85% survived to 30 dph when stocked at 6 dph in 36L tanks at a density of 4 larvae/L and fed *Artemia* at a density of 500/L, adjusted twice daily (Houde and Lubbers 1986). Similarly, 80% of striped bass larvae survived over a period of 24 days starting from 6 dph when stocked in 8L tanks at a density of 3 larvae/L and fed *Artemia* at a density of 500/L, with food and water changed daily (Eldridge et al. 1981). About 60% of the 24 dph larvae survived after being reared in 15L tanks at a density of 4 larvae/L and fed 500 *Eurytemora affinis* nauplii/L for 14 days, the prey density being restored twice a day (Tsai et al. 1991). Although these studies were not specifically designed to test the stocking density effect on striped bass larvae, better survival at lower stocking densities and in larger rearing volumes was a recurring finding. A broad range of stocking densities need to be systematically evaluated to define the optimal stocking density among different stages of striped bass larvae. A primary objective of this thesis was to identify a stocking density range for striped bass larvae to serve as a valuable guideline for prospective farmers. Therefore, a 'range finding' approach with no replicates was used to compare a large range of stocking densities and ration sizes in the initial trials (Trial 1 to 3) presented in this thesis. Trial 4 and 5 further explored the main and interaction effect of stocking density and ration size on larval striped bass cannibalism, survival and growth.

Chapter 3: General Materials and Methods

3.1 Striped bass eggs and larvae rearing

Striped bass eggs were collected late in the ebb tide from the Stewiacke River estuary, Nova Scotia, about 1 km upstream of the confluence with the Shubenacadie River (DFO Scientific Licence 325881). The eggs (ca. 3.5mm diameter) were caught with a plankton net (1 mm mesh) then carefully transferred into coolers (Coleman 12L) filled with river water and kept suspended with a gentle air supply via a silica diffuser and a small aquarium air pump. Eggs were trucked 20km to the Dal-AC within 2h of capture, then transferred to upwelling hemispherical incubators (ca. 106L Aquabiotech, Coaticook. QC) with a flow-through supply of brackish water (2-3ppt) at 17-18℃. Water quality was maintained by removing buoyant dead eggs several times a day and removing the autogenous oil by cleaning the water surface and inside tank rim with paper towel. Eggs hatched in about 36-48 hours. Swim bladder inflation of striped bass occurs between 4 to 8 days post-hatching depending on the rearing temperature (Bailey and Doroshov 1995). To inflate the swim bladder, larvae gulp air from the water surface which passes through the pneumatic duct, the connection between the oesophagus and the swim bladder. The pneumatic duct exists only for a short period of time among physoclists, and once degenerated, the swim bladder inflation cannot occur (Bennett et al. 1987). At 3 dph, larvae were transferred in water to a 1.5m diameter tank (depth about 40cm, around 700L) in preparation for swim bladder inflation and first feeding. Water in the 1.5m tank was maintained at 18^{o}C, and 2-3ppt salinity. The light intensity at the water surface was about 1 lux until about 10 dph. A well

screen centre pipe (4 inches in diameter; slit size =500µm, Welpro Supply Limited, Truro) was used as an outlet filter. A black rubber air diffuser wrapped around the base of the centre screen emitted micro-bubbles which prevented larvae from getting close enough to be drawn onto the outlet screen. A single 180° perimeter nozzle (Hummert International, St. Louis, Missouri; Clayton and Summerfelt 2010) was set up over the tank which supplied a fine spray aligned with the direction of water flow to break down the water surface tension. At 3 dph, clay (6-50 porcelain clay) was autoclaved for 20 minutes at 121 ºC dry cycle, then 80 grams was mixed with 1L distilled water in a 2L plastic bucket, the mixture was homogenized for 2-3 minutes by a kitchen hand-blender (Sunbeam, Model No. 4192), then the slurry was filtered through a nylon foot sock, then gently poured in a zig-zag pattern in the rearing tank to achieve turbidity of about 150 Nephelometric Turbidity Units (NTU). Addition of clay slurry was repeated every eight hours or so. The water surface was towelled hourly from 9:00 to 21:00h to remove oil, a condition critical for swim bladder inflation. Swim bladder inflation rate was checked 24h after adding clay. Larvae (20 to 30 individuals) were randomly scooped out by a 3-inch plastic kitchen sifter, with larvae placed in a petri dish and euthanized by MS222 (tricaine methanesulfonate; Syndel Canada, Nanaimo, BC). Larva were examined under a dissecting scope; an inflated swim bladder was easily visible as a gas bubble. If the incidence of inflation was lower than 80%, the clay treatment was continued up until 9dph.

Stage I *Artemia* (Biomarine Aquafauna; California) were offered every two to three hours between 07:00 and 22:00h from 5 to 12 dph. Within 15 minutes post feeding, a large percentage of larvae had an orange coloured distended abdomen indicating they were successful in capturing the *Artemia* prey. Dead *Artemia* and larvae were siphoned daily. At

about 12 dph, larvae were weaned onto enriched stage II *Artemia*. Rearing water was maintained at 20°C and 3-5ppt salinity.

3.2 *Artemia* production

The process was divided into three steps: decapsulation, incubation and harvesting.

3.2.1 Preparation

1. Decapsulation solution: in a 10L glass beaker, 66g NaOH pellets (Fisher chemical, Ottawa, ON, Canada) were dissolved in 4.2L distilled fresh water (FW), to which was added 1.4L Javex bleach (5.25% hypochlorite).
2. 0.3M sodium thiosulphate rinse solution: in a flask, 47.3g of sodium thiosulphate (Fisher chemical, Ottawa, ON, Canada) was dissolved in 1L distilled water.

3.2.2 Decapsulation

1. About 5600ml of decapsulation solution and 200g *Artemia* cysts were gently mixed together in a 10L bucket, using a wooden spoon. The progression of the decapsulation was examined under a dissecting scope every few minutes. After 8-15 minutes, decapsulation completed when the cysts colour turned from brown to orange.
2. Decapsulated cysts were rinsed with FW in a 100-125μm sieve for 5-10 minutes until the water became clear.
3. 0.3M sodium thiosulphate was poured over the cysts to neutralize the toxic hypochlorite, followed by a FW rinse for 5-10 minutes
4. Decapsulated cysts were transferred into several 100ml plastic containers, covered with FW and stored at 4°C. The FW level was checked every two days and more added as

needed to prevent the cysts drying.

3.2.3 Incubation

1. Artemia were cultured in an incubator cabinet (185 x 185 x 50cm) with two platforms, bottom (185 x 125 x 50cm) and upper (185 x 65 x 50cm). In the bottom platform, four Perspex 20L cone incubators (Aquatic Ecosystems Inc.) were each partly filled with 15L seawater (SW), each cone equipped with an compressed air supply and silica diffuser. To maintain 28℃, hot air was provided by two domestic space heaters (Seabreeze Electric Corporation, Toronto, Ontario, Canada, Model: SF10), with the temperature thermostatically regulated by an extractor fan mounted in the cabinet ceiling and a temperature sensor placed in one of the incubator cones. High light intensity was provided by four fluorescent tubes mounted on the back wall of the cabinet. In the upper platform, eight plastic fruit-juice containers (15.5cm diameter, 3.5L volume) were held inverted in a plastic frame. Oxygen was injected into each vessel via a micropipette tip inserted into the rubber bung in the neck of the container.
2. To each incubator of SW at 28℃ were added 25g of decapsulated cysts. The clumps needed breaking up by hand. Vigorous aeration maintained the cysts in suspension. Aeration, temperature, and re-suspending the cysts which adhered to the wall of the cone incubator at the water surface were checked every few hours.

3.2.4 Harvesting

1. The cysts hatched in 20-24h; hatching rate was checked by pipetting some stage I nauplii into a petri dish and examining them under a dissecting scope.
2. The air stone was removed, then each cone incubator was manually lifted from the

chamber and placed on the lab floor.

3. To concentrate the stage I *Artemia*, the room lights were switched off and the cone incubator covered with a black plastic bag. The positively phototactic *Artemia* were concentrated at the base of the cone over 10 minutes by a large flashlight beam directed horizontally.
4. Using a 3/8-inch diameter PVC pipe, the *Artemia* were siphoned into a shallow white bucket (10L). As a further purification step, the bucket was placed at an angle under a high light intensity desk lamp for up to 10 minutes, then the clean *Artemia* poured off into a 100-125μm sieve leaving the cyst debris in the bucket. The *Artemia* in the sieve were gently rinsed with FW for up to 5 minutes to reduce bacterial contamination.
5. Using SW, the stage I *Artemia* were transferred from the sieve into a 2L plastic container at a concentration of 2000-3000 *Artemia* per ml with aeration provided by a small silica diffuser.
6. To produce enriched stage II *Artemia*, the inverted 3.5L plastic juice containers were filled with 750ml SW, to which 150ml of concentrated stage I *Artemia* were added and held in suspension by oxygen injected through the base via a small silica diffuser.
7. Enrichment powder (0.12g; Algamac 3050, Aquafauna Bio-Marine, Hawthorn, California) was homogenized in 100ml SW with a kitchen hand blender (Sunbeam, Model No. 4192), then added to each container and incubated for 10-12h at about 20°C. Oxygen was checked every few hours. Stage II *Artemia* were harvested by pouring them through a sieve and rinsing off the residual enrichment powder and storing a 2L plastic container.
8. To quantify the concentration of the stage II *Artemia*, three 20μl samples were pipetted

into a separate petri dish with 2-3ml of formalin (Fisher Scientific) coloured pale pink with Rose Bengal dye (Sigma Chemicals) added to the petri dish to euthanize and dye the *Artemia*. The number of *Artemia* in each sample was then counted under a dissecting scope, and the mean of the three samples used to estimate the density in the 2L container. To hold the *Artemia* at stage II, the 2L containers were stored in a water bath at 6 ^{o}C, with gentle aeration to each container.

3.3 Fish counting, and tank set up

Larvae for the trials were harvested from the 1.5m diameter tank by first shining a flashlight to attract the positively photo-tactic fish into a small area. Larvae were then siphoned from the tank using a flexible corrugated plastic pipe (2.5cm diameter) obliquely into a 120µm sieve (21cm diameter), immersed in water in a clean shallow wide bucket with a side drain. Larvae were then transferred in water to an insulated cooler (Coleman, 50 x 30 x 35cm) about half-filled with rearing water. The black eyes of the larvae made them easily visible against the white liner of the cooler facilitating their capture. Up to six volunteers, each equipped with a clicker counter (Fisher Scientific Part MDL2 07905) and a 120ml plastic sample cup (Fisher Scientific Part NCS60210*), were seated around a large table (179 x 64 x 74cm) with the cooler of larvae in the middle. Larvae were gently scooped out of the cooler using the sample cup, then gently poured and counted into a white plastic container (2L ice cream, Ampak, Dartmouth) in batches of 200 fish in Trials 1-3 (Figure 3.1). In Trial 4 and 5, two people each gently decanted the larvae from the cooler into a 90ml specimen container (Wide-Mouth Bio-Tite, Thermo Scientific-Samco, Ottawa, ON),

then a batch of 15 counted out individually in water using a white plastic spoon into a hemispherical white coffee mug (Rossy, Truro, NS, Canada). Each batch of 15 larvae was then gently transferred in water to their assigned tank.

Figure 3.1. Trial 1. Counting 16 dph striped bass (*Morone saxatilis*) larvae. Larvae were stocked in a insulated cooler and each volunteer equipped with a 120ml plastic sample cup, a clicker counter and a white plastic container.

In 2018, the system for Trials 1 and 3 was an 8-tank recirculation aquaculture system (RAS) with 4000L capacity. The rearing tanks were made of dark green fiberglass (1m diameter and 65cm depth), with a flat bottom and a 5cm diameter centre drain. Water depth was 22-25cm (volume ca. 200L) maintained by an external standpipe. The white centre well-screen (slit size 500 µm; Welpro Supply Limited, Truro, NS, Canada) was 15cm diameter and 75cm high. To provide an upwelling water current, a black microbubble

aquarium diffuser (45cm long, Aqua-Life, Mail Order Pet Supplies; Hamilton, ON, Canada) supplied with compressed air was wrapped around the base of the centre screen. Water flow rate to each tank was 4.8-5.1L/minute creating a water velocity of about two revolutions per minutes. Water entered each tank horizontally beneath the water surface via a 2.54cm diameter 90° elbow in a vertical inlet manifold (2.5cm diameter white schedule 40 PVC) pipe with a throttle valve to adjust the flow. Salinity was maintained at 2-4ppt, temperature 20-21℃, pH 7.4-7.6 and oxygen saturation 85-100%. The effluent from the eight tanks was recirculated through a sand filter and a biofilter tower, then gravity fed through an oxygen diffuser, then back to the tanks. Make-up water rate to a central sump was about 2-2.5L per minute, a mixture of freshwater from a well and seawater trucked in from Sandy Cove (NRC Institute of Marine Biosciences; Sandy Cove, NS, Canada, seawater filtered to 4µm). The rearing water alkalinity was 100-116 parts per million (ppm), NH_3 0.002-0.009 ppm, TAN 0.13-0.15 ppm, NO_2^- 0.005-0.012 ppm, NO_3^- 2.0-3.3 ppm, pH 7.6-8.2. Illumination for each tank was from a single LED household bulb (10 watts; Philips) inside a clear glass safety screen mounted centrally in a circular roof to the tank, 1.2m above the water surface. Light intensity at the water surface was 10 to 12 lux measured by a SPER scientific broad range lux/fc meter (model L632244, Scottsdale, AZ, U.S.A.), and was adjusted via a dimmer switch and brown paper hand towel wrapped around the glass safety screen. A lightproof plastic hood with an access window on each tank minimized extraneous light and disturbance. The photoperiod in 2018 was simulated natural day length (Latitude 45°N), identical to the rest of the wet lab, controlled by a computer timer with no dawn or dusk

period; photoperiod length was around 15h during the experiment period. The photoperiod in 2019 was LD 24:0. Fish were fed stage II enriched *Artemia*. Visible larvae mortalities were siphoned using a 3/8-inch diameter PVC pipe into a 20cm diameter sifter within a shallow bucket where they were counted. To catch a random sample of live larvae required the skill and speed of two people due to their rapid escape response. In Trial 1, larvae were initially caught by the author holding a plastic kitchen sifter (20cm diameter) in each hand, quickly moving each sifter in a semi-circle from the back to the front of the tank. However, this sampling procedure yielded a high percentage of non-inflated swim bladder larvae. To get a more representative sample, a revised method was used: two people worked together, one crowded the larvae up using two kitchen sifters, then the other person quickly scooped up a single sample with another sifter. This sampling method was used later for Trial 1 to 3. Larvae were held alive in a bucket of rearing water, then euthanized a few at a time (MS222, 0.1g/L) and examined under a dissecting scope (Leica, Model EZ4), to record TL to the nearest mm, swim bladder inflation rate and evidence of cannibalism (fish in mouth or stomach). Then BW was recorded to nearest 0.001g (Mettler Toledo, Model XS603S)

In 2018, Trial 2 was conducted in a six-tank RAS with 700L capacity. The rearing tanks were cylindrical (60cm diameter, and 40cm deep) made of green fiberglass. Water depth was 35cm, giving a rearing volume of ca.100L. The white centre well-screen (slit size 500μm, Welpro Supply Limited, Truro, NS, Canada) was 9cm in diameter and 48cm high and at its base was rubber diffuser to provide an upwelling water current of air bubbles. Water entered the tanks beneath the water surface via a row of 4 holes (0.5cm diameter) on

the vertical inlet pipe (3cm white PVC) with a PVC ball valve to adjust the flow to 3.5L per minute creating a water velocity of about two revolutions per minute. Mean temperature was 20.3°C and salinity 3.2ppt. Illumination was from 4 LED household bulbs (10 watts; Philips) installed 2 meters above the tanks, pointing towards the white ceiling. Light intensity at the water surface was ca.10 lux and was adjusted via a dimmer switch. The photoperiod was LD 15:9.

In 2019, Trials 4 and 5 were conducted in twelve black plastic buckets, herein referred to as 'tanks' (5 US gallons, 18.9L, MH R05, Ampak Inc., Laval, QC). The trials were conducted in a quiet lightproof room (4.5x4.5m, 20.25m^2, ceiling height=3m). Air temperature, and hence rearing temperature, was maintained at 20-23°C by a ceiling-mounted large radiator/fan and a smaller household utility heater mounted on the bench (1500W, Power Zone, BNT-15B2, Orgill, Collierville, TN, USA). Light intensity at the water surface was 4-5 lux provided by two ceiling-mounted domestic fluorescent lights, each shaded with an opaque black plastic sheet on a 24h light (LD 24:0) photoperiod. The tanks were arranged on the floor in two rows of six, each row about 2.5m apart. The rearing volume was 15L (water depth 25cm, diameter 28cm), each tank about 60% full. Aeration in each tank was provided by a flexible black rubber diffuser suspended vertically against the tank wall controlled by a small microvalve (30cm long, Aqua-Life, Mail Order Pet Supplies; Hamilton, ON, Canada). Air was supplied to each tank via a manifold of PVC tubing (2.5cm) connected to the main wet-lab compressor. A reservoir tank (250L, Xactics, Cornwall, ON, Canada) of 3ppt salinity brackish water, sat in the middle of the room. It

was refilled daily using two hose pipes supplies, freshwater and seawater (30ppt) and allowed to equilibrate to room temperature before use.

3.4 *Artemia* clearance rates in RAS

In 2018, *Artemia* clearance rates were estimated in two RAS systems using the same water depths, flow rates and aeration as the main trials. The clearance rates were compared both with zero fish and with either 1500 larvae/tank (32 dph) in RAS 2 or 600 larvae/tank (32 dph) in RAS 11.

Artemia were evenly added to the tank across the water surface, and swirled gently by hand, the same method as a regular feeding during the main trials. Then two replicate samples, each about 100ml, were collected at 0, 15, 30, 45 and 60 minutes in 120 ml plastic sample containers. The samples were taken at 10cm depth in 180 ° arc, about half the radius of the tank. To preserve the contents, about 10 ml of 10% buffered formalin was added to each sample. The *Artemia* were enumerated by first collecting them on a mesh sieve (80μm), recording the volume of the filtrate to the nearest ml. The *Artemia* were then rinsed into a petri dish and counted under a dissecting scope. The density of *Artemia* nauplii per ml was calculated. The *Artemia* clearance rate (change%/minute) was calculated as follows: Clearance rate= $\frac{(\textit{Artemia}\text{ density at T1}-\textit{Artemia}\text{ density at T2})}{\textit{Artemia}\text{ density at T1}}$/(T2-T1)x100

In the absence of striped bass larvae, *Artemia* density in RAS 2 decreased gradually and were still present 60 minutes following their addition. In the first 15min, The *Artemia* clearance rate was low, ranging from 3.9 to 4.5%/min, and between 15 and 30min, was 2.6%

to 4.6%/min (Table 3.1). In contrast, when the tank was stocked with 1500 larvae (7 larvae/L), the *Artemia* clearance rate was much higher at 6.7%/min in the first 15min and by 15min all the *Artemia* were gone (Table 3.1).

In RAS 11 with zero larvae, *Artemia* density decreased slowly. The clearance rate in the first 15min ranged from 2.2 to 4.2%/min, and between 15 and 30min post-addition, was 3.4 to 4.2%/min (Table 3.2). By comparison, when stocked with 600 larvae (6 larvae/L), the clearance rate during the initial 15min post feeding was much higher, ranging from 6 to 6.1%/min (Table 3.2). In conclusion, the results demonstrated the striped bass larvae were efficient predators confirming previous studies (Duston and Astatkie 2012, MacIntosh and Duston 2007), and losses of prey down the outlet drain were sufficiently low to be considered insignificant, and are not considered further in this thesis.

Table 3.1 RAS 2. *Artemia* clearance rate (% min^{-1}) in tanks with either 0 or 1500 striped bass larvae (*Morone saxatilis*, 32dph) within a recirculation system. Each tank had a volume of water of ca.200L and the water inflow rate was 5L/min. At 0, 15, 30, 45 and 60min after *Artemia* addition, two 120ml water samples were collected and the *Artemia* density was calculated. / indicates *Artemia* were absent. Negative values indicate the *Artemia* density at T2 is greater than T1.

Larvae	Time (min)	Tank 1	Tank 2	Tank 3
0 larvae	0-15	4.5	3.9	4.3
	15-30	4.6	2.6	3.3
	30-45	2.3	-0.6	3.9
	45-60	-3.0	5.8	6.7
1500 larvae/tank	0-15	6.7	6.7	6.7
	15-60	/	/	/

Table 3.2 RAS 11. *Artemia* clearance rate (% min^{-1}) in tanks with either 0 or 600 striped bass larvae (*Morone saxatilis,* 32dph) within a recirculation system. Each tank had a volume of water of ca.100L and the water inflow rate was 3.5L/min. At 0, 15, 30, 45 and 60min after *Artemia* addition, two 120ml water samples were collected and the *Artemia* density was calculated. / indicates *Artemia* were absent. Negative values indicate the *Artemia* density at T2 is greater than T1.

Larvae	Time	Tank 1	Tank 2	Tank 3
0 larvae	0-15	4.0	4.2	2.2
	15-30	3.4	3.8	4.2
	30-45	0.5	0.6	2.6
	45-60	1.7	0.2	3.1
600 larvae/tank	0-15	6.0	6.1	6.4
	15-30	4.3	5.7	2.6
	30-45	2.2	2.9	6.7
	45-60	0.4	-21.1	/

3.5 Response variables

In all five trials, mortality due to cannibalism was estimated as the initial number of fish stocked in each tank minus the survivors at the end of trial and minus the sum of the daily retrieved mortalities, and accounted for any fish removed during sampling. The incidence of cannibals (%) at the end of each trial was calculated as the total number of cannibals divided by the total number of survivors in each treatment. Cannibals were primarily identified by their large body size. Survival rate was calculated as the number of survivors at the end of trial divided by the initial larvae number and accounted for any fish removed during sampling. In Trial 4 and 5, the specific growth rate (%BW or TL/day) was calculated as $\frac{\ln(\text{body size final}) - \ln(\text{body size initial})}{\text{T(days)}} \times 100$. For all ANOVA analyses, three assumptions: normality, constant variance and independence were established before accepting the analysis. Normality of the error terms was checked by conducting a normal

probability plot of the residuals. Residuals versus fits plot was used to ensure a constant variance. Independence was met since the treatments were randomly assigned to each tank. Statistical significance was set $\alpha=0.05$ for all analyses. The specific statistical methods are described in materials and methods section for each trial.

Chapter 4: Effect of ration size on larval striped bass cannibalism, survival and growth

4.1 Introduction

Among the three published studies in which prey density was an experimental factor, survival and growth of striped bass larvae generally improved as prey availability increased, but the results differed considerably (for review *see* Chapter 2, page 19-20). Briefly, larvae (6mm) stocked in 8L of static water (18°C, changed daily) at 3 larvae/L offered 10 *Artemia*/L once daily lost weight from 7 to 30 dph and only 50% survived (Eldridge et al. 1981). Survival increased to around 80% among 500 and 1000 *Artemia*/L per day and the highest survival was 90% among larvae offered 5000 *Artemia*/L per day with growth rate in TL of 0.22mm/day (Eldridge et al. 1981). By comparison, survival was very good (>90%) and growth rate was 0.36mm/day from 5 to 25 dph among larvae reared at a density of 4/L in 32L of static water (19°C), the prey density was adjusted to 100/L several times per day (Chesney 1989). When offered *E. affinis* (70% nauplii and 30% copepodites) at either 100 or 250/L, survival was also relatively good at 60 and 87% respectively, and the growth rate was between 0.33 and 0.4mm/day (Chesney 1989). In the third study, 60% survival was achieved by offering *E. affinis* nauplii at a density of 1112/L from 9 to 24 dph at a density of 4 larvae/L in 15L of static water (19°C), the prey density was restored twice daily (Tsai 1991). Together these data serve to emphasize the optimal feeding regime for larval striped

bass is unresolved.

At Dal-AC, feeding trials in past years did not yield survival and growth estimates needed for intensive culture, since they were only of 1h duration followed by lethal sampling to quantify prey in the gut (MacIntosh and Duston 2007; Duston and Astatkie 2012; Sampson et al. 2013). In 2016, a key step forward was a successful 18 day trial comparing two *Artemia* enrichment products in eight tanks, each 300L and stocked with 1200 larvae (4 larvae/L; 13 dph, TL 7mm, BW 4mg; Jing Lu and Jim Duston, 2016 *unpubl. data*). A ration of 150 *Artemia*/larva/meal was offered five times daily for 9 days, increasing to 200 *Artemia*/larva/meal for the next 9 days, all based on an initial stocking density of 1200 larvae per tank. Survival was excellent in all tanks, >90% at 31 dph, with no evidence of abnormally large 'cannibals'. Final mean (SE) BW and TL at 31 dph was 49(1.3)mg and 19mm, the overall growth rate was 0.7mm/day was independent of enrichment treatment (J. Lu and J. Duston 2016, *unpubl. data*). In 2017, a trial in the same rearing system compared two ration levels either 125 or 250 *Artemia*/larva/meal starting with 22 dph larvae (TL 11mm, BW 13mg; J. Duston, D. Roberts and S. Qiu, *unpubl, data*). The stocking density was 4 larvae/L and the fish were offered five meals daily, the same as in 2016. The ration after 10 days was increased in the low/high ration treatments to 150/300 *Artemia*/larva/meal for the last 4 days to 36 dph. Survival 88% was very good, and independent of ration. Final mean BW, by contrast, was about two-fold greater in the high ration treatment, 75 vs. 45mg respectively (Duston, Roberts and S. Qiu, *unpubl. data*). The growth rate in TL was similarly very good at 0.5 to 0.8mm/day in low and high ration

treatments respectively. Estimated losses due to cannibalism were very low, only 8.3 and 7.5% in the low and high ration treatments respectively.

In 2018, the start of my thesis practical work, to better define the relationship between ration level and both growth and survival, the decision was made to take a 'range-finding' approach by testing up to eight ration levels with no replicates within each of the available RAS either 6 or 8 tanks. A design with four ration levels, each with two replicates was carefully considered, but seemed too restrictive given the limited state of knowledge. The same large tank system was utilized in 2018, following the successful trials the previous two years, but the rearing volume was lowered to 200L to make husbandry and food production more manageable. For Trial 1, disappointingly, the swim bladder inflation rate of the only larvae available with a large population was very low (41%) but since there was no alternative, the trial proceeded. The inclusion of larvae with no swim bladder, abbreviated herein as NSB, yielded some interesting results. Larval fish that failed to inflate their swim bladder resulted in poor survival and growth associated with a loss of neutral buoyancy, and a reduction in predatory efficiency (Hunter 1972, Martin-Robichaud and Peterson 1998,). Towards the goal of achieving the most cost-efficient rearing protocol, Trial 1 tested a relatively lower ration compared to the 2016 and 2017 trials ranging from 70 to 140 *Artemia*/larva/meal offered at five meals per day for 14 days. In addition, Trial 2 started with 24 dph larvae from a second cohort with a much higher swim bladder inflation rate (98%), but there were only sufficient larvae to stock a smaller 6-tank (ca.100L) RAS each with 600 fish. Because older and relatively larger larvae were used, the ration size for

Trial 2 ranged from 100 to 350 *Artemia*/larva/meal in increments of 50, offered for 14 days. Ration size was based on initial number of larvae stocked in each tank and remained fixed throughout each trial.

4.2 Materials and methods

Husbandry, *Artemia* production, fish counting, and the experimental rearing system are described in **Chapter 3**. The specific procedures used in Trial 1 and 2 are described below.

4.2.1 Trial 1: Cannibalism, survival and growth of 16 dph larvae stocked at 7/L offered eight *Artemia* rations (range: 70 to 140 *Artemia*/larva/meal) for 14 days.

The swim bladder inflation rate of the larvae used was 41% (overall 75 of 183 examined at 5, 6, 7, 8 and 16 dph). The reason for the poor inflation rate is unknown, since all the standard rearing protocols were followed (*see* Chapter 3). Separating inflated from non-inflated larvae was not practical. On June 19, 2018, at 16 dph, 12,000 larvae were counted. A total of 15 cannibals were removed; they were identified by their relatively large body size (mean (SE) BW 19 (1.0)mg; TL 13 (0.2)mm). Among the rest of the population, the overall mean BW and TL was 4 (0.5)mg and 9 (0.2)mm, CV_{BW}=50.5%. The difference in the mean (SE) body size of larvae with an inflated vs. non-inflated swim bladder (ISB vs. NSB), was close to being significant for BW (6 (1.2) vs. 3 (0.3)mg; p =0.095, T-test), and very significant for TL (9.6 (0.2) vs. 8.7 (0.2)mm; p=0.003, T-test).

Within a RAS with eight tanks (each 1m diameter, 0.26-0.27m water depth, 204-212L volume), the experimental unit was a tank and each stocked with 1500 larvae, a density of 7 /L. The rearing water averaged 20.7°C and 5.2ppt salinity and the water inflow rate was 5L/min per tank. A microbubble aquarium diffuser supplied with compressed air was wrapped around the base of the centre well-screen (slit size 500μm) to provide an upwelling water current. By error, the photoperiod was simulated natural day length (Latitude 45°N), with photoperiod of LD 15:9. The intended photoperiod was LD 24:0, which would have matched the 2016 and 2017 trials.

The following day (17 dph), feeding of enriched stage II *Artemia* commenced, the number of *Artemia*/larva/meal across the eight tanks ranged from 25 to 250 (25, 50, 75, 100, 125, 150, 200, 250) based on 1500 larvae/tank. The next day (18 dph) following concerns there was too little food in some tanks and too much in others, as indicated by the incidence of distended orange abdomens, the ration range was changed to 70, 80, 90, 100, 110, 120, 130, 140 *Artemia*/larva/meal based on 1500 larvae per tank. This fixed ration was then offered five times daily (09:00, 12:00, 15:00, 17:00, and 19:00h) for 14 days. Visible mortalities on the tank floor were removed each afternoon use a siphon with the aid of a phone light. After seven days (25 dph), a sample of larvae from each tank was caught by the author holding a plastic kitchen sifter (ca. 20cm diameter) in each hand, quickly moving each sifter in a semi-circle from the back to the front of the tank. A minimum of 25 larvae from each tank were held in a small bucket of tank water then euthanized (MS222, 0.2g/L) individually to record BW (to 0.001g), TL (to nearest 0.5mm), swim bladder inflation rate,

and identification of cannibals. This sampling procedure yielded a high percentage (88 to 96% per tank) of larvae without an inflated swim bladder (NSB) in some treatments, suggesting they were easier to catch than larvae with an inflated swim bladder (ISB). To get a more representative sample, on the afternoon of the same day (25 dph; June 27), the 'two person" method (see Chapter 3.3, page 36) was used to catch at least 19 fish (range 19 to 24, ISB ranged 85 to 100%). Both sets of data were pooled hence the total number of larvae sampled from each treatment after seven days was up to 48.

After 14 days (32 dph), a random sample of ca. 40 (range 29 to 64) larvae from each treatment were caught used the same 'two-person' method of day 7. On both day 7 and 14 the sampled larvae were euthanized individually, and body size was measured, swim bladder inflation assessed, and cannibals identified. Then, on day 14 all the remaining larvae in each tank were then euthanized and counted and incidence of cannibals and swim bladder inflation recorded. The cannibals were identified by their distended belly and large BW. Among those cannibals with prey in their mouth, the prey was gently removed using forceps, the orientation of the prey was recorded, either head or tail toward to the cannibal's mouth (head-first and tail-first), then the BW and TL of both cannibal and prey was measured.

4.2.2 Trial 2: Cannibalism, survival and growth of 24 dph larvae stocked at 6 larvae/L offered six *Artemia* rations (range: 100 to 350 *Artemia*/larva/meal) for 14 days.

3600 larvae (24 dph) were randomly distributed among six tanks on July 9, 2018.

Mean(SE) BW was 27(0.8)mg, and TL 14(0.2)mm, CV_{BW}=27.9%. Their initial BW was about five-fold greater than fish used in Trial 1. Swim bladder inflation rate was 98% (overall 114 of 120 examined at 16 and 22 dph). During the counting, only one cannibal was detected (BW 77mg, TL 20mm). The experimental unit was tank, each with a rearing volume of 100L. The initial stocking density was 6 larvae/L. The rearing water was 20.3℃ and 3.2ppt salinity, at an inflow rate of 3.5L/min per tank. Six fixed rations were offered: 100, 150, 200, 250, 300, 350 *Artemia*/larva/meal, based on 600 larvae per tank. Five meals were offered daily (09:00, 12:00, 15:00, 17:00, and 19:00h) for 14 days. Visible mortalities were removed each afternoon by siphoning and were counted with the aid of a phone light. On day 7 and 14 (31 and 38 dph), ca. 25 (range 25 to 29) fish were randomly sampled from each tank to determine body size (see 4.2.1 Trial 1 for detailed procedures). All survivors were counted on day 14, and cannibals were identified by their distended belly and large BW; the BW and TL of cannibals was recorded.

4.2.3 Statistical analysis

In both Trial 1 and 2, the losses to cannibalism (%) and incidence of cannibals (%) was calculated (*See* **Chapter 3.5** for details)

In Trial 1, the Kruskal-Wallis test was used to compare the median BW among the eight treatments, and the Mann-Whitney test was used sparingly to make pair-wise comparisons (Minitab 18). In both Trial 1 and 2, one-way ANOVA was used to compare the difference in BW means among the treatments (Minitab 18). To satisfy the assumptions

of normality and constant variance, a logarithm base 10 transformation was applied to the mean BW on day 7 in Trial 1. Two sample T-test was used to compare the mean BW and TL between NSB and ISB larvae in Trial 1. Anderson-Darling Normality test was used to test the normality of mean BW distribution and regression was used to analyze the survival rate and losses due to cannibalism (%) among treatments (Minitab 18). The descriptive statistics and the BW distributions were also obtained with Minitab 18.

4.3 Results

4.3.1 Trial 1

After seven days (25 dph), the mortalities retrieved ranged from 1 to 32 per tank, less than 2.1% of the 1500 stocked (Table 4.1). Mean BW increased between four- and six-fold to between 18 and 25mg compared to the initial 4mg (Table 4.1). Among larvae with an inflated swim bladder (ISB), BW of the smallest was 14 to 15mg in all eight treatments, and many were clustered around 25mg (Figure 4.2 upper panel). The mean TL of ISB and NSB failed to indicate a clear effect of ration, ranging from 13 to 14mm and 11 to 12mm respectively (Table 4.1). The BW CV was relatively low, ranging from 24 to 52% because of the small number of cannibals among the sampled larvae in each treatment ranging from 1 to 4 and total of 19 (Figure 4.2, upper panel). Among these, three ‘head-first’ and two ‘tail-first’ cannibals were evident in the three lowest ration treatments (Table 4.2). The cannibals were about 4 to 5 times heavier and 1.5 times longer than the prey (Table 4.2, Figure 4.1). The prey were partially decomposed, preventing the determination of swim

bladder inflation status. Putative cannibals, fast-growing larvae around 40mg BW, were numerous and contributed to the asymmetric body size distributions. Consequently, the 95% confidence intervals of mean BW were large, and an effect of ration size was significant only between the lowest and highest ration treatments, as their respective confidence limits do not overlap (ANOVA, $F_{7, 227}$=2.25, p=0.031, Figure 4.3 upper panel). Median BW of ISB larvae on day 7 ranged from 21 to 27mg and was similar among the seven treatments fed between 70 to 130 *Artemia*/larva/meal (Kruskal-Wallis, p=0.372; Figure 4.3, lower panel). Among all eight treatments, the median BW on day 7 was significantly different (Kruskal-Wallis, p=0.004), due to the difference between the lowest and highest ration, 21 vs. 27mg (Mann Whitney, p<0.001, Figure 4.3, lower panel). NSB larvae were about 50% smaller than ISB larvae, their mean BW ranged from 12 to 15mg (Table 4.1). The percentage of NSB larvae in the samples on day 7 ranged from 20 to 58% with a mean of 36%, compared to the day 1 estimate of 59% (Table 4.1). The incidence of NSB larvae among the four lowest ration treatments pooled on day 7 was lower than the four highest ration treatments, 27 vs. 45%, and almost significant (2 sample T-test, T=2.38, p=0.064).

Table 4.1. Trial 1, day 7 (24 dph). Mean (SE) body weight (BW, mg) and total length (TL, mm) of striped bass larvae (*Morone saxatilis*) with either an inflated or non-inflated swim bladder (ISB, NSB) among eight tanks offered a fixed ration ranging from 70 to 140 enriched stage II *Artemia*/larva/meal, five times daily based on the initial stocking density of 1500 larvae/tank. The percentage of NSB larvae among the total sampled on day 7 is shown. Mortalities retrieved are cumulative to day 7. Initial mean (SE) BW and TL at 16 dph of all larvae: 4 (0.52)mg, 9 (0.2)mm, ISB: 6 (1.2)mg, 9.6 (0.2)mm, NSB: 3 (0.3)mg, 8.7 (0.2)mm. Rearing volume 212L at 20.7°C and salinity 5.2ppt.

Ration	70	80	90	100	110	120	130	140
Mortalities retrieved	1	32	26	27	7	8	28	18
Larvae sampled (n)	46	42	41	44	48	44	44	41
NSB (%)	26	41	20	20	30	58	51	42
All larvae BW	20(0.9)	20(1.6)	25(2)	25(2.2)	23(1.6)	18(1.3)	19(1.3)	24(2.4)
ISB BW	22(0.9)	25(2.2)	28(2.2)	28(2.6)	28(1.8)	25(2.1)	26(1.6)	32(3.2)
NSB BW	13(0.5)	12(0.5)	12(0.7)	15(0.7)	14(0.5)	13(0.5)	13(0.3)	12(0.6)
ISB TL	13(0.2)	14(0.3)	14(0.3)	14(0.3)	14(0.2)	13(0.1)	14(0.2)	14(0.3)
NSB TL	11(0.2)	11(0.1)	11(0.2)	12(0.1)	11(0.1)	11(0.1)	11(0.1)	11(0.1)

Table 4.2. Trial 1. Day 7 (24 dph). The body weight (BW, mg) and total length (TL, mm) of five cannibals and prey (cannibalism victims) and prey/cannibal total length ratio among the striped bass larvae (*Morone saxatilis*). Two types of cannibalism were recorded: head-first (H) and tail-first (T). 1500 larvae were stocked in each of the eight tanks and offered a fixed ration ranging from 70 to 140 enriched stage II *Artemia*/larva/meal, five times daily based on the initial stocking density of 1500 larvae/tank. Each column is a single cannibalism event.

Ration	70	80	90	70	90
Cannibalism type	H	H	H	T	T
Cannibal BW	36	39	52	34	30
Cannibal TL	15	16	17	15	14
Prey BW	9	10	8	7	7
Prey TL	11	11	11	8	9
Prey/cannibal TL ratio	0.73	0.69	0.65	0.53	0.64

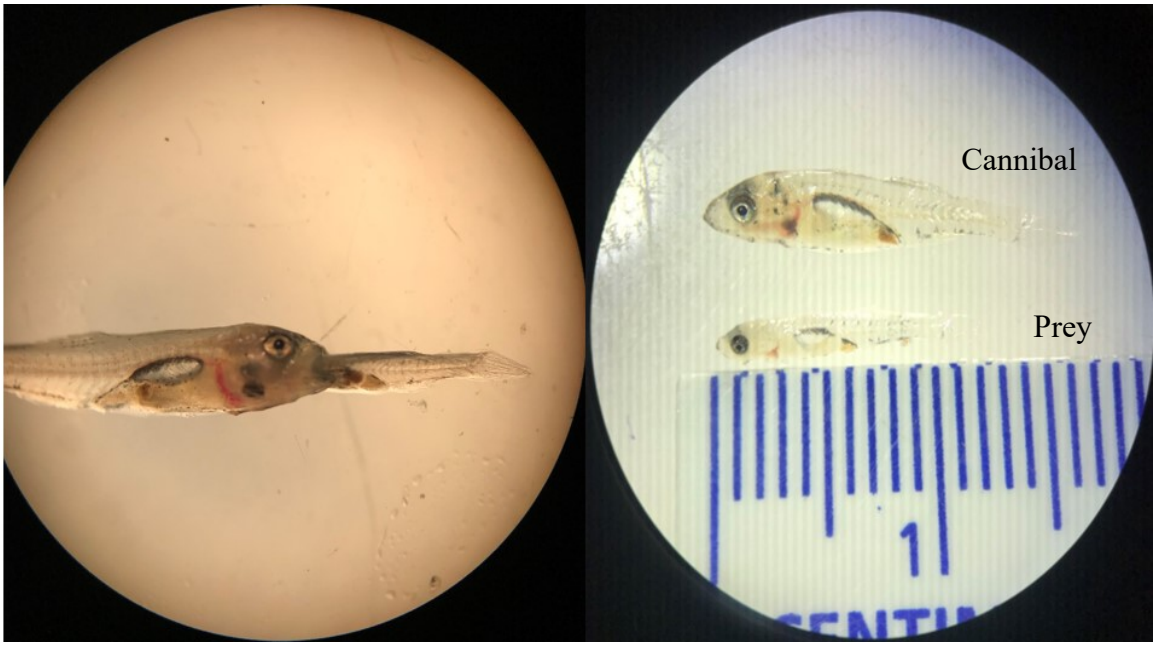

Figure 4.1. Trial 1. Head-first cannibalism among 24 days post-hatch (dph) striped bass (*Morone saxatilis*) larvae, both photos show the same pair of fish. The cannibal and prey were separated and measured. The body weight and total length of the cannibal was 52mg, 17mm and prey 8mg, 11mm. At 16 dph, 1500 larvae were stocked in a tank and offered a fixed ration 90 enriched stage II *Artemia*/larva/meal, five times daily based on the initial stocking density. Rearing volume 212L at 20.7℃ and salinity 5.2ppt.

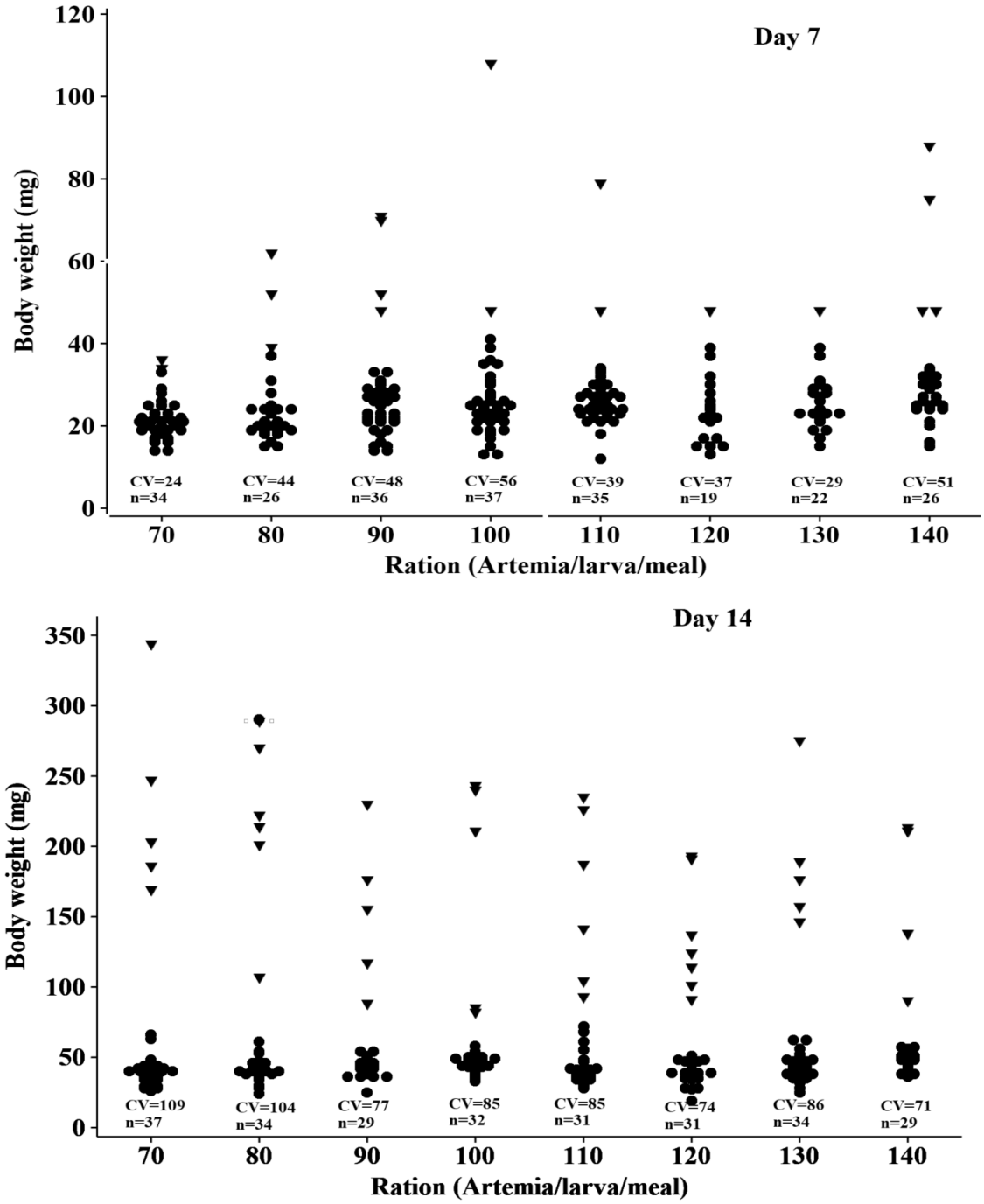

Figure 4.2. Trial 1. Body weight distribution and coefficient of variation (CV) of a sample of striped bass (*Morone saxatilis*) larvae with an inflated swim bladder after 7 (upper panel) and 14 days (lower panel) reared in eight tanks and offered a fixed ration ranging from 70 to 140 enriched stage II *Artemia*/larva/meal, five times daily based on the initial stocking density of 1500 larvae/tank. Triangles indicate confirmed cannibals. Initial mean (SE) body weight was 6 (1.2)mg at 16 days post-hatch. Rearing volume 212L at 20.7ºC and 5.2 ppt salinity.

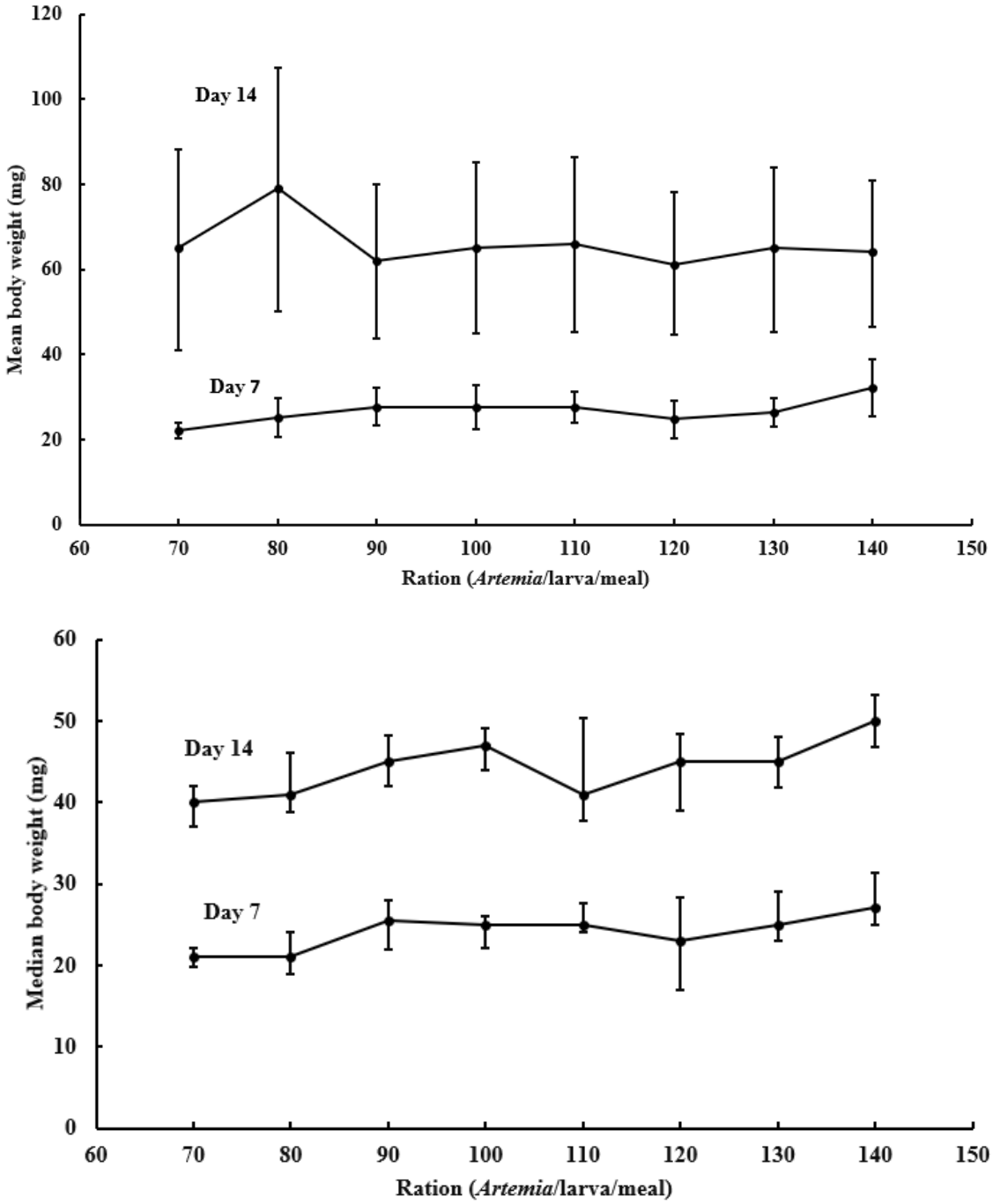

Figure 4.3. Trial 1. Mean (upper panel) and median body weight (lower panel), both with 95% confidence intervals, of inflated swim bladder striped bass (*Morone saxatilis*) larvae after 7 and 14 days reared in eight tanks and each offered a fixed ration ranging from 70 to 140 enriched stage II *Artemia*/larva/meal, five times daily based on the initial stocking density of 1500 larvae/tank. Initial mean (SE) body weight was 6 (1.2)mg at 16 days post-hatch. Rearing volume 212L at 20.7ºC and 5.2 ppt salinity.

At the end of the trial (day 14, 31dph) the survival rate among ISB larvae was positively related to ration size, ranging from 66 to 98% (Table 4.3). The survival of ISB larvae was curvilinear, reaching an asymptote of around 95% among the three highest ration treatments (Figure 4.4). NSB larvae survival, by contrast was very poor, ranging from 0 to 5% (Table 4.3). Since the number of retrieved mortalities was very low (<3%), it is concluded that cannibalism contributed to most of the losses. The overall losses to cannibalism was inversely related to ration, ranging from 49% among larvae offered 130 *Artemia*/larva/meal to 69% among the lowest ration 70 *Artemia*/larva/meal treatment (Table 4.3).

The BW distribution of the ISB larvae on day 14 was highly asymmetric in all treatment groups (Figure 4.2 lower panel). The BW CV on day 14 among the eight groups were larger than on day 7, ranging from 71 to 109% since the number of cannibals among sampled fish in each treatment was higher ranging from 4 to 7 (Figure 4.2, lower panel). The incidence of cannibals (include cannibals among smapled larvae) ranged from 4.4 to 7.5% of final total survivors in each treatment (Table 4.3). The BW of the cannibals ranged from 82 to 381mg, an increase of 16- to 76-fold since day 1. The TL of the cannibals ranged from 21 to 34mm. The BW specific growth rate was 20 to 30%/day and the growth in TL was 0.8 to 1.7mm/day. The estimated number of larvae consumed by each cannibal in each treatment on avarage ranged from 24 to 43 during the 14 day trial.

The median BW of ISB larvae on day 14 ranged from 40 to 50mg, the effect of ration was significant (Kruskal-Wallis, p=0.028; Figure 4.3 lower panel). The median BW of the

group offered the lowest ration was significantly lower than the highest ration, 40 vs. 50mg (Mann-Whitney, $p<0.001$; Figure 4.3 lower panel). The effect of ration on mean BW of ISB larvae was not evident, ranging from 61 to 79mg (Figure 4.3 upper panel). The mean TL of ISB larvae was similar among the eight treatments about 18 to 19mm. The overall growth in length of ISB larvae was similar across the eight treatments, ranging from 0.6 to 0.7mm/day. The NSB larvae did not exhibit spinal deformity at this stage and their morphology was similar to normal ISB larvae, however, their mean BW was only about half that of ISB larvae, ranging from 25 to 31 mg.

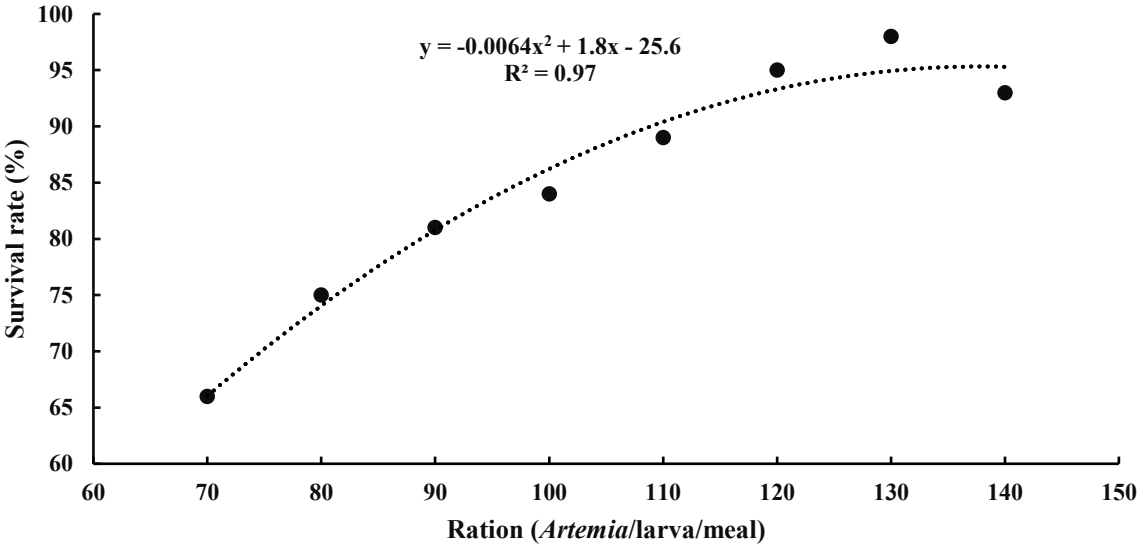

Figure 4.4. Trial 1. The survival rate of inflated swim bladder (ISB) striped bass (*Morone saxatilis*) larvae after 14 days (31 days post-hatch, dph) reared in eight tanks, each offered a fixed ration ranging from 70 to 140 enriched stage II *Artemia*/larva/meal, five times daily based on the initial stocking density of 1500 larvae/tank. Initial mean (SE) body weight was 6 (1.2)mg at 16 days post-hatch. Rearing volume 212L at 20.7℃ and 5.2ppt salinity.

Table 4.3. Trial 1, day 14 (31 dph). Inventory overview among eight tanks of striped bass (*Morone saxatilis*) larvae offered a fixed ration of 70 to 140 enriched stage II *Artemia*/larva/meal five times daily based on initial stocking density of 1500 larvae/tank. The estimated initial number of larvae either with an inflated or non-inflated swim bladder (ISB, NSB) was 615 and 885 in each ration treatment, based on an inflation rate of 41%. Mortalities are the cumulative sum retrieved from day 1 to 14. The incidence of cannibals is the total number of cannibals divided by total survivors. The losses due to cannibalism (%) are calculated as estimated number of mortalities due to cannibalism divided by initial larvae number. Rearing volume 212L at 20.7℃ and 5.2ppt salinity.

Ration	70	80	90	100	110	120	130	140
Number sampled	83	78	74	78	86	101	109	80
Mortalities retrieved	2	41	27	28	9	9	32	19
Survivors (ISB+NSB)	383	440	469	473	526	611	624	563
ISB survivors only	354	418	444	448	481	537	553	520
% ISB survivors	66	75	81	84	89	95	98	93
NSB survivors	0	0	1	0	10	42	43	18
% NSB survivors	0	0	0.1	0	1.1	4.9	5.0	2.1
Cannibals (n)	29	22	24	25	35	32	28	25
Incidence of cannibals	7.5	5.0	5.1	5.3	6.6	5.2	4.5	4.4
Losses to cannibalism (%)	69	62	62	61	58	52	49	56

4.3.2 Trial 2

The number of mortalities retrieved up to day 7 (31 dph) ranged from 7 to 17 in each tank, less than 2.9% of the 600 striped bass larvae stocked (Table 4.4). From the sample of 25 to 29 larvae in each treatment on day 7, only two cannibals were retrieved, one from each of the two lowest ration treatments (BW, 65 and 69mg). Among the rest of the 'non-cannibals' the BW distributions were normal (Anderson-Darling normality test, P>0.05, Figure 4.5, upper panel). The mean BW on day 7 was significantly different among treatments (ANOVA, $F_{5, 155}$=36.6, p<0.001, Table 4.4). The mean BW of the larvae offered the two highest rations were similar, and they were significantly larger than other four treatments (Table 4.4). Larvae offered the lowest ration gained on average, only 6mg in the

first 7 days with a mean BW of 34mg, while the fish offered the highest ration doubled their size compared to the initial BW of 27mg, reaching a mean BW of 63mg (Table 4.4). Similarly, mean TL on day 7 was significantly different among treatments (ANOVA, $F_{5, 153}$=42.2, p<0.001,) ranging from 17mm in the two lowest ration treatments to 20mm in the two highest (Table 4.4). The mean TL among larvae in the two highest ration treatments were similar (p>0.05), and both were significantly higher than other four treatments, while larvae in the two lowest ration treatments had a similar TL that was significantly lower than three highest ration treatments (Table 4.4)

At the end of the trial on day 14, the survival rate was positively related to ration, increasing from 58% among larvae offered 150 *Artemia*/larva/meal to between 90 and 92% among the three highest ration treatments (Table 4.4). The total number of mortalities retrieved from each tank ranged from 9 to 22, 1.5 to 3.7% of 600 stocked (Table 4.4). The overall losses assumed due to cannibalism were inversely related to ration, decreasing from 37% in the 150 *Artemia*/larva/meal treatment to 6% in the highest ration treatment (Table 4.4).

The distribution in BW of larvae sampled on day 14 in each of the six treatments was normal (Anderson-Darling Normality test, p>0.05), fast-growing cannibals were absent among the sampled larvae in all ration levels (Figure 4.5, lower panel). The coefficient of variation in BW on day 14 was highest in the two lowest ration treatments (27%) (Figure 4.5). The mean (SE) BW on day 14 was significantly different among treatments, ranging from 36 (1.9) to 76 (2.6)mg in the lowest and highest ration treatments respectively

(ANOVA $F_{5, 153}$=43.52, p<0.001, Table 4.4). The mean TL was also significantly different among treatments, ranging from 18 to 22mm in the lowest and highest ration treatments (Table 4.4). The overall growth rate in TL over the 14-day trial ranged from 0.3 to 0.6mm/day. Among the remaining larvae in the six tanks, n=2557, a total of only 8 cannibals were identified, all in the three lowest ration treatments, 3, 4 and 1 in 100, 150 and 200 *Artemia*/larva/meal treatments respectively (Table 4.4). The body size of cannibals ranged from 253 to 1363mg BW and 28 to 50mm TL (Table 4.5). The mean BW specific growth rate and growth rate in TL ranged from 17 to 29%/day and 1.3 to 2.9mm/day. On average, the estimated number of larvae consumed by each cannibal in each treatment ranged from 60 to 91 during the 14-day trial.

Table 4.4. Trial 2. The inventory overview and mean (SE) body weight (BW, mg), total length (TL, mm) and growth in length (GIL, mm/day) of a sample of striped bass (*Morone saxatilis*) larvae after both 7 and 14 days among six tanks offered a fixed ration ranging from 100 to 350 enriched stage II *Artemia*/larva/meal, five times daily based on the initial stocking density of 600 larvae/tank. Initial mean (SE) BW and TL was 27 (0.8)mg, 14 (0.2)mm at 24 dph. Rearing volume 100L at 20.3℃ and 3.2ppt salinity. Within a row, means sharing the same letter are not significantly different (α>0.05). The incidence of cannibals is the total number of cannibals divided by total survivors on day 14. The losses due to cannibalism (%) is calculated as estimated number of mortalities due to cannibalism divided by initial larvae number.

Ration	100	150	200	250	300	350
Mortalities day 1-7	11	17	11	7	9	12
BW day 7	34(2.0)^{d}	37(1.6)^{cd}	43(1.2)^{bc}	46(1.7)^{b}	56(2.2)^{a}	63(2.3)^{a}
TL day 7	17(0.3)^{d}	17(0.2)^{cd}	18(0.2)^{bc}	19(0.2)^{b}	20(0.3)^{a}	20(0.2)^{a}
Mortalities day 8-14	11	0	6	7	0	2
BW day 14	36(1.9)^{d}	49(2.6)^{c}	48(1.9)^{c}	61(2.2)^{b}	71(2.4)^{a}	76(2.6)^{a}
TL day 14	18(0.3)^{d}	19(0.3)^{c}	19(0.2)^{c}	20(0.2)^{b}	21(0.3)^{a}	22(0.3)^{a}
GIL day 1-14	0.3	0.4	0.4	0.4	0.5	0.6
Number sampled	50	50	52	50	51	82
Final survivors	357	335	482	517	522	523
% Survivors	62	58	84	90	91	92
Cannibals (n)	3	4	1	0	0	0
Incidence of cannibals (%)	0.8	1.2	0.2	0	0	0
Losses to cannibalism (%)	33	37	12	7	7	6

Table 4.5. Trial 2. Body weight (BW, mg) and total length (TL, mm) of eight cannibal striped bass (*Morone saxatilis*) larvae at the end of a14 day trial among three ration treatments. Larvae were offered a fixed ration ranging from 100 to 350 enriched stage II *Artemia*/larva/meal, five times daily based on the initial stocking density of 600 larvae/tank. Initial mean (SE) BW and TL was 27 (0.8)mg, 14 (0.2)mm at 24 dph.

Ration	100	100	100	200	150	150	150	150
Individual	1	2	3	4	5	6	7	8
BW	451	253	444	954	572	1363	486	283
TL	34	28	36	45	38	50	36	30

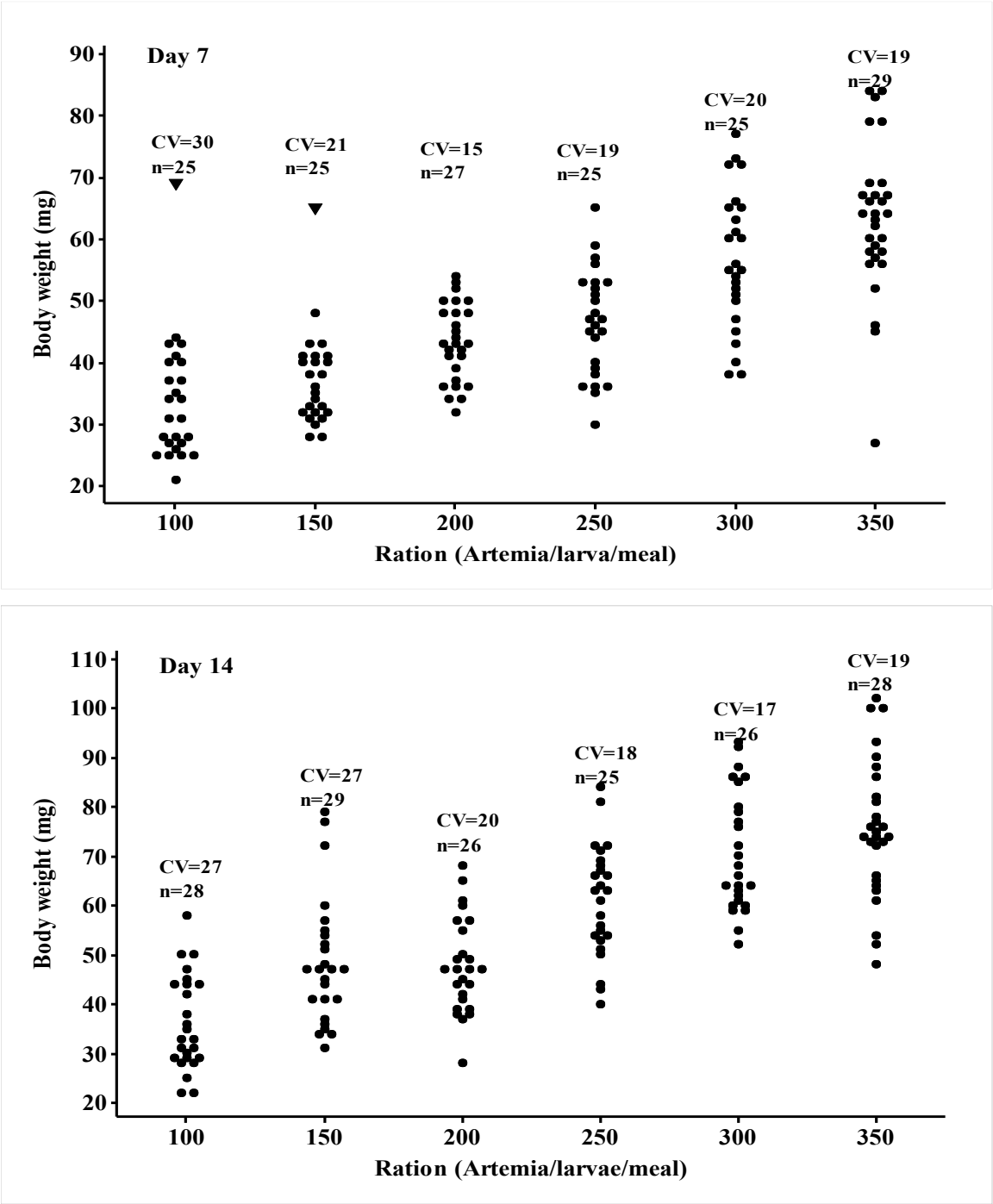

Figure 4.5. Trial 2. Body weight distribution of a sample of striped bass (*Morone saxatilis*) larvae after 7 days (31 days post-hatch, dph; upper panel) and 14 days (38 dph; lower panel) reared in six tanks, each offered a ration size ranging from 100 to 350 enriched stage II *Artemia*/larva/meal, five times daily based on 600 larvae/tank. Triangles indicate confirmed cannibals. Rearing volume 100L, each tank initially stocked with 600 larvae age 24 dph, mean (SE) body weight 27 (0.8)mg. Temperature was 20.3°C and salinity 3.2ppt.

4.4 Discussion

In Trial 1, the overall survival rate of 16dph larvae (BW 4mg, TL 9mm) was low due to the high mortality among larvae with an uninflated swim bladder. Among larvae with an inflated swim bladder, by contrast, the survival rate was relatively good and was positively correlated with ration size (70 to 140 *Artemia*/larva/meal). The estimated losses to cannibalism was high (49 to 69%) and inversely related to ration size in Trial 1, and appeared to be the main cause of mortality, with NSB fish being the main prey. In Trial 2, the overall survival rate was positively related to ration sizes (100 to 350 *Artemia*/larva/meal) with >80% in all except the two lowest ration treatments which experienced about 60% survival rates. The losses due to cannibalism and the incidence of cannibals was relatively low compared to Trial 1. The growth of ISB larvae were very good 0.6-0.7mm/day and 0.4-0.6mm/day in Trial 1 and 2 respectively. The growth rates of cannibals were extremely high in both trials with overall BW SGR of 17 to 30%/day and growth in TL of 0.8 to 2.9mm/day.

The high losses to cannibalism in Trial 1 (49 to 69%) was 5- to 9-fold higher compared to the cannibalism losses in 2017 (7.5 to 8.3%). Such cannibalism rates observed in Trial 1 were higher than the 30 to 44% losses to cannibalism among striped bass larvae offered an excess of *Artemia* seven times daily from 7 to 23 dph (Braid and Shell 1981). However, it is difficult to make meaningful comparisons since Braid and Shell (1981) used 7.6L McDonald hatching jars, and a higher stocking density (79 larvae/L) compared to Trial 1

(initial 7 larvae/L and less than 4/L at the end of the trial) and offered excess live *Artemia* more frequently (7 times daily) than the present study (5 times daily). Larvae with an un-inflated swim bladder are clearly highly vulnerable to cannibalism. Prey capture rate of 13 dph ISB striped bass larvae was three-times higher than NSB larvae at 80 and 25 *Artemia*/larva/h respectively under same prey density treatment (Duston and Astatkie 2012). Lower prey capture rate of NSB larvae resulted in a longer vulnerable period and higher susceptibility to predation. On day 7, ISB larvae were about two times bigger than NSB larvae in each of the eight treatments in Trial 1. Similarly, the growth of yellow perch (*Perca flavescens*) in the first two weeks after hatching was significantly faster among ISB than NSB larvae at 0.5 and 0.32mm/day respectively (Czesny et al. 2005). The growth difference was due to the significantly higher prey capture rate among ISB larvae, which was at least two times higher than NSB larvae (Czesny et al. 2005). In addition, NSB larvae yellow perch had a higher oxygen consumption compared to ISB larvae, indicating they devoted more energy to overcome the negative buoyancy which could not be allocated to somatic growth (Czesny et al. 2005). Striped bass larvae with an un-inflated swim bladder exhibited a relatively high tail-beat frequency as an energetic expense that was similarly associated with poor growth (Meng 1993). In an early study on striped bass larvae, the incidence of non-inflated swim bladders ranged from 30 to 90%, and NSB larvae were rapidly consumed by their conspecifics prior to 30 dph if not separated (Doroshev 1970). The swim bladder inflation rate of striped bass larvae at Dal-AC was generally between 80 to 100%, the extremely low inflation rate among larvae in Trial 1 was unknown because

all the established protocols were followed.

The greater range in BW among the larvae in Trial 1 on day 7 and 14, as indicated by the larger BW coefficient of variation (CV), likely greatly contributed to the higher incidence of cannibalism. On day 1, a higher incidence of cannibals was recorded in Trial 1 than Trial 2 (0.125 vs. 0.05%) during the counting process indicated a larger disparity among larvae used in Trial 1. The higher incidence of cannibals in Trial 1 than Trial 2 contributed to the higher BW CV on day 7 and 14, which further facilitated cannibalism. Higher losses due to cannibalism were recorded among the treatments with a higher BW CV at the end of both Trials 1 and 2. Similarly, losses to cannibalism among North American burbot (*Lota lota maculosa*) was positively related to TL CV, and was highest in the group with the largest TL CV on day 1 (Barron et al. 2013). A minimum difference in body size is required for head-first cannibalism to occur, for example, a 25% difference in length between cannibals and prey enable complete ingestion among Atlantic cod larvae (Folkvord 1997). The maximum ratio of prey length to predator length is species specific: 0.56 for flagtail grouper (*Cephalopholis urodeta*), 0.41 for breckled hawkfish (*Paracirrhites forsteri*; Mihalitsis and Bellwood 2017) and 0.59 for perch (*Perca fluviatilis*, Dörner, and Wagner 2003). In Trial 1 on day 7, the ratio of larval striped bass prey to predator length ranged from 0.53 to 0.73, similar to the three species cited above. In the present study, a lower ration resulted in more competition for *Artemia* and greater risk of cannibalistic behavior. The estimated number of larvae consumed by each cannibal ranged from 24 to 43 in Trial 1, which is comparable to burbot, a total of 43 burbot (21mm TL)

presumed consumed by two cannibals during a 15 day trial (Barron et al. 2013). In Trial 2, by comparison, the number of conspecific larvae consumed by cannibals was higher ranging from 60 to 91 larvae per cannibal associated with larger difference in BW between the non-cannibal fish and the cannibals. The largest cannibal in Trial 2 was 1363mg, about 28 times larger than the mean body size of its non-cannibal conspecifics. The SGR of cannibals was high in Trial 1 and 2 ranging from 17 to 30%/day. High daily ration and SGR was also reported among cannibals of the catfish (*Pseudoplatystoma punctifer*, 100mg in dry mass, DM), they can consume 40% DM daily, and grew at 25% DM/day (Baras et al. 2011). The high growth and daily ration requirement of the cannibals contributed to the severe loss of siblings within a catfish cohort. However, the larvae presumed to have been consumed by the catfish cannibals may not have been fully ingested. In tail-first cannibalism, only the posterior part of prey body was ingested, the anterior proportion of the carcass was discarded (Baras et al. 2003). The discarded body parts may be consumed by other conspecifics, promoting the emergence of putative cannibalistic fast-growing larvae such as the 40mg larvae from Trial 1 on day 7 in the present study. Residual small body parts of striped bass larvae were not detected in large tanks and relatively deep water and could be completely decomposed before the next mortality retrieval the next day. In the future studies a more frequent retrieval of mortalities may be helpful to estimate more accurately the losses due to cannibalism.

The growth of striped bass larvae was good in both Trials 1 and 2. Striped bass larvae efficiently captured *Artemia* prey in the recirculation system; most prey were consumed

within 15 min of being offered (details *see* Chapter 3.4). The growth and survival of striped bass larvae among the three highest ration treatments (250, 300 and 350 *Artemia*/larva/meal) in Trial 2 were comparable to the 2016 trial with >90% survival and 0.7mm/day growth rate when offered 150 and 200 *Artemia*/larva/meal in the first and second 9 days (J. Lu and Duston unpubl. data). The ISB larvae in Trial 1 also exhibited a good survival rate (89-98%) and growth rate (0.6-0.7mm/day) among 110 to 140 *Artemia*/larva/meal treatments. While the actual ration in each treatment maybe higher than the proposed ration because NSB larvae experienced high mortality, the ration was fixed throughout Trial 1. The LD 15:9 photoperiod in Trial 1 appeared to have negligible effect on growth and survival as the 2016 trial run at LD 24:0 resulted in similar survival and growth of ISB striped bass larvae. Generally, longer photoperiods improve larvae growth rates as larvae continuously feed in daylight if prey is always available (Hubbs and Blaxter 1986). However, longer photoperiods also extend the duration of foraging, therefore, 24h light might not prove beneficial for larvae growth if energy ingested does not exceed the energy expended searching for prey. For example, BW of snapper (*Pagrus auratus*) larvae (11-31 dph) was significant greater in LD 18:6 than LD 24:0 (Fielder et al. 2002). Survival, however, was not affected by photoperiod, as has been reported in many species, such as larval sole (*Solea solea*, 12, 18 and 24h light, Fuchs 1978), and larval barramundi (*Lates calcarifer*, 8, 16 and 24h light, Barlow et al. 1995). In an early study (Eldridge et al. 1981), striped bass larvae (7-31 dph) offered 5000 *Artemia*/L once daily (equal to 1600 *Artemia*/larva/day) had a similar survival (>90%) but a lower growth rate (0.22mm/day) compared to Trial 2.

Even though the larvae used in Trial 2 were older and bigger than larvae used by Eldridge et al. (1981), they grew better when offered 300 *Artemia*/larva/meal (equal to 1500 *Artemia*/larva/day) in Trial 2 possibly because the ration was equally divided into 5 meals rather than only one meal per day offered by Eldridge et al. (1981). Adding *Artemia* into a small tank once daily may not be good for water quality nor the vitality of *Artemia*. In Trial 2, the broader range and higher ration (100 to 350 *Artemia*/larva/meal) compared to Trial 1 (70 to 140 *Artemia*/larva/meal) and older age and larvae body size contributed to a larger effect of ration on BW growth. However, the high incidence and mortality rates of NSB larvae in Trial 1 made the analysis of results more complicated, because the *Artemia* ration increased as the NSB larvae decreased while the NSB can be the extra prey for other ISB and cannibals. The mean BW of ISB larvae in Trial 1 was independent of ration provided, but the mean BW of larvae in Trial 2 was positively correlated to ration. Offering a larger ration resulted in a higher instantaneous prey density, prey capture capacity of striped bass was positively correlated to larvae age and prey density. When offered *Artemia* at 50, 200, and 800/L to 9 dph (TL 9mm) striped bass, the capture rate was 3, 7, and 15 *Artemia*/larva/hour, and increased to 26, 92, and 220 *Artemia*/larva/hour at 22 dph (TL 15mm, Duston and Astatkie 2012). In 100 *Artemia*/L density, the capture rate increased from 5 *Artemia*/larva/hour among 9-11 dph to over 100 *Artemia*/larva/hour at 22 dph (MacIntosh and Duston 2007). Comparing the larvae in Trial 2 vs. Trial 1, they were older 24 vs. 16 dph and had a higher swim bladder inflation 98 vs. 41%, and the instantaneous *Artemia* densities were higher 600 to 2100/L vs. 495 to 995/L, therefore, larvae captured

prey more efficiently which likely contributed to the more homogenous growth, higher survival rate and a lower cannibalism rate in Trial 2.

Survival rather than growth is the priority through the larval stage, to maximize the yield of juveniles seedstock. Offered 250 *Artemia*/larva/meal, five meals daily for larvae age 24 to 38 dph can achieved 90% survival and less than 10% of losses due to cannibalism. Overall consideration of the results from Trial 1, 2016, and 2017 trials indicates 150 *Artemia*/larva/meal, five meals daily between 16 to 31 dph can achieve 90% survival.

Chapter 5. Effect of stocking density on striped bass larvae survival and growth

5.1 Introduction

The encounter rate between larval fish and their prey, be it copepods or conspecifics, is dependent on the number of individuals per unit volume of water. Hence, the stocking density of the larvae and the number of *Artemia* provided per day (ration), are two potentially critically important factors affecting survival and growth. In the two trials described in Chapter 4, the single experimental factor was ration, with the larval stocking density being fixed. Here, in Chapter 5, stocking density was the single experimental factor. No publications in the primary literature on larval striped bass have quantified the relationship between stocking density and survival and growth. In an unpublished trial conducted in 2017 comparing 17 dph larvae stocked at 7 and 14/L, larvae were offered 70 *Artemia*/larva/meal, five meals daily. The final mean BW of the larvae after 14 days was independent of stocking density (38 vs. 40mg), but survival was significantly higher at the lower stocking density, 54 vs. 45% (ANOVA, p=0.02), and losses due to cannibalism was estimated at 49.5 and 41.3% in the high and low stocking density treatments respectively (Duston *unpubl. data*). By comparison, among striped bass larvae stocked at 79/L at 7 dph in 7.6L McDonald jars, the overall survival rate was very low (10.4 to 12.6%) and the losses to cannibalism was 36% (range 30-43.9%) after 17 day despite being fed an excess ration of *Artemia* (Braid and Shell 1981). Very small rearing tanks of 7-8L and high stocking density were implicated in the poor survival. By comparison, striped bass larvae

reared in 15L fiberglass tanks (4 larva/L, 19℃) fed 500 *Artemia*/L from 5 to 19 dph exhibited 70% survival (Tsai 1991). An even higher survival rate of 85% was recorded in a larger rearing volume (36L, 19℃) at the same stocking density (4 larvae/L) when offer 500 *Artemia*/L from 6-30 dph (Houde and Lubbers 1986). To better define the effect of stocking density on survival and growth, Trial 3 described here adopted the same 'broad range finding' approach as the previous chapter, up to eight levels of stocking densities (1 to 9/L, age 23 dph) were compared with no replicates, ration was fixed at 100 *Artemia*/larva/meal, five meals daily in consideration of the *Artemia* production capacity.

5.2 Materials and methods

Husbandry, *Artemia* production, fish counting, and experimental rearing system are described in **Chapter 3**. The specific procedures used in Trial 3 are described below.

5.2.1 Trial 3: Effect of eight stocking densities (range: 1 to 9 larvae/L) on survival and growth of striped bass larvae from 23 to 37 dph. Ration fixed at 100 *Artemia*/larva/meal, five meals daily.

On June 25, 2018, at 23 dph, 7200 striped bass larvae were counted. A total of 24 cannibals were removed, their mean (SE) BW 62(4.3)mg and TL 17(0.4)mm. The larvae selected for the trial had a mean BW(SE) 11(0.6)mg and TL 12(0.2)mm. The incidence of swim bladder inflation was 100% (50 of 50 examined at 23 dph). Within a RAS of eight tanks (each 1m diameter, 0.22-0.23m water depth, 173-180L), each stocked with 200 to

1600 larvae in increments of 200 larvae/tank, to achieve densities of 1 to 9 larvae/L. The rearing water was 20℃ and 2.9ppt salinity at an inflow rate of 5L/min per tank. The photoperiod was simulated natural day length (Latitude 45°N, LD 15:9). Light intensity measured above the water surface was about 10.5 lux.

The next day (24 dph), feeding of enriched stage II *Artemia* commenced. *Artemia* were offered to each tank at 100 *Artemia*/larva/meal based on the initial stocking numbers. This fixed ration was offered five times daily (09:00, 12:00, 15:00, 18:00, and 21:00h) for 14 days. After 7 days (30 dph), a random sample of 25 larvae (range 25 to 28) from each tank was taken. Larvae were then euthanized individually (MS222, 0.2g/L) to record BW (to 0.001g) and TL (to nearest 0.5mm). On day 14 (37 dph), a random sample of 25 (range 25 to 28) larvae from each tank were caught, then they were euthanized individually, and their body size was measured. All the remaining survivors in each tank were counted. The cannibals were identified by their large BW and distended belly. All cannibals from each treatment were caught and their body size was recorded.

5.2.2 Statistical analysis

The estimated losses due to cannibalism (%) and incidence of cannibals (%) was calculated (*See* **Chapter 3.5** for details).

One-way ANOVA was used to compare the difference in BW and TL means among treatments (Minitab 19). To satisfy the assumptions of normality and constant variance, a logarithm base 10 transformation was applied to the mean BW on day 7. The normality of

the BW distribution was tested by Anderson-Darling normality test (Minitab 19).

5.3 Results

The number of mortalities retrieved from day 1 to 7 ranged from 0 to 10 in each tank, less than 2% of the initial number of larvae stocked (Table 5.1). The BW of larvae on day 7 in all but three treatments were normally distributed (Anderson-Darling normality test, $P>0.05$, Figure 5.1, upper panel). The exceptions were the 800, 1600 and 1000 larvae/tank density treatments (Anderson-Darling normality test, $P<0.005$). Among sampled fish in 1000 larvae/tank treatment on day 7, one cannibal with a prey in the mouth was found and weighed 95mg and TL 21mm, 4.5 times heavier and 1.6 times longer than the mean of other larvae within the same treatment. The body size of the prey removed from the mouth of the cannibal 14mg BW and 11mm TL. Mean BW on day 7 was significantly different among treatment ranging from 17 to 21mg (ANOVA, $F_{7, 204}=5.37$, $p<0.001$, Table 5.1). An effect of stocking density on BW on day 7 was not evident, ranging from 17mg among larvae in 1000 and 1400 larvae/tank treatments to 22mg in 400 larvae/tank treatment (Table 5.1). Similarly, mean TL were independent of stocking density ranging from 13 to 14mm (Table 5.1).

On day 14, the cumulative total number of mortalities retrieved from each tank ranged from 0 to 23, up to 1.4% of the initial larvae stocked (Table 5.1). The highest survival rate was 99% in the lowest stocking density treatment. Among the other seven treatments, survival ranged from 56 to 86%; an effect of stocking density was not evident (Table 5.1).

The estimated losses due to cannibalism also failed to indicate a clear effect of stocking density among treatments ranging from 1 to 41% in the lowest (1 larva/L) and highest stocking density (9 larvae/L) treatments, and among the other six stocking density treatments ranged from 14 to 31% in 1400 and 400 larvae/tank treatments (Table 5.1). The final mean BW was significantly different among treatments, ranging from 25 to 36mg in 1400 and 400 larvae/tank treatments, respectively (ANOVA, $F_{7, 212}$=5.08, p<0.001, Table 5.1). Mean BW of larvae stocked in 400 larvae/tank treatment was significantly larger than all other stocking density treatments except the 1600 larvae/tank treatment (Table 5.1). Mean TL of larvae on day 14 was independent of stocking density ranging from 15mm to 16mm (Table 5.1). Striped bass larvae BW in all treatments were normally distributed (Anderson-Darling normality test, p>0.05, Figure 5.1, lower panel) with CVs ranging from 18 to 28%. Cannibals were present in all except the lowest density treatment, their incidence ranged from n=3 to 11, 0.4% to 1.4% of the total survivors in each treatment (Table 5.1). The mean BW of the cannibals ranged from 231 to 364mg, about 8 to 10 times larger than non-cannibal larvae (Table 5.2)

Table 5.1. Trial 3. Inventory, mean (SE) body weight (BW, mg), total length (TL, mm) and growth in length (GIL, mm/day) among 23 and 37 dph striped bass (*Morone saxatilis*) larvae stocked at eight densities ranging from 200 to 1600 larvae/tank (1 to 9 larvae/L). Larvae were offered a fixed ration of 100 enriched stage II *Artemia*/larva/meal, based on the initial stocking density five times daily for 14 days. Losses due to cannibalism are calculated as total estimated mortalities due to cannibalism divided by initial larvae number. Initial mean (SE) BW and TL was 11 (0.6)mg and 12 (0.2)mm. Rearing volume 173 L at 20°C and 2.9ppt salinity.

Stocking density	200	400	600	800	1000	1200	1400	1600
Exact density (larvae/L)	1	2	3	5	6	7	8	9
Mortalities day 1-7	0	8	0	9	1	4	5	10
BW day 7 (23dph)	20(0.7)^{abc}	22(0.9)^{a}	18(0.8)^{bc}	21(0.8)^{ab}	17(0.8)^{c}	19(0.8)^{abc}	17(1.0)^{c}	20(0.8)^{ab}
TL day 7 (23dph)	14(0.2)^{a}	14(0.2)^{a}	13(0.2)^{a}	14(0.2)^{a}	13(0.2)^{a}	14(0.2)^{a}	13(0.2)^{a}	14(0.2)^{a}
Mortalities day 8-14	0	8	3	5	6	0	4	13
Number sampled	55	51	52	51	53	53	51	53
Final survivors	144	208	434	514	724	851	1151	862
%Survivors	99	62	80	70	77	75	86	56
Losses to cannibalism (%)	1	31	19	28	22	24	14	41
BW day 14 (37dph)	29(1.3)^{b}	36(1.5)^{a}	29(1.2)^{b}	30(1.1)^{b}	30(1.6)^{b}	28(1.2)^{b}	25(1.0)^{b}	30(1.3)^{ab}
TL day 14 (37dph)	15(0.3)^{a}	16(0.3)^{a}	15(0.2)^{a}	16(0.2)^{a}	16(0.3)^{a}	16(0.2)^{a}	15(0.2)^{a}	16(0.2)^{a}
GIL day 1 to 14	0.2	0.3	0.2	0.3	0.3	0.3	0.2	0.3
Cannibals (n)	0	3	3	6	5	5	5	11
Incidence of cannibals (%)	0	1.4	0.7	1.2	0.7	0.6	0.4	1.3

Table 5.2. Trial 3. The number, mean (SE) body weight (BW, mg) and total length (TL, mm) of striped bass (*Morone saxatilis*) larvae cannibals on day 14 (38 dph) among 8 different stocking density treatments ranging from 200 to 1600 larvae/tank; larvae were offered 100 stage II *Artemia* per larvae per meal, 5 times a day for 14 days. Rearing volume was 173L at 20°C and 2.9ppt salinity.

Stocking density	200	400	600	800	1000	1200	1400	1600
n	0	3	3	6	5	5	5	11
BW (mg)	/	364(75)	231(25)	289(48)	234(41)	284(36)	249(69)	282(42)
TL (mm)	/	33(2.4)	28(1.0)	29(1.8)	27(1.6)	31(1.0)	29(2.7)	28(1.5)

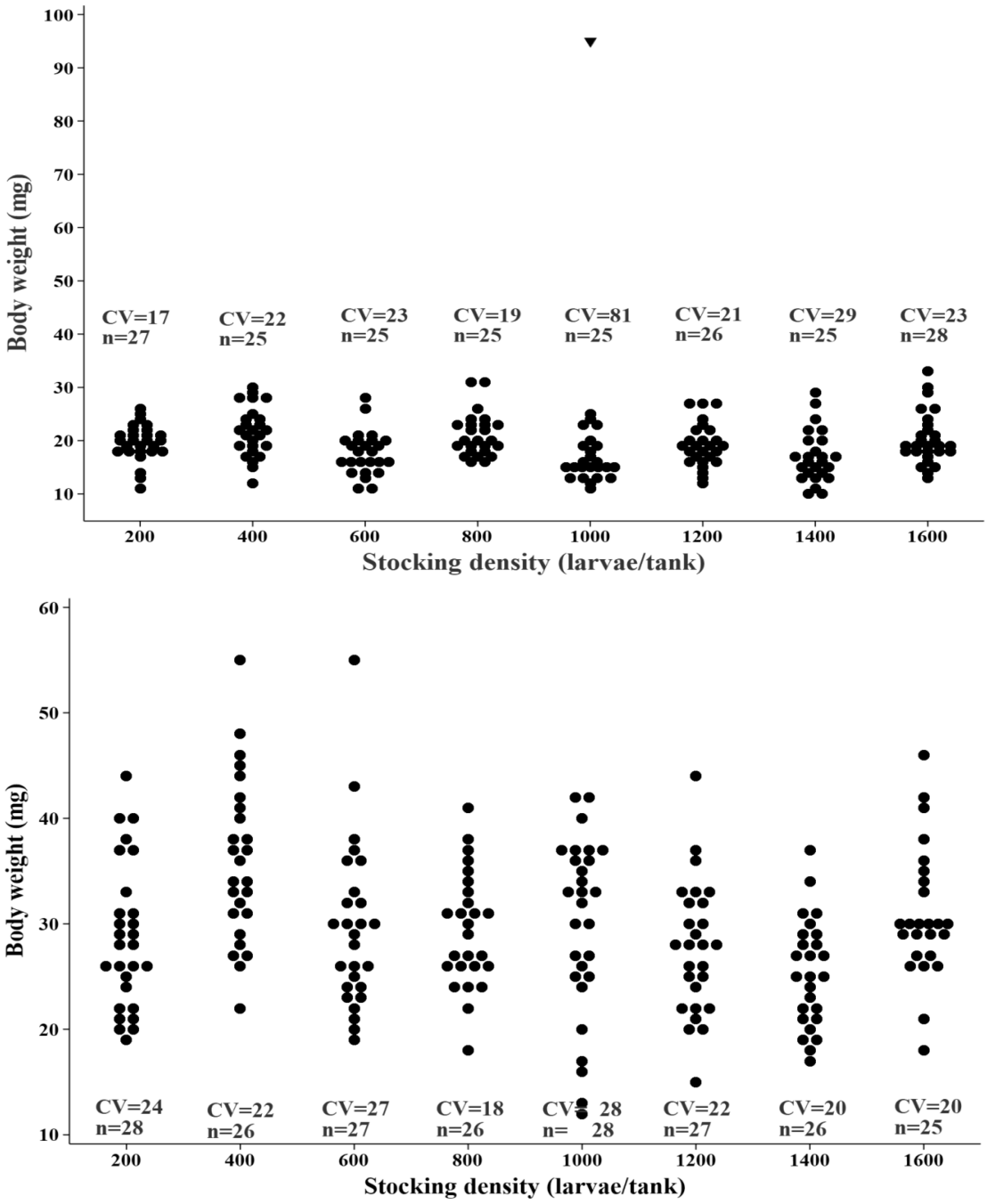

Figure 5.1. Trial 3. Body weight distribution of a sample of striped bass (*Morone saxatilis*) larvae after 7 days (30 days post-hatch, dph; upper panel) and 14 days (37 dph; lower panel) reared in eight tanks, stocking density ranged from 200 to 1600 per tank (1-9 larvae/L). Larvae were offered a ration of 100 enriched stage II *Artemia*/larva/meal, five times daily based on initial stocking number. The single triangle indicates a confirmed cannibal. Rearing volume 173L, temperature was 20℃ and salinity 2.9ppt. Initial mean (SE) body weight 11 (0.6)mg.

5.4 Discussion

Stocking density (1 to 9 larvae/L) had no effect on BW, TL or survival rate among 23-37 dph striped bass larvae based on an overall comparison of the eight levels. However, comparing only the lowest and highest stocking density (200 vs. 1600/tank) suggested losses to cannibalism contributed to differences in the survival rate. Cannibalism was the principal cause of mortality in all treatments except the lowest stocking density treatment.

Survival rates of larvae among all density treatments (range 56 to 99% in 9 and 1 larvae/L treatments) were higher than the 2017 trial (54 and 45% in 7 and 14 larvae/L treatments). The highest losses to cannibalism in the 1600/tank treatment (9 larvae/L) was similar to the high stocking density treatment (14 larvae/L) in 2017, at 41.3 vs. 41%. In the 2017 trial, the growth of striped bass larvae was independent of stocking density and the survival rate was significantly higher in low stocking density (7/L). By comparison, in Trial 3, survival and growth were both independent of stocking densities ranging from 1 to 9 larvae/L. The growth of European sea bass larvae was also independent of stocking densities among 50, 100, 150 and 200 larvae/L when offered a mixed ration of rotifer and *Artemia*. Among early larvae (1-30 dph) survival and growth was independent of stocking density (range 50 to 200 larvae/L), the main cause of mortality was an inability to feed or swim bladder hypertrophy and cannibalism was not evident (Hatziathanasiou et al. 2002). In the present study, the incidence of swim bladder inflation was 100%, with cannibalism appearing to be the main cause of mortality. Despite no statistically significant effect of stocking density on cannibalism in the present study, the highest losses to cannibalism were

nonetheless recorded in the highest stocking density treatment, suggesting a critical 'threshold' may have been exceeded. The increased incidence of cannibalism at higher stocking densities is maybe attributable to higher encounter rates between individual fish during foraging. African sharptooth catfish larvae exhibited a similar response, which exhibited no significant differences in the rates of cannibalism over the range of 50-150 fish/L, but a high rate of cannibalism in the highest stocking density treatment only (Haylor 1991). Nile tilapia (*Oreochromis niloticus*) fry (16mg BW) stocked in a RAS at densities of 3, 5, 10, 15, and 20 fry/L exhibited reasonably good survival at all treatments ranging from 90% to 100%, fry at 3 and 5/L densities experienced significantly higher survival rates and specific growth rates (El-Sayed 2002). The relatively higher mortality in the highest Nile tilapia density was due to cannibalism with decreased growth rates associated with chronic stress response at higher stocking density (Barcellos et al. 1999, El-Sayed 2002). Among northern pike, cannibalism losses were lower at higher stocking densities due to a perceptual confusion effect; when cannibals encounter too many prey, they were less efficient to select a single prey and mis-estimated the size of the prey, making cannibalistic attacks less successful (Kucharczyk et al. 1998). The confusion effect also caused the cannibals to misestimate the size of the prey and attack the prey that are slightly larger than their mouth gape, species such as perch, striped bass have spiny fin-rays that make regurgitation impossible and the cannibals will eventually suffocate (Baras et al. 2003). No clear relationship between BW CV and stocking density was exhibited by the striped bass larvae in Trial 3. In some treatments, the BW CV was smaller on day 14 than

day 7, perhaps due to losses of the smallest individuals due to cannibalism. Similarly, BW CV of giant gourami was independent of stocking density when stocked 0.6 to 19.2 larvae/L for 21 days (Arifin et al. 2019).

In the present study, TL growth rates of striped bass larvae were similar among larvae offered 100 *Artemia*/larva/meal in Trial 2 at 0.2 to 0.3mm/day; slower than the 0.5mm/day exhibited by 22 dph larvae (TL 11mm, BW 13mg) offered 125 *Artemia*/larva/meal, five meals daily at a density of 4 larvae/L (Duston, D. Roberts and Qiu, unpubl, data). In an earlier study, striped bass larvae (7-31 dph) had a similar growth rate (0.22mm/day) as the present study, but a higher survival rate (>90%) at 4 larvae/L and offered 1600 *Artemia*/larvae/day (Eldridge et al. 1981). Higher growth rate among larvae offered a higher ration in Trial 2 indicated that food may became a limiting factor as striped bass larvae grow. Growth of larval sunshine bass had a highly significant, negative linear relationship with stocking density, because each tank received the same amount of food, lower growth rates at higher stocking densities suggested food became a limiting factor as stocking density increased (Ludwig and Lochmann 2007). Similarly, among 3 dph Atlantic cod larvae stocked at density 300 fish/L, growth was lower when offered one million than two million rotifers per day. In contrast, no difference in specific growth rates were found in cod larvae stocked at 50, 100, 200, 300 fish/L when excess food was offered, indicating food availability rather than stocking density is the primary factor affecting the growth (Baskerville-Bridges and Kling 2000). In comparison, the ration in the current study was calculated based on the number of individual fish, so ration offered to each tank was

proportional to stocking density to ensure each individual from different stocking density treatment had same amount of food at each meal. No clear relationship between stocking density and growth was found in the present study but a negative relationship between growth, survival and stocking density has been recorded in other species. Spotted sand bass (*Paralabrax maculatofasciatus*) larvae offered rotifers, *Artemia* and copepods, exhibited a significantly higher specific growth rate and standard length when stocked at 50 and 100 larvae/L compared to higher densities 150 and 200 larvae/L (Alvarez-González et al. 2001). Survival rate of spotted sand bass was significantly higher at the lowest density because low oxygen concentration and cannibalism impaired survival at higher stocking densities (Alvarez-González et al. 2001). A similar negative relationship between growth, survival and stocking density were also observed in African catfish (*Clarias gariepinus*) fry (5-12 larvae/L; Kareem and Olanrewaju 2015) and vundu larvae (*Heterobranchus longifilis*) (5-50 larvae/L; Imorou Toko et al. 2008). The different results between the current study and other studies are possibly due to stocking density in the present study (range 1 to 9 larvae/L) being too low to affect the growth and survival of striped bass larvae. The highest stocking density in the current study was 9 larvae/L, much lower than other studies. A broader range of stocking densities needs to be tested to determine the relationship in striped bass larvae between survival, growth and stocking density. The availability of striped bass larvae was limited at Dal-AC; and tanks available for experiment were relatively large, the smallest volume being 100L making it relatively hard to experimentally achieve higher stocking densities like other studies. To better test a broad range of stocking densities, smaller

volume tank systems are needed to achieve higher stocking densities, while allowing treatment replication applied in the limited floor area. Based on the present experimental results, 9 larvae/L can be safely used to culture striped bass age 23 to 37dph, however, further work was needed to explore stocking density exceeding 10 larvae/L. In the next chapter, stocking density of 15 larvae/L was tested.

Chapter 6. The main and interaction effects of ration size and stocking density on striped bass larvae survival and growth

6.1 Introduction

In 2018, effect of ration size (70 to 140 and 100 to 350 *Artemia*/larvae/meal, five meals daily, Trial 1 and 2) and stocking density (1 to 9 larvae/L, Trial 3) on striped bass larvae survival, growth and cannibalism were conducted in a RAS with relatively large tank volumes of 100 to 200L/tank (Chapters 4 and 5). Survival and growth were positively correlated with ration size, with an 85% survival rate achieved when offered 200 *Artemia*/larva/meal, a response which plateaued when ration size was further increased. Stocking densities of 1 to 9 larvae/L, by comparison, did not affect growth or survival. However, density dependence is common influencing on survival and growth of larval and juvenile striped bass in the wild (Kimmerer et al. 2000, Martino and Houde 2012). Since the influence of stocking density may be dependent on the amount of food available, the interaction between these two factors was investigated in 2019.

The 2018 experiments narrowed down the stocking density and ration size range for optimized striped bass rearing to around 150 *Artemia*/larva/meal and 250 *Artemia*/larva/meal at a density of 9 larvae/L for 17-31 dph and 24-38 dph larvae, respectively. The relatively large rearing volumes and large numbers of striped bass larvae made husbandry difficult. Moreover, mortalities were possibly not being detected during

daily husbandry checks because it was difficult to see them in the large green tanks and deep water. To improve the precision of the experiments in 2019, two trials were conducted in smaller tanks, static water and a smaller number of larvae and three replicates per treatment. Both shared the same 2x2 factorial experimental design and started with larvae of the same age, 12 dph, but from different cohorts. The two levels of stocking density were the same for both trials, 3 and 15 larvae per litre, in a rearing volume of 15L (45 and 225 larvae per tank). These stocking densities were chosen because the total number of larvae was low enough to be manageable in terms of counting, but since the static tanks were untested, so water quality and husbandry were a concern at the planning stage. 15 larvae/L was higher than the highest density 9 larvae/L in 2018 trials and the five-fold difference between the two densities in Trial 4 and 5 were aimed to reveal differences in the response variables, survival, growth and incidence of cannibalism. The ration size differed by several-fold between the two trials. In the first trial (designated Trial 4) the two ration levels, 'medium' and 'high' were 50 and 200 *Artemia*/larva/meal. Since this ration size resulted in good growth and survival and negligible cannibalism, a second trial (designated Trial 5) compared 10 and 50 *Artemia*/larva/meal, referred to in the text as 'low and medium'.

6.2 Materials and methods

Husbandry, *Artemia* production, fish counting, and the experimental rearing system are described in **Chapter 3**. The specific methods are described here. Newly fertilized eggs were collected from the Stewiacke River estuary on June 2, 3 and 11, 2019. The eggs

collected June 2 and 3 were pooled together to form cohort 1 used in Trial 4; the June 11 eggs were cohort 2 and used in Trial 5.

Both trials were a 2x2 randomized factorial design; the two factors being stocking density and ration size, each with two levels. Each of the four treatment combinations had three replicates. Trial duration was 16 days and both trials commenced with 12 dph larvae. Stocking density was either 3 or 15 larvae/L for both trials, abbreviated here as S_3 and S_{15}. Ration size for Trial 4 was either 50 or 200 *Artemia*/larva/meal, 'medium' and 'high', abbreviated as R_{50} and R_{200}. For Trial 5 the ration was either 'low' or 'medium', 10 or 50 *Artemia* /larva/meal, abbreviated as R_{10} and R_{50}. Ration size was based on the initial number of larvae stocked into each tank and remained fixed throughout each trial, regardless of decreases in the number of fish due to mortality. The initial *Artemia* density at feeding in $R_{10}S_3$, $R_{10}S_{15}$, $R_{50}S_3$, $R_{50}S_{15}$, $R_{200}S_3$, $R_{200}S_{15}$ treatments were 30, 150, 150, 750, 600 and 3000/L. The experimental unit was a black plastic bucket (5 US gallons, 18.9L, MH R05, Ampak Inc., Laval, QC) herein referred to as 'tank'. The black interior of the tanks made visualization of the mortalities easier, since they turned white post-mortem. The rearing volume was 15L of static water (water depth 25cm, diameter 28cm).

In preparation for stocking with larvae, each tank was partly filled with about 7.5L of brackish water (salinity 3.5ppt). The 12 tanks were stocked in an assigned random order in batches of n=15 larvae. At the end of the stocking process, the rearing volume in each tank was adjusted to 15L. To determine the initial body size of the larvae, three random samples of 15 fish were collected from the cooler during the stocking process, then euthanized

individually (MS222, 0.2g/L) and measured (TL to nearest 0.5mm; BW to 0.001g). The morning after stocking, any mortalities in each tank were removed with a turkey baster, counted and replaced with larvae from the same cohort that had been held in the cooler overnight adjacent to the trial tanks. Visualizing the white bodies of the dead larvae was facilitated by a small LED headlight (Energizer HD. C322). Feeding then commenced; larvae in each tank were offered a set ration of enriched stage II *Artemia* five meals per day (09:00, 12:00, 15:00, 18:00, 21:00h), the same timing as previous trials. The rearing water was partially exchanged each day between 07:00 and 08:00h by first removing the air diffuser, then decanting 5L from each tank, taking care not to catch larvae. Mortalities were then removed from the tank floor using a turkey baster. During removal of mortalities, which took about 5 minutes per tank, any evidence of cannibalism among the other larvae was noted, including biting attacks, and larvae with another larva in their mouth. The rearing volume was returned to 15L by carefully pouring in water from the reservoir tank. A second mortality check and removal was conducted daily around 17:00 h. Twice daily, temperature, oxygen concentration and salinity were measured in each tank (YSI Pro 2030 Yellow Springs, OH, USA), and pH (Orion 9107BNMD, Orion Star A121, Thermo Scientific, Beverly, MA, USA).

On day 7 in Trial 4 and day 9 in Trial 5, the mean body length of larvae in each tank was estimated non-lethally. A random sample of n=10 larvae from each tank in Trial 4 and n=6 in Trial 5 were transferred in rearing water into a Petri dish (diameter=8.5cm) with a plastic ruler (1mm increments) placed underneath, and then an image was taken (Apple

iPhone 7 plus). The fish were then returned to the rearing tank. From the image, the TL of each larva was estimated using Image J software (National Institutes of Health and the Laboratory for Optical and Computational Instrumentation, University of Wisconsin). At the end of each trial, on day 16 (28 dph for both trials), 7L of water was removed from each tank. The larvae were collected by quickly pouring the remaining water through a large plastic kitchen sifter, then quickly euthanized in ice-cold MS222 solution. All visibly large larvae, putative cannibals, were counted and their individual TL (to 0.5mm) and BW (to 0.001g) was recorded, and gut contents noted. The remaining larvae were counted, and a random sample of 30 were measured if survival was >30 (all tanks from Trial 4), or if survival was <30 (all three tanks of $R_{10}S_3$ treatment in Trial 5), all larvae were measured (TL to 0.5mm, BW to 0.001g). The specific growth rate (SGR, %/day) in total body length and losses to cannibalism was estimated. The cumulative mortality rate (%) for each tank was based on the mortalities retrieved each day divided by the initial number of larvae stocked. Some of these mortalities had missing tails, eyes or heads indicating they were victims of a cannibal attack. But since they were not ingested, they were not counted as a loss to cannibalism, following the criteria defined by Baras et al. (2003). The total mortality was the sum of mortalities retrieved and the losses to cannibalism. The cannibals were identified by their relatively large body size and distended abdomen, then their mean TL and BW was calculated separately from the other fish sampled from the tank.

6.2.1 Trial 4: Ration: medium or high (50 or 200 *Artemia*/larva/meal); stocking density: 3 or 15 larvae/L

Larvae from cohort 1, age 12 dph, were stocked into the 12 tanks between 09:45 and 10:50h on June 17, 2019. Six tanks were each stocked with 45 larvae (3/L), and six with 225 larvae (15/L), a total of 1620 fish. Initial mean (SE) BW and TL, was 2.5 (0.12) mg and 7.7 (0.07) mm. The swim bladder inflation rate was 98% (44 of 45 examined at 12 dph). Larvae were offered four meals each of 100 *Artemia*/larva on June 17. The following morning, 08:50h, a total of 63 mortalities were replaced, between 2 to 11 larvae per tank, overall 3.9% mortality. Then the trial started, larvae were offered five meals per day of either 50 or 200 *Artemia*/larva/meal based on the initial number of larvae stocked into each tank. On day 7 (June 24, 19 dph), TL was estimated non-lethally. On day 16 (July 3, 28 dph), all surviving larvae were euthanized, counted, and the body size of a random sample of n=30 was measured. Water quality parameters during the trial (mean and range) were as follows: salinity 3.8ppt (2.8 to 4.5), temperature 19.7℃ (18 to 21.6), oxygen saturation 92% (67 to 103), and pH 8.1 (7.7 to 8.3).

6.2.2 Trial 5: ration: low or medium (10 or 50 *Artemia*/larva/meal); stocking density: 3 or 15 larvae/L

Larvae from cohort 2, age 12 dph were stocked into the 12 tanks between 09:00 to 10:20h on June 26, 2019, following the same procedures as Trial 4, but a new randomization plan to stock six tanks with 45 larvae (3/L) and six tanks with 225 larvae

(15/L), a total of 1620 larvae. Initial mean (SE) BW and TL were 3.1 (0.31)mg and 8.6 (0.16)mm. Swim bladder inflation rate was 94% (overall 66 of 70 examined at 11 and 12 dph). On June 26 the larvae were offered four meals of either 10 or 50 *Artemia*/larva/meal. The following morning, 09:25h, a total of 36 mortalities were replaced, range 0 to 12 per tank, 2.2% overall mortality. Then the trial started, larvae were offered five meals daily for 15 days of either 10 or 50 *Artemia*/larva/meal based on the initial number of larvae stocked into each tank. On day 9 (July 5, 21 dph), the TL of larvae was estimated non-lethally. On day 16 (July 11, 28 dph), all survivors were euthanized, counted, and a sample measured. Water quality parameters during the trial (mean and range) were as follows: salinity 3.9ppt (3.4 to 4.2), temperature 20.5°C (18.1 to 21.2), oxygen saturation 96% (81 to 99), and pH 8.3 (8 to 8.4).

6.2.3 Statistical analysis

With tank as the experimental unit, the response variables of mean BW, TL, SGR and the mean number of losses to cannibalism were compared by ANOVA using the GLM function in Minitab version 18, followed by Tukey's pairwise mean comparison test (Minitab, LLC. State College, Pennsylvania). Cumulative mortality was analyzed using the PROC CATMOD procedure in SAS (SAS 9.4, 2013. Cary, NC). Three assumptions: normality, homogeneity of group variance and independence were established before accepting the analysis. To analyze the variation in growth between treatments, the final BW data of the three replicates within each treatment were pooled and the coefficient of

variation (CV) was calculated. Pair-wise comparison of CV between treatments was conducted during the approximate F-test in MedCal (MedCalc Statistical software 19.1, 2019, Ostend, Belgium). Log F was calculated using the following equation (Forkman 2009): $\log F = \log {c_1}^2(1 + \frac{(n_1-1){c_1}^2}{n_1})^{-1} - \log {c_2}^2(1 + \frac{(n_2-1){c_2}^2}{n_2})^{-1}$, C is the coefficient of variation and *n* is the number of samples. To avoid the type I error: the rejection of a true null hypothesis as a result of a test procedure, calculated *p* values were corrected by Bonferroni adjustment and express as p_{adj}. The normality of BW distribution was tested by Anderson-Darling normality test in Minitab 19.

6.4 Results

6.4.1 Trial 4: Ration: medium or high (50 or 200 *Artemia*/larva/meal); stocking density: 3 or 15 larvae/L

The cumulative mortality up to day 7 was very low (<3%) in all treatments (Figure 6.1). Mean (SE) TL on day 7 ranged from 11.1 (0.32)mm to 13.1 (0.32)mm in the $R_{50}S_{15}$, and $R_{200}S_3$ treatments, respectively (Table 6.1). Mean TL on day 7 was highly significantly dependent on ration (F=23.67; *p*=0.003), but independent of stocking density (F=3.11; *p*=0.128), and there was no interaction (F=1.88; *p*=0.219). Specific growth rate (SGR) in total body length up to day 7 ranged from 5.2%/day in the $R_{200}S_{15}$ group to 7.6%/day in the $R_{50}S_3$ group, and was significantly dependent on ration (F=23.1, *p*=0.003; Table 6.1). The excess Artemia was found among the groups offered a high ration at the end of the day.

From day 8 onwards the cumulative mortality among the two groups fed a medium

ration (R_{50}) increased steadily, reaching a mean of 12.6% on day 16 at low stocking density ($R_{50}S_3$) and 19.3% at high stocking density ($R_{50}S_{15}$; Figure 6.1). In the high ration groups (R_{200}), by comparison, cumulative mortality was only 4.4% in the high stocking density ($R_{200}S_{15}$) and 3.0% in the low stocking density ($R_{200}S_3$; Figure 6.1). The first cannibal attack was observed on day 12 (23 dph) in the $R_{50}S_{15}$ group. From day 13 to the end of the trial on day 16, the cannibal attacks were evident four times during routine husbandry checks, and 16.7% (11/66) of the mortalities were missing eyes and/or their tail suggesting they were victims of a cannibal attack. On day 16, the final mean cumulative mortality rate was highly significantly dependent on ration ($p<0.001$), but independent of stocking density ($p=0.20$, Figure 6.1) and there was no interaction ($p=0.57$) between ration level and stocking density.

In the high ration/low stocking treatment ($R_{200}S_3$), total mortality was very low, a mean of 3.5% (5/141), with no evidence of cannibalism (Table 6.2). A similar low total mortality rate of 5% (34/675) occurred in the high ration/high stocking density group ($R_{200}S_{15}$; Table 6.2). By contrast, mortality was relatively high in the medium ration/high density group ($R_{50}S_{15}$) replicates each stocked with 225 fish; losses were consistently high across all replicates and overall was 21.8% (147/675), and in the medium ration/low density ($R_{50}S_3$) treatment mortality was 14.1% (19/135) (Table 6.2). Losses attributed to cannibalism in the $R_{200}S_3$, $R_{50}S_3$, $R_{200}S_{15}$, $R_{50}S_{15}$ treatments were 0.74 (1/135), 1.5 (2/135), 0.59 (4/675) and 2.5% (17/675) respectively (Table 6.2), it was not significantly affected by either ration ($F=1.88$, $p=0.207$) or stocking density ($F=0.21$, $p=0.659$).

The final mean BW at 28dph (day 16) ranged from 18mg in both low ration groups $R_{50}S_{15}$ and $R_{50}S_3$ to 55mg in high ration groups $R_{200}S_{15}$, and $R_{200}S_3$ (Figure 6.2). The mean TL of larvae ranged from 15mm in $R_{50}S_{15}$ to 19mm in the $R_{200}S_{15}$ group (Table 6.1). Final mean BW was significantly affected by ration ($F= 845.8$; $p<0.001$) but was independent of stocking density ($F=0.16$, $p =0.70$), and there was no interaction ($F=0.07$, $p=0.79$). Fed a high ration, the larvae were about 300% heavier and 130% longer than those larvae fed the medium ration (Table 6.1, Figure 6.2). The SGR day 1-16 was significantly affected by ration ($F=904$, $p<0.001$) but not stocking density ($F=0.01$, $p=0.92$; Table 6.1). The growth in length ranged from 0.43mm/day in two medium ration (R_{50}) treatments to 0.73mm/day in the $R_{200}S_{15}$ treatment. Body size frequency distribution on day 16 provided no indication of cannibalism since all were normally distributed with no outliers with a large BW (Anderson-Darling normality test, $p>0.05$, Figure 6.2). Both high ration groups ($R_{200}S_{15}$ and $R_{200}S_3$) were normally distributed with most larvae between 45 to 60mg BW with a coefficient of variation (CV) of 20 and 21% respectively (Figure 6.2). Among the medium ration groups, by comparison, final BW ranged between 15 and 30mg with a CV of 25 and 23% in $R_{50}S_{15}$ and $R_{50}S_3$ treatments respectively (Figure 6.2). The multiple pairwise comparisons between the four treatment CVs indicated they were not significantly different, since the $p_{adj.}$ was always higher than 0.46.

Table 6.1. Trial 4. Mean (SE) total body length (TL, mm) of striped bass (*Morone saxatilis*) larvae at 28 days post-hatch (dph) in a 2x2 factorial trial of 16 days duration testing the effect of stocking density (3 or 15 larvae/L, S_3, S_{15}) and ration (50 or 200 *Artemia*/larvae/meal based on initial stocking density, five meals daily, R_{50}, R_{200}). Initial (12 dph) mean(SE) TL 7.7 (0.07)mm, BW of 2.5 (0.12) mg, rearing volume 15L, 3.8ppt salinity, 19.7°C. Means were derived from 3 replicates of each treatment. The number (n) of fish sampled from each treatment on day 7 and day 16 are shown in parentheses in the treatment column. SGR=specific growth rate in total body length (% TL/day). Growth in length (GIL, mm/day) from day 1 to day 16 is also shown. Within each column, means sharing the same superscript letter are not significantly different (α=0.05).

Treatment	TL, day 7	SGR day 1-7	TL, day 16	SGR day 1-16	GIL day 1-16
$R_{50}S_{15}$(37,92)	11(0.3) ^{b}	5.2(0.26) ^{b}	15(0.1) ^{b}	4.0(0.01) ^{b}	0.43
$R_{200}S_{15}$(54,90)	13(0.3) ^{a}	7.4(0.04) ^{a}	19(0.1) ^{a}	5.8(0.05) ^{a}	0.73
$R_{50}S_{3}$(38,90)	12(0.3) ^{b}	6.4(0.51) ^{ab}	15(0.1) ^{b}	4.0(0.02) ^{b}	0.43
$R_{200}S_{3}$(25,104)	13(0.3) ^{a}	7.5(0.16) ^{a}	19(0.2) ^{a}	5.7(0.10) ^{a}	0.72

Table 6.2. Trial 4, day 16 (28 dph). Inventory of striped bass (*Morone saxatilis*) larvae survivors and retrieved mortalities in each of 12 rearing tanks in a 2x2 factorial trial of 16 days duration testing the effect of stocking density (3 or 15 larvae/L; S_3, S_{15}) and ration (50 or 200 *Artemia*/larva/meal, based on the initial stocking density, five meals daily; R_{50}, R_{200}) starting at 12 days post-hatch. Mortalities were removed twice daily. Tank rearing volume 15L, 3.8ppt salinity, 19.7°C.

Stocking density	Low			Low			High			High		
Ration	High			Medium			High			Medium		
Code	$R_{200}S_3$			$R_{50}S_3$			$R_{200}S_{15}$			$R_{50}S_{15}$		
Replicate	1	2	3	1	2	3	1	2	3	1	2	3
Stocked day 1	45	45	45	45	45	45	225	225	225	225	225	225
Mortalities retrieved	2	1	1	5	4	8	7	13	10	44	49	37
Loss to cannibalism (n)	1	0	0	0	0	2	0	4	0	8	7	2
Total mortalities	3	1	1	5	4	10	7	17	10	52	56	39
Mortality rate	7	0	0	9	7	13	1	5	1	23	25	17
Survivors (n)	42	51	45	41	42	39	222	212	223	173	169	186
% Survival	93	100	100	91	93	87	99	94	99	77	75	83
Loss to cannibalism (%)	2	0	0	0	0	4	0	2	0	4	3	1

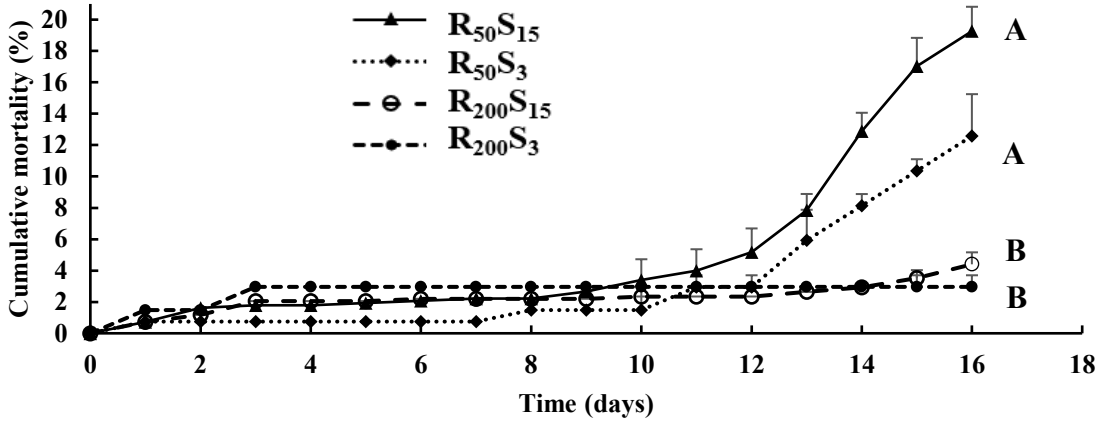

Figure 6.1. Trial 4. Mean (SE) cumulative mortality among striped bass (*Morone saxatilis*) larvae (Day 0=12 days post-hatch) in a 2x2 factorial design trial. Stocking density was either 3/L (S_3) or 15/L (S_{15}) in 15L rearing volume (n=45 or 225 larvae per tank). Ration was either 50 or 200 *Artemia*/larva/meal (R_{50}, R_{200}) based on the initial stocking density, five meals daily for 16 days. Salinity 3.8ppt and temperature 19.7°C. Each coordinate is the mean mortality of three replicate tanks. Final mean mortality values on day 16 sharing the same letter are not significantly different (α=0.05).

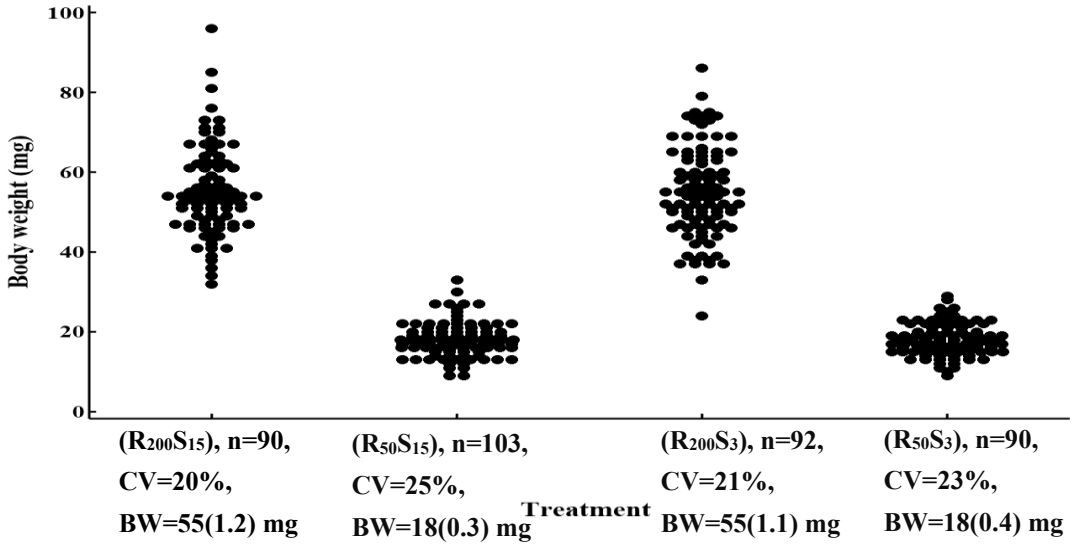

Figure 6.2. Trial 4. Body weight distribution and coefficient of variation (CV) of striped bass (*Morone saxatilis*) larvae at 28 days post-hatch (dph) after 16 days rearing in four treatments in a 2x2 factorial design: either low (S_3) or high (S_{15}) stocking density (3 or 15 fish/L) and offered either medium (R_{50}) or high (R_{200}) ration (50 or 200 *Artemia*/larvae/meal based on initial stocking density, five meals daily) for 16 days. About 30 fish were randomly sampled from each replicate tank and the data from 3 replicate tanks are pooled. The rearing volume was 15L brackish water at 3.8ppt and 19.7°C.

6.4.2 Trial 5. ration: low or medium (10 or 50 *Artemia*/larva/meal); stocking density: 3 or 15 larvae/L

Trial 5, relative to Trial 4, resulted in higher mortality, a higher incidence of cannibalism, and greater variations in body size, due in part to the low ration of 10 *Artemia*/larva/meal. In addition, there was a substantial 'cohort' effect, as evidenced by a two-fold higher mortality in the two treatment groups repeated in both trials, $R_{50}S_3$ and $R_{50}S_{15}$.

Cumulative mortality up to day 10 was independent of stocking density, but was significantly affected by ration (F=7.2, p=0.003); ranging from 7.4 to 14.4% in high ration groups compared to 25.5 to 29.6% in the low ration groups (Figure 6.3). Specific growth rate in TL up to day 9 ranged from 1.6%/day in $R_{10}S_3$ groups to 4.7% in $R_{50}S_3$ groups (Table 6.3). Mean TL of larvae on day 9 was highly significantly dependent on ration (F=11.8, p=0.009) but independent of stocking density (F=0.2, p=0.65), and there was no interaction effect (F=2.2, p=0.18). Mean (SE) TL on day 9 ranged from 9.9 (0.32)mm in $R_{10}S_3$ to 13.1 (0.27)mm in the $R_{50}S_3$ group (Table 6.3).

Cumulative mortality between day 9 and 16 increased steadily in all groups. In the two groups fed a low ration (R_{10}), mortalities reached a final mean of 37.8% and 28.3% in the low (S_3) and high (S_{15}) density group, respectively (Figure 6.3). In the medium ration treatments (R_{50}), by comparison, total mortalities retrieved was lower, 24.7 and 21.5% in the high (S_{15}) and the low (S_3) density group respectively (Figure 6.3).

The total mortality including the presumed losses due to cannibalism in the medium ration groups ranged from 25.2% (34/135) in $R_{50}S_3$ to 44.1% (298/675) in the $R_{50}S_{15}$ treatment (Table 6.4). The two groups fed a low ration, by comparison, exhibited a significantly higher mortality rate, ranging from 76% (102/135) in $R_{10}S_3$ to 80.1% in $R_{10}S_{15}$ ($F=107$, $p<0.001$; Table 6.4).

Cannibals were first detected on day 3 when five attacks were observed during daily husbandry among the three tanks in the low ration, low stocking density treatment ($R_{10}S_{15}$). Thereafter, from day 10 (22dph) onwards, missing eyes and tails were increasingly evident among the retrieved mortalities, 21.9% (29/132) of the mortalities exhibited signs of cannibalistic attacks and 10 attacks were observed during either mortality checks or feedings. At the end of the 16 day trial, the mean cumulative mortality rate was highly significantly dependent on ration ($F=7.2$, $p=0.028$), but independent of stocking density ($F=0.7$, $p=0.426$), and there was no interaction effect ($F=3.0$, $p=0.121$). Disappearance of larvae, assumed due to cannibalism, was significantly affected by ration ($F=28.5$, $p=0.001$), but independent of stocking density. Losses attributed to cannibalism in the $R_{10}S_{15}$, $R_{50}S_{15}$, $R_{10}S_3$, $R_{50}S_3$ treatments were 52 (350/675), 19 (131/675), 38 (51/135) and 4% (5/135) respectively (Table 6.4).

The CV in BW on day 16 among the low ration treatments was high, 169 and 117% in $R_{10}S_{15}$ and $R_{10}S_3$ treatments, respectively (Figure 6.4). In the other two medium ration treatments by comparison, the coefficient of variation was 72 and 23% in the $R_{50}S_{15}$ and $R_{50}S_3$ treatment respectively (Figure 6.4). The CV was significantly different among most

of the multiple pairwise comparison between the treatments (p_{adj}=0.001), with the exception between $R_{10}S_{15}$ and $R_{10}S_3$ (F=1.27, p_{adj}=1.00), $R_{50}S_{15}$ and $R_{10}S_3$ (F=0.58, p_{adj}=0.293). There were 9, 6, 2 and 0 cannibals found in the $R_{50}S_{15}$, $R_{10}S_{15}$, $R_{10}S_3$, $R_{50}S_3$ treatments respectively (Figure 6.4) and their mean(SE) TL and BW was 21(0.8), 22(1.0), 17(2.3)mm, and 76(11.7), 115(15.6) and 64(5.5)mg. The mean BW of the cannibals was independent of stocking density (F=3.19, p=0.096) and ration (F=4.27, p=0.058), while the mean TL was significantly affected by stocking density (F=8.03, P=0.013) but independent of ration level (F=1.5, p=0.241).

The specific growth rate in BW and growth in length ranged from 1.9% to 3.5%/day and 0.19 to 0.41mm/day in $R_{10}S_3$ and $R_{50}S_{15}$ respectively from day 1 to 16 (Table 6.3). At the end of the trial, the larvae fed a medium ration (R_{50}) were on average 250% heavier and 130% longer than larvae fed a low ration (R_{10}). The final mean (SE) BW (excluding outliers) ranged from 9(0.3)mg in $R_{10}S_3$ to 23(0.7)mg in $R_{50}S_{15}$ groups (Figure 6.4). The mean (SE) TL of larvae ranged from 12(0.1)mm in $R_{10}S_3$ to 15(0.1)mm in the $R_{50}S_{15}$ group (Table 6.3). The final mean BW was significantly affected by both ration (F=97.5, p<0.001) and stocking density (F=5.6, p=0.046) but there was no interaction effect (F=1.3, p=0.282). Mean final TL was highly dependent on ration (F=65.6, p<0.001), but was independent of stocking density (F=3.1, p=0.115), and there was no interaction (F=0.8, p=0.398).

Table 6.3. Trial 5. Mean (SE) total body length (TL, mm) of striped bass (*Morone saxatilis*) larvae at 28 days post-hatch (dph) following 16 days rearing either at low (S_3) or high (S_{15}) stocking densities (3 or 15 fish/L) and offered either low (R_{10}) or medium (R_{50}) ration (10 or 50 *Artemia*/larvae/meal based on initial stocking density, five meals daily. Initial (12 dph) mean TL 8.6 (0.16)mm, BW of 3.1 (0.32) mg. Rearing volume 15L, 3.9ppt salinity, 20.5°C. Means were derived from 3 replicates of each treatment, the number (n) of fish sampled from each treatment on day 9 and day 16 are shown in parentheses in the treatment column. SGR=specific growth rate in total body length (%per day). Growth in length (GIL, mm/day) is also shown. With each column, means sharing the same superscript letter are not significantly different (α=0.05).

Treatment	TL, day 9	SGR day 1-9	TL, day 16	SGR day 1-16	GIL day 1-16
$R_{50}S_{15}$(30,85)	13(0.3) [a]	4.4(0.15) [a]	15 (0.1) [a]	3.5(0.19) [a]	0.41
$R_{50}S_3$(28,91)	13(0.3) [a]	4.7(0.36) [a]	14 (0.3) [a]	3.2(0.08) [a]	0.36
$R_{10}S_{15}$(28,91)	11(0.4) [b]	2.6(0.42) [b]	12(0.1) [b]	2.0(0.12) [b]	0.21
$R_{10}S_3$(20,31)	10(0.2) [b]	1.6(0.58) [b]	12(0.1) [b]	1.9(0.07) [b]	0.19

Table 6.4. Trial 5, day 16. Inventory of striped bass (*Morone saxatilis*) larvae survivors and retrieved mortalities in each of 12 rearing tanks in a 2x2 factorial trial lasting 16 days duration testing the effect of stocking density (3 or 15 fish/L; S_3, S_{15}) and ration (10 or 50 *Artemia*/larva/meal, based on the initial stocking density, five meals daily; R_{10}, R_{50}) starting at 12 days post-hatch. Mortalities were removed twice daily. Tank rearing volume 15L, 3.9ppt salinity and 20.5°C.

Stocking density	Low			Low			High			High		
Ration	Medium			Low			Medium			Low		
Code	$R_{50}S_3$			$R_{10}S_3$			$R_{50}S_{15}$			$R_{10}S_{15}$		
Replicate	1	2	3	1	2	3	1	2	3	1	2	3
Stocked day 1	45	45	45	45	45	45	225	225	225	225	225	225
Mortalities retrieved	11	10	8	21	12	18	60	58	49	62	50	79
Loss to cannibalism (n)	1	4	0	7	23	21	44	43	44	125	128	97
Total mortalities	12	14	8	28	35	39	104	101	93	187	178	176
Survivors (n)	33	31	37	17	10	6	121	124	132	38	47	49
% survival	73	69	82	38	22	13	54	55	59	17	21	22
Mortality rate (%)	27	31	18	62	78	87	46	45	41	83	79	78
Loss to cannibalism (%)	2	9	0	16	51	47	20	19	20	56	57	43
Cannibals (n)	0	0	0	0	1	1	3	3	3	3	2	1

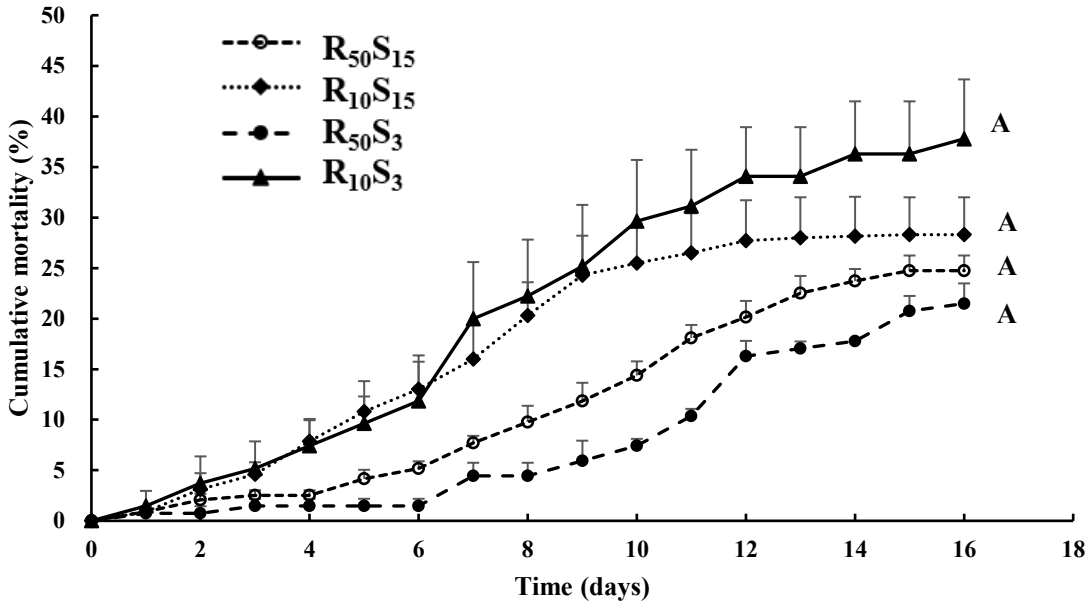

Figure 6.3. Trial 5. Mean (SE) cumulative mortality among striped bass (*Morone saxatilis*) larvae (Day 0=12 dph) in a 2x2 factorial design trial. Stocking density was either 3/L, (S_3) or 15 /L (S_{15}) in 15L rearing volume (n=45 or 225 larvae per tank). Ration was either 50 or 200 *Artemia*/larva/meal (R_{50}, R_{200}) based on the initial stocking density, five meals daily for 16 days. Salinity 3.9ppt and temperature 20.5°C. Each coordinate is the mean (SE) mortality of three replicate tanks. Final mean cumulative mortality values on day 16 sharing the same letter are not significantly different (α=0.05).

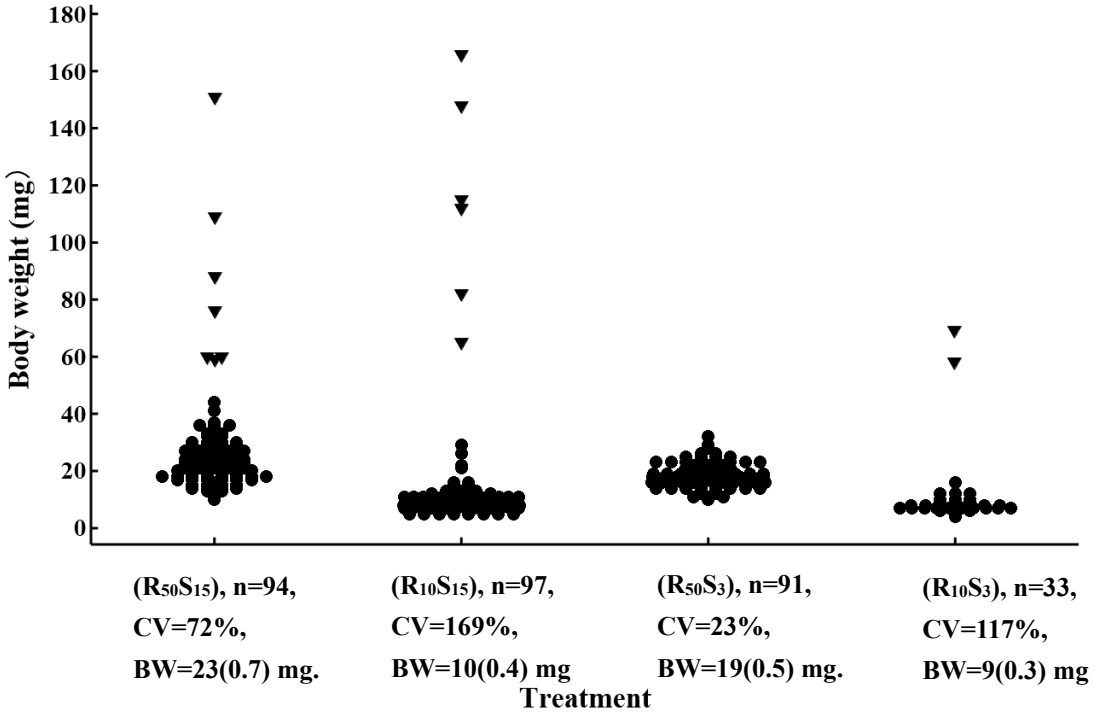

Figure 6.4. Trial 5. Body weight distribution and coefficient of variation (CV) of striped bass (*Morone saxatilis*) larvae age 28 days post-hatch (dph) after 16 days rearing on 2x2 factorial design trial four treatments, either low (S_3) or high (S_{15}) stocking density (3 or 15 fish/L) and offered either low (R_{10}) or medium (R_{50}) ration (10 or 50 *Artemia*/larvae/meal based on initial stocking density, five meals per day) for 16 days. Data for each treatment are pooled from three replicate tanks. Triangles indicate confirmed cannibals. Initial (12 dph) mean TL 8.6 (0.16)mm, BW of 3.1 (0.32) mg. The rearing volume was 15L brackish water at 3.9ppt and 20.5°C.

6.5 Discussion

The new experimental approach of using small volume tanks and static water proved an effective method to explore factors influencing the survival and growth of larval striped bass. Ration was clearly the key factor to achieve the goal of high growth rate and high survival. Provided with sufficient *Artemia* the problem of cannibalism can be largely

eliminated. A ration of 200 *Artemia*/larva/meal in Trial 4 ensured that food supply was not a limiting factor and may have been excessive. The optimum ration was somewhere between 50 and 200 *Artemia*/larva/meal; significantly higher survival rate and growth in BW in high ration treatments relative to medium ration treatments indicated that high ration was needed to sustain good growth and survival. Similar to Trial 3 (1-9 larvae/L), stocking densities between 3 and 15 striped bass larvae/L resulted in similar performance, indicating higher densities should be tested to identify the most efficient density for mass production. Ration was clearly more important than stocking density in regulating the growth-survival-cannibalism dynamic.

Very good survival (99%) and negligible cannibalism (0.3%) were achieved among larvae offered 200 *Artemia*/larva/meal in Trial 4, this was the first time such high survival and low cannibalism was recorded because the prey was excess and always available for larvae. In other trials, excess prey was never achieved due to the following reasons because large number of larvae used, excess *Artemia* ration was exceeding our *Artemia* production. Loss to cannibalism was lower among larvae offered 50 *Artemia*/larva/meal in Trial 4 compared to all ration treatments in Trial 1 and 2. The mortalities retrieved in static water tanks were higher than RAS systems indicating the mortalities from the larger thanks were underestimated. This might be due to the decomposition of mortalities between each mortality check and difficult to visualize the tiny mortalities in a big tank with running water in Trial 1 and 2. The smaller experimental static water system, by comparison, with the aid of a LED headlight, mortalities retrieved was easier and the estimates of the extent

of cannibalism was reliable, this system was more suitable for investigating the cannibalism among striped bass larvae.

Larvae can capture prey more efficiently when offered higher ration because of higher instantaneous prey density at feeding. Capture success increases with prey density and improves rapidly with experience and morphological development. Seventy-three percent of 2 dph bay anchovy (*Archosargus rhomboidalis*) foraged successfully when offered 50 prey per litre density which increased to 96% when offered 1000 prey per litre; these percentages increased to 98% and 100% respectively when larvae were 9 dph (Houde and Schekter 1980). The capture rate of striped bass larvae on *Artemia* in a 60-minute feeding trial in the dark was significantly affected by the interaction between prey density and larval age. Among 9 dph larvae, capture rate ranged from 4 to 20 prey/larva/hour at 50 and 800 prey/L and increased to 26 and 220 prey/larva/h at 22 dph (Duston and Astatkie 2012). The capture rate of 8 dph striped bass larvae in 100 prey/L density was 5 *Artemia*/larva/hour, which increased to 142 *Artemia*/larva/hour at the age of 22 dph (MacIntosh and Duston 2007). The improved prey capture rate was associated with the increased proportion of twin cone photoreceptors as larvae grew (MacIntosh and Duston 2007). In the present study, the lowest prey density at feeding was 30 *Artemia*/L in $R_{10}S_3$ treatments, while the highest prey density treatment $R_{200}S_{15}$ was 100 times higher at 3000/L. The BW, TL, and growth in length of striped bass larvae were consistently higher in higher ration treatments (R_{200} in Trial 4, R_{50} in Trial 5) within identical stocking densities (S_3 or S_{15}), which probably contributed by the higher capture rate in higher prey density and higher ration on an

individual basis. Likewise, 5 dph striped bass larvae stocked at 4 larvae/L and offered 50, 100 and 250 *Eurytemora affinis*/L and 100 *Artemia*/L, the growth in length during the 16 day trial was increased as prey density increased, from 0.3mm/day to 0.4mm/day in 50 and 250 *E.affinis*/L respectively (Chesney 1989). The same growth in length 0.36mm/day was recorded in 100 *Artemia*/L treatment in Chesney (1989) and $R_{50}S_3$ treatment in Trial 5 since they shared similar experimental conditions: stocking density was 4 and 3/L, the prey density was 100 and 150 *Artemia*/L, the duration was 16 days. In agreement with the former study, growth rate of striped bass larvae (8L tank, 12.5 larvae/L) in TL was positively correlated with food concentrations, rations greater than 100 *Artemia*/L (8 prey/larva/day) resulted in better growth rates, the highest growth rate observed was in the highest prey density 5000 *Artemia*/L (400 prey/larva/day; Eldridge et al. 1981). Despite the daily ration and prey density being higher, the growth in length of 0.22mm/day in the 5000 *Artemia*/L treatment from 9 to 28 dph (400 prey/larva/day) in Eldridge et al. (1981) was lower than the $R_{50}S_{15}$ treatment in Trial 5 of 0.41mm/day from 12 to 28 dph. The difference might be because a larger rearing tank was used in Trial 5 and the food was equally divided into 5 meals daily rather than the single meal offered by Eldridge et al. (1981). In both Trials 4 and 5, survivors in all treatments experienced positive growth as the lowest ration was 50 *Artemia*/larva/day, which is 6 times higher than the threshold ration (8 prey/larva/day) for positive growth in Eldridge et al. (1981). The 9 dph striped bass larvae (4 larvae/L,15L tank) were offered three stages of *Eurytemora affinis* at five densities, adults (5, 10, 25, 50, and 100/L), copepodites (10, 20, 50, 100, and 200/L) and nauplii (50, 100, 250, 500, and

1000/L; Tsai 1991). The growth of striped bass larvae consistently demonstrated a linear relationship with prey density in spite of some significant differences in slopes among regression lines of the three different food types (Tsai 1991). Among the studies cited above only Eldridge et al. (1981) estimated the daily ration requirement for striped bass. The daily ration requirement was estimated according to the feeding and digestion rate which increased with larvae size and age and prey density. At 12 dph, daily ration requirement for individual striped bass larvae ranged from 111 to 115 *Artemia* under 1000 and 5000 *Artemia*/L density, and increased to 151 and 247 at 21 dph (Eldridge et al. 1981). In present study Trial 4 $R_{200}S_{15}$ treatment, prey density at feeding was 3000/L, so the daily ration requirement for individual larva in this group should be between 115 to 151 *Artemia*, while the larvae were actually offered 1000 *Artemia*/day, I found excess food was left in this treatment at the end of the day and relatively lower incidence of cannibalism and higher survival rate. All other treatments in Trial 4, the daily ration was higher than the recommend ration by Eldridge et al. (1981), therefore, a relatively high survival rate was achieved in these treatments.

Cannibalism was observed earlier and was more evident in Trial 5 than Trial 4 and cannibalistic attacks observed during the feeding and mortality checks were higher in Trial 5 than Trial 4 (10 vs 4). Belly bites were observed earlier and most frequently in both trials, the victims can usually escape from the attacks, but had a high probability of dying after the attacks. Tail attacks, by comparison, were found in the later period of the trial. Similarly, a mean of 92% of cannibalistic attacks by walleye larvae (4-10 dph) were trunk attacks

compared to 8% tail attacks, but nearly 98% resulted in the escape of the victim. However, 19% of the trunk attack victims die within 24 hours; with the tail attacks, by comparison, victims were nearly always ingested (Loadman et al. 1986). Among northern pike (*Esox lucius*), by comparison, victims of tail attacks died a few days after being injured (Kucharczyk et al. 1998). In present study, the mortalities due to trunk attacks were usually harder to identify than the tail attacks, the latter usually resulted in a missing tail.

The risk of cannibalism among larval fish can be greatly reduced by increasing availability of copepod prey. For example, walleye larvae, when excess *Artemia* (17 to 120 *Artemia*/larva/meal, 14 meals daily) was provided, there was no effect of stocking density (0.3, 1, 1.7 and 2.3 larvae/L) on cannibalism from 1 to 19 dph (Cuff 1977). Only two larvae loss to cannibalism during the experiment; the low incidence of cannibalism was not as a consequence of less cannibalistic attacks but due to the successful escape by well-fed larvae in all different stocking density treatments (Cuff 1977). Similarly, in Trial 1, low feeding efficiency and growth rate of NSB larvae resulted in their poor escape ability from cannibalistic attacks and experienced high mortality. In Trial 4 and 5, aggression and cannibalism was influenced by hunger and food availability because a mean of 57 and 55% of the mortalities in Trial 4 and 5 were retrieved in the morning following 12h without feed; the highest percentage of mortality (66%) found in medium ration and high stocking density treatments ($R_{50}S_{15}$) also occurred in the morning during Trial 5. Similarly, 94% of the fat snook was found dead in the morning, following 15h without food (Corrêa and Cerqueir 2007). In 7 dph spotted seatrout, aggression and cannibalism were mainly

triggered by hunger because the aggressive behaviors were significantly increased with increasing time after feeding, irrespective of stocking densities (15, 30, and 60 fish/L; Manley et al. 2014). When food is not adequate to satiate fish populations, spotted seatrout become aggressive and consumed each other as an alternative food source (Manley et al. 2014). In the present study, due to the limited of personnel, no meals were offered between 22:00h and 8:00h next day. In future studies a peristaltic pump could be used to continuously deliver live *Artemia* to larvae during the night hours to possibly reduce cannibalism triggered by hunger (Baras et al. 2003)

The BW CV was consistently larger in the lower ration and higher stocking density treatments, which contributed to the establishment of hierarchy within the population and promoted a switch from tail-first to head-first cannibalism. Food quality and ration size were the two most important factors influencing growth depensation among larval striped bass. Striped bass larvae fed a lower ration *Artemia* (100-600 *Artemia*/larva/day) with formulated feed (0.3g/fish daily), fish eggs (0.2-0.6ml/fish daily) diverged in size as the CV of length increased from 12.8% at stocking to 23.3% after 21 days resulting in a bimodal length-frequency distribution (Paller and Lewis 1987). Fish fed large quantities of *Artemia* (1000-5000 *Artemia*/fish daily), in contrast, did not show growth depensation as the mean CV was decreased from 10.7% to 8.8% after 21 days, and was significantly lower than the low *Artemia* ration treatments (Paller and Lewis 1987). Cannibalism was only observed in tanks that received small amounts of *Artemia* with a higher CV (21.2%) but not in tanks receiving higher dietary rations with associated lower BW CV (8%), which

indicates size variation is an important factor affecting the rate of cannibalism (Paller and Lewis 1987). Small differences in initial BW CV among Atlantic cod larvae(28 vs 37.5%) resulted in significantly different rates of cannibalism (0.03 vs 0.39%/day) in a 16-day experiment starting from 0.5g BW when excess dry feed was offered (Folkvord and Otterå 1993). North American burbot (*Lota lota maculosa*) with Lower BW CV from size grading resulted in a significantly higher survival rate due to the reduction of cannibalism in (Barron et al. 2013). The control group with an initial CV of 18.1% resulted in 29% cannibalism, significantly higher than the graded group with an initial CV of 11.7% that yielded only 1% cannibalism (Barron et al. 2013). The overall survival rate was also significantly different, with 59.3% of the ungraded control and 93.3% of the graded treatment surviving, respectively (Barron et al. 2013). In the present study, the treatments with a higher BW CV consistently experienced higher losses to cannibalism, the Trial 5 $R_{10}S_{15}$ treatment, for example, with a BW CV=169% had a significantly higher loss to cannibalism (52%) compared to $R_{50}S_{15}$ (CV=72%) with 20% loss.

Stocking density had no significant effect on cannibalism in the present study; higher cannibalism rates occurred in higher stocking density treatments, which can be due to a greater probability of encounter rate between cannibals and potential prey. When food was not a limiting factor, a higher cannibalism rate and lower survival rate was found when striped bass were stocked at relatively higher stocking densities. Seven day old striped bass larvae were stocked at a density of 79 larvae/L, and sufficient *Artemia* was offered to ensure that adequate food was available for all larvae, the overall mean survival was low at 16.2%

and cannibalism was high at 36% (Braid and Shell 1981). Comparedto Trial 4, Trial 5 ration was also excessive in the high ration (R_{200}) treatments, the mean survival rate was high at 95.6% with less than 1% of mortalities due to cannibalism. The biggest differences between Trial 4 and the study of Braid and Shell (1981) were the stocking densities (15 vs 79/L) and the volume of rearing tanks (15 vs 7.6L), which both contributed to a higher encounter rate between cannibals and prey. A similar result was found in African sharptooth catfish, where 13 dph larvae stocked at the density of 150, 100 and 50 larvae/L, offered excess commercial trout starter feed, noted cannibalistic attacks increased with increasing stocking density (Haylor 1991). In 100 and 150 larvae/L treatments cannibalism was the principle cause of mortality and accounted for 44.1 and 56.5% of the total mortalities, while cannibalism only accounted for 21% of total mortality at a lower stocking density of 50 larvae/L (Haylor 1991). However, among perch (*Perca fluviatilis*) larvae, stocked at densities of 10, 31.6, and 100 larvae/L and offered excess *Artemia* for 21 days starting from 1 dph, ration was increased each day to ensure fish were fed in excess throughout the experiment (Baras et al. 2003). The mortality rate, cannibalism rate and CV in BW at the end of experiment was significantly higher at the lowest stocking density as increasing stocking density postponed commencement of cannibalism, lowered proportion of cannibals and lowered the rate of cannibalism per capita (Baras et al. 2003). These results, markedly different from the present study, illustrates the complexity of growth-survival-cannibalism dynamics of different species and rearing conditions.

In this study, there was a clear cohort effect' as mortalities and losses to cannibalism

in the two treatment groups ($R_{50}S_3$ and $R_{50}S_{15}$) repeated in Trials 4 and 5 yielded different responses. Specifically, the rate of total mortality, including losses to cannibalism was about two-fold higher in Trial 5 compared to Trial 4 (25.2vs 14.4% and 44.1 vs 21.8% in $R_{50}S_3$ and $R_{50}S_{15}$ respectively). The losses to cannibalism were also higher in Trial 5: 19.4 vs 2.5% and 3.7 vs 1.4% in $R_{50}S_{15}$ and $R_{50}S_3$ respectively. The specific growth rate of striped bass larvae in repeated treatments was also higher in Trial 4 than Trial 5: 4.0 vs 3.5%/day and 4.0 vs 3.2%/day from day 1 to 16 in $R_{50}S_{15}$ and $R_{50}S_3$ respectively. Similarly, growth in body length was better in Trial 4, 0.43 vs 0.41mm/day and 0.43 vs 0.36mm/day in $R_{50}S_{15}$ and $R_{50}S_3$, respectively. The only thing that was different between the otherwise identical repeated treatments in both trials were the swim bladder inflation rate, being 4% higher among larvae used in Trial 4 than Trial 5 (98% vs. 94%). Whether a 4% difference in swim bladder inflation rate is sufficient to produce differences in cannibalism, total mortality, and indices of somatic growth are unknown.

In conclusion, ration size, but not stocking density, had a significant effect on striped bass larvae growth, survival, and cannibalism. During the period of 12-28 dph, 200 *Artemia*/larva/meal, five meals daily and 15 larvae/L resulted in best survival and growth under the conditions of this study.

Chapter 7. Conclusion

Striped bass larvae can grow well in both the RAS and small static water systems when offered sufficient *Artemia* prey. Ration rather than stocking density was more important in regulating survival, growth and cannibalism among intensively cultured striped bass larvae. Growth and survival were positively correlated with ration levels among 12-38 dph striped bass larvae; stocking density from 1 up to 15 larvae/L resulted in a similar performance. In static water systems, even 200 *Artemia*/larva/meal, five meals daily resulted in good survival, growth and negligible cannibalism, up to 50% of water needs to be changed daily to maintain water quality which is not feasible on a large-scale production. Similarly, in the earlier studies used prey density as an experimental factor in static water systems; a specific prey density that needs to be maintained throughout the day to achieve a desire survival and growth rate. This is not an efficient way to produce striped bass larvae because a large quantity of *Artemia* is needed to maintain the prey density but only a small portion of prey were consumed by larvae, moreover, it is not feasible to maintain the prey density if a RAS is used. For commercial scale production of striped bass larvae, RAS system and 250 *Artemia*/larva/meal, five meals daily is sufficient for farmers since a good survival (90%) and low cannibalism (7%) were achieved. Ration recommended in this thesis based on larvae number is easy to calculate and offering prey more frequently at five times a day ensure most of *Artemia* were consumed by the larvae.

Rearing striped bass larvae at 15/L appears to be safe, and higher stocking densities deserve to be explored. Using a RAS and offer ration based on larvae number is a more efficient way to produce striped bass larvae since less labour is needed to maintain water quality, *Artemia* cysts and production. The new knowledge presented here can potentially reduce the problem of cannibalism and this is a significant step forward toward the goal of commercialization of striped bass aquaculture in Nova Scotia.

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