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The effect of temperature and fish size on growth of juvenile lumpfish (*Cyclopterus lumpus* L.)

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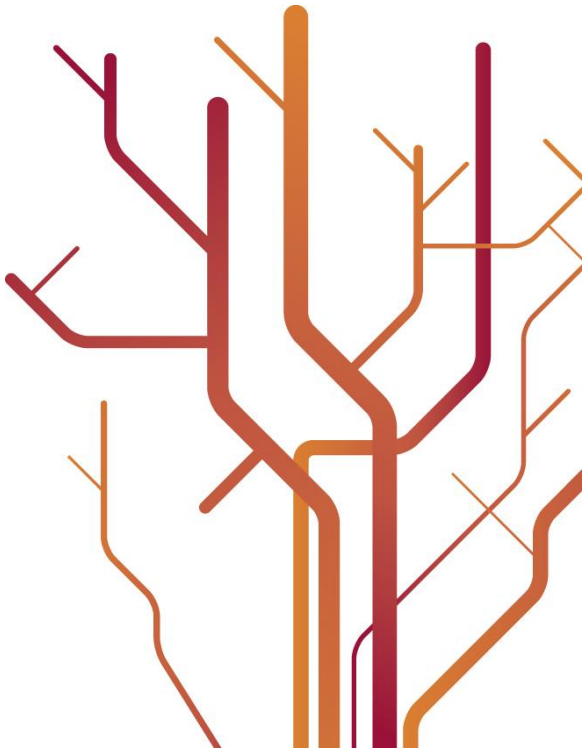

Preface

This thesis was written in the period August 2012 – August 2013, aided by supervisors, friends and colleagues who each and all deserve my greatest gratitude. First of all, to my supervisor, professor Albert Kjartansson Imsland who spent dark hours guiding me through the jungle of articles, temperature optimums and statistical obstacles, a great thank you for all your help and motivation. To my other supervisor, professor Inger-Britt Falk-Petersen, thank you for your patience, your feedback and your helpful guidance, and to Dr. Atle Foss for shipping in from Bergen to teach me how to pit-tag and sample my 348 new found pets. To the ladies in the office and to the boys at Troms Marin Yngel; I could not have done without you!

Kjørsvikbugen, 10. Augsut 2013.

Ane Vigdisdatter Nytrø.

Contents

Abstract

The aim of this study was to investigate the effect of temperature and fish size on growth response and feeding parameters of juvenile Atlantic lumpfish *Cyclopterus Lumpus* L. Two experiments were carried out, experiment 1 with emphasis on temperature related growth and experiment 2 as a satellite group to examine the effect of temperature on feeding parameters. On 11 April 2013 a number of 348 juvenile lumpfish (mean initial weight 26.5 g, S.E. ± 0.6) were individually tagged and randomly distributed alongside a sub-group of 337 untagged specimens (mean initial weight 21.9 g, S.E. ± 0.04) between 10 experimental units. This produced the outline for experiment 1 and further growth analysis. Fish were adapted to temperatures of 4, 7, 10, 13 °C and ambient (average temperature 5.8 °C, S.E. ± 0.04) before rearing at constant temperature throughout the experimental period. Experiment 2 consisted of 241 unmarked fish (mean initial weight 46.7 g, S.E. ± 1.0) reared at ambient temperature (average temperature 5.8 °C, S.E. ± 0.04) and 13 °C in two replicate groups for each temperature (n = 60 per replicate group). Feed was collected to estimate feeding parameters for each temperature group.

Higher temperatures increased final weight and length, overall specific growth factor and feed intake, but minor effects were observed for feed conversion efficiency between the two treatment groups of experiment 2. The optimal temperature for growth decreased with increasing size. The negative effect of suboptimal temperatures was most significant for the highest temperatures 10 and 13 °C with increasing weight.

This study demonstrated that temperatures 7, 10 and 13 °C for weight classes 25 – 200 g induces an overall increase in growth for juvenile lumpfish relative to that of temperatures of ambient and 4 °C. An ontogenetic shift in temperature preference for growth with increasing size was observed. The results indicate that an increase in temperature does not have any significant effect on feed utilization and that temperatures below ambient (5.8 °C, S.E. ± 0.4) restricts growth.

1. Introduction

The production of Atlantic salmon *Salmo salar* (L.) in Norway reached 1.5 million tons in 2009, making Norway the greatest producer of captive Atlantic salmon in the world (Torrissen et al. 2011). The parasitic sea louse *Lepeophteirus salmonis* (Krøyer) has been reported to cause increased cortisol levels, alterations in physiological homeostasis, osmotic imbalance and mortality in salmonids (Grimnes & Jakobsen 1996; Bjorn et al. 2001; Heuch et al. 2005; Sivertsgard et al. 2007) and salmon farms are assumed to be among the main causes of mortality in juvenile wild salmonids, constituting a contributing factor to decreasing stocks of wild fish (Bjorn et al. 2001; Krkosek et al. 2007; Costello 2009). The high concentration of potential hosts for lice in sea pens has been a problem since the onset of the salmon farming industry in the 1970s. Attempts to reduce the concentration of lice in sea pens has been a main goal ever since, not only as an economic concern, but also in terms of animal welfare and potential costs for the eco-system (Brandal et al. 1976; McVicar 1997; Asche et al. 2005; Krkosek et al. 2006). Due to the persistence of re-infections of sea louse, efficient control management strategies are hard to find (McVicar 2004). Today the density of lice is mainly regulated by the aid of chemoterapeutants, but increasing resistance to delousing agents such as avermectins (SLICE ®) (Burridge et al. 2010), organophosphates (Salmosan ®) (Fallang et al. 2004) and pyrethroids (Excis ®, Betamax ® and AlphaMax ®) (Sevatdal & Horsberg 2003; Burridge et al. 2010) is an emerging problem in aquaculture (Jimenez et al. 2012). Furthermore, such medical treatments are often expensive (Costello 2009), stressful to fish (Burka et al. 1997) and hazardous to the environment (Burridge et al. 2010).

1.1 The role of cleaner-fish in aquaculture

The biological control of sea lice through the use of "cleaner fish" has recently become a feasible option due to the increased occurrence of resistant lice, the reduced public acceptance of the use of chemotherapeutants in food production and the urgent need for an effective and sustainable method of parasite control in Atlantic salmon aquaculture (Denholm et al., 2002; Treasurer, 2002). Today cleaner-fish present the only environmentally friendly alternative to chemical de-lousing of salmonids (Cowx et al. 1998; Treasurer 2002). Their use has a lower impact on the environment, reduces cortisol levels of farmed fish, and is often found to be a cheaper alternative to chemical treatments (Treasurer 2002). Cleaner fish have been utilized

as a predator of sea louse in salmon net pens for several years, efficiently grazing on ecto-parasites; mobile pre-adult and adult lice (Groner et al. 2013). In northern parts of Norway low temperatures are a challenge for cleaner fish species such as ballan wrasse (*Labrus bergylta*, Asxanius) and goldsinny wrasse (*Ctenolabrus rupestris*, L.), limiting their distribution in northern waters (Cowx et al. 1998; Lein et al. 2013). Furthermore, Lein et al. (2013) showed a decrease in the appetite of ballan wrasse for lice with decreasing temperatures. This calls for an alternative cleaner-fish species with a natural northern distribution and a better adaptation to cold temperatures. Observations from pilot experiments have indicated that juvenile lumpfish, *Cyclopterus lumpus* (L.) potentially will graze on pre-adult and adult stages of sea lice attached to salmon (Willumsen 2001; Andersen & Vestvik 2012; GIFAS 2012).

1.2 The lumpfish

The lumpfish is a scale-less, short and thick fish, easily distinguishable by their high dorsal crest, which covers the first dorsal fin entirely. The pelvic fins are modified to constitute a ventral suction disc, allowing specimens to rest on vegetation, rocky substrate and algae (Bigelow & Schroeder 1953; Davenport 1985).

The species is commonly found in the arctic margins of the North Atlantic; in the east from 80° north off Spitsbergen and Nova Zemlya in the north to Portugal in the south. In the west the species has been frequently found along the coasts of America from Cape Cod to Canada and the coasts of Greenland 70° north in the north-west (Cox & Anderson 1922; Andrijasev 1964; Blacker 1983). The migratory behavior of lumpfish is similar to that of coastal benthic teleosts, but adults are also frequently found mid-water in open oceans outside the spawning season (Cox & Anderson 1922; Andrijasev 1964; Myrseth 1971; Davenport & Kjorsvik 1986). As a semi-pelagic species, it spends most of its life at offshore feeding grounds at deep water before conducting an active migration over great distances towards shallow coastal waters to spawn between winter and early spring (Myrseth 1971; Blacker 1983; Davenport 1985; Goulet et al. 1986; Holst 1993; Mitamura et al. 2012).

The first one to two years post hatch juveniles spend summer and autumn in the upper surface waters of tide pools in the intertidal zone (Moring 1990) but some juveniles have also been

observed at deep sea (Daborn & Gregory 1983; Davenport 1985). Tide pools offer the young shelter and food in the form of seaweed, other algae and a fluctuating availability of prey between tidal cycles (Moring 1990). Juveniles are typically observed in association to algae, either attached or nearby. Algae are shelter to harpacticoids, a preferred prey species of juvenile lumpfish among planktonic animals and phytal species (Ingolfsson & Kristjansson 2002). As they outgrow the protective shelters of tide pools at 4-5 cm they are forced to migrate to deeper water to avoid predations (Moring 1990; Albert 2000), decreasing water temperatures and hence declining availability of algae (Moring 1990). The Norwegian sea is believed to be an important nursery area for lumpfish originating from spawning grounds off the North Atlantic coast (Holst 1993).

1.3 Lumpfish, a species suited for aquaculture?

Attempts to breed lumpfish to produce spawning fish for roe were attempted by Klokseth and Øiestad (1999) and Haaland (1996). Early pilot scale experiments confirmed that adult lumpfish could with ease be reared and would spawn in captivity (Klokseth & Øiestad 1999; Andersen & Vestvik 2012), but that further research was needed to obtain better results in terms of growth (Benfey & Methven 1986; Havforskningsinstituttet 2003). Despite these results, most research on lumpfish has mainly been focused on wild populations for documenting the spawning migrations, its peculiar spawning behavior and larval- and juvenile trade-offs in foraging behavior, as these factors have been of concern to lumpfish roe fisheries (Holst 1993; Torstensen 1998; Sunnanå 2007a). An early attempt to create a production protocol for the domestication of lumpfish has been piloted by Schaer and Vestvik (2012) for Arctic Cleanerfish AS.

1.4 Growth and feeding in juvenile marine species

Foraging-predator tradeoffs and feeding behavior have been discussed for juvenile lumpfish in the wild (Moring 1989; Ingolfsson & Kristjansson 2002), but little effort has been spent on estimating the growth efficiency of the juvenile lumpfish in captivity. The metabolic cost of foraging and trade-off strategies in juvenile and larval lumpfish has been discussed (Brown et

al. 1997; Killen et al. 2007; Jobling et al. 2012), but with an emphasis on live feeds rather than formulated.

According to Jobling (1997) the preferred temperature of a species is often closer to the temperature optimum for growth rather than the optimal temperature for food consumption and – conversion. The temperature-related growth rates of juvenile fish have been studied for several marine species including spotted wolfish *Anarhichas minor* (Òlafson) (Imsland et al. 2006), cod *Gadus morhua* (L.) (Bjornsson et al. 2001; Imsland et al. 2005), turbot *Scophthalmus maximus* (L.) (Imsland et al. 2007a), halibut *Hippoglossus hippoglossus* (L.) (Hallaraker et al. 1995; Jonassen et al. 1999), plaice *Pleuronectes platessa* (L.) and flounder *Platichthys flesus* (L.) (Fonds et al. 1992).

Temperatures above that of the temperature optimum for growth rapidly decreases the specific growth rate in several species. Growth rates for juveniles have commonly been found to follow a bell shaped curve for several species, with increasing growth (SGR % day^{-1}) until reaching a maximum at the temperature optimum and decreases rapidly with further increase in temperature (Otterlei et al. 1999; Bjornsson et al. 2001). Furthermore, an ontogenetic shift in optimum temperatures for growth can be observed in several species. Investigating specific growth rates and feed conversion ratios enables us to examine the optimal temperature for ingested food used for growth. The close relationship between ingestion rate and growth rate should also be stressed, as growth is also determined by food supply (Jobling 1997).

Land based aquaculture utilizes the possibility of maximizing the effect of temperature manipulation on growth and feed efficiency. Optimal temperatures for growth rates have been shown to be higher than the temperature optimum for ingestion rate (Jobling 1997). Finding the temperature optimums for the two factors is hence crucial to increase production efficiency, as temperature is interpreted as the most important external factor for growth (Brett & Groves 1979; Jobling 1997).

1.5 The physiology of temperature related growth in juvenile fish

Increasing temperature causes two main counteracting effects on growth:

1. Increase in the energy cost for maintenance metabolism, hence decreasing the scope for growth.
2. Increase in the efficiency of food energy transformation to net energy (Brett & Groves 1979; Elliott 1982; Pörtner et al. 2001; Van Ham et al. 2003), thus increasing the scope for growth.

Temperatures have a secondary effect in terms of increasing oxygen consumption at high temperatures (Hallaraker et al. 1995; Jonassen et al. 2000b), and causes energy costs to decrease at continuous light and at lower temperatures (Imsland & Jonassen 2001). In terms of feed costs a maximum- and maintenance ration (R_{max} and R_{maint}, respectively) can be established, defining the available energy for somatic growth and metabolic functions. R_{max} and R_{maint} both decrease with increasing fish size, but R_{maint} at a slower rate than R_{max}, reducing the available energy for somatic growth (Brett & Groves 1979). This causes more developed specimens to allocate energy mainly for maintenance purposes (R_{maint}), while younger have a larger capacity for distributing energy towards growth. At higher temperatures the increased metabolic rates will eventually exceed that of possible gain by the means of increased food intake (Hallaraker et al. 1995; Jonassen et al. 2000b) which in the end will reduce the final possible growth rate (Jonassen et al. 1999). These factors yield the dome shaped curve for T_{opt} FCE and T_{opt} SGR observed for juvenile fish of several marine species (Fonds et al. 1992; Bjornsson & Tryggvadottir 1996; Jonassen et al. 1999; Otterlei et al. 1999; Bjornsson et al. 2001; Imsland et al. 2005; Imsland et al. 2006).

1.6 Objectives

In the following described experiments the effects of temperature on growth and feed conversion efficiency of juvenile lumpfish were studied. The main objective of the current study was to compare growth- related parameters between juvenile fish at temperatures of 4, 7, 10 , 13 °C and ambient temperature (5.8 °C ± S.D. 0.3). Furthermore, a satellite study was carried out with an subordinate objective of comparing feed utilization at ambient temperature (5.8 °C ± S.D. 0.1) and 13 °C.

2. Materials and methods

2.1 Fish stock and rearing conditions

Lumpfish milt and roe was collected from wild caught brood fish during the first two weeks of June 2011. The broodstock consisted of 20 females and 1 male lumpfish. Fertilized egg clusters were treated with Buffodine (2.5 ml / l water for 10 minutes) prior to incubation in hatching trays. This treatment was repeated twice throughout the incubation period. The hatching trays (40 x 40 cm, Sterner Fish Tech, Ski, Norway) were submerged at a water depth of 12.5 cm in raceways measuring 360 x 40 x 17 cm (Sterner Fish Tech, Ski, Norway). All larvae were pooled upon hatch, creating a genetic material of half-siblings. Temperature was ambient with an increase from 4 °C to 5.5 °C throughout the incubation period, reaching a maximum of 8 °C during start feeding. Light intensity was kept dim (< 10 lux) during incubation, with an increase in light intensity during the juvenile stage (< 90 lux). The larvae and juveniles were reared at continuous light (LD 24:0) from hatching and throughout the experimental period.

The hatching period commenced at ~250 day degrees and persisted for two successive weeks. Larvae were subsequently transferred to shallow raceways 360 x 40 x 17 cm (Sterner Fish Tech, Ski, Norway) after hatching and start-fed with Artemia nauplii enriched with Multi Gain (BioMar, Brande, Denmark). After three weeks, weaning on Anglo Norse formulated feed (300-500 µm) was initiated, and 14 days later 500-800 µm dry feed pellets (Gemma Wean Diamond, Skretting, Norway) were introduced to the diet. One month later pellet size was increased to 0.8 mm and feed type changed to Gemma Diamond (Skretting, Norway). At experimental start-up the lumpfish had reached approximately 30 g, and pellet size was increased accordingly to 1.5 mm (Gemma Diamond, Skretting, Norway).

The experimental tank set up consisted of twelve quadratic 70 x 70 cm gray fiber-glass units (Bia Miljø, Herdla, Norway), each with a rearing volume of 245 l. Automatic feeders (Billund Aquakulturservice, Billund, Denmark) were installed for each tank, and a computer program (SB 1500, Torp Aquateknik, Denmark) estimated daily feeding rates.

In order to obtain the appropriate temperatures for the different temperature groups, ambient seawater (5.8 °C, S.D. ± 0.3), cooled seawater (4.2 °C, S.D. ± 0.4) and heated seawater (12.9 °C, S.D. ± 0.7) were mixed in four header tanks. Each header tank supplied two replicate

experimental units. Temperature was adjusted by daily measurement of the temperature in the experimental units. In both experiments the temperatures were adjusted to remain within S.D. ± 0.3 °C (TABLE 1, Appendix II) during the experimental period. From 12 May oxygen was added to the holding tanks of the highest temperature groups, T10, T13 and S13. Oxygen saturation and temperature were measured daily with a hand-held Oxyguard Handy Alpha (Sterner Aquatech, Ski, Norway) in the effluent water in each unit and was maintained above 80 % (TABLE II, Appendix II).

Water for the ambient seawater temperature groups (T-AMB and S-AMB) was supplied directly from the main header tank of the research facility without further temperature manipulation. Seawater was pumped from 60 meters below sea level and was UV treated and particle filtered before entering the header tanks. Water flow was set to 17 l min^{-1} in each experimental unit, but was increased to 20 l min^{-1} in all groups by May 5.

Daily routines included monitoring of fish behavior, cleaning and flushing excess feed and faeces in all tanks. On sampling dates the tanks were thoroughly scrubbed if required, and outlet drain sieves were changed in accordance to the increase in feed pellet size to prevent clogging.

2.2 Experimental design

The experiments in this study were conducted at Troms Marin Yngel, Kraknes, Norway (69° 45" 53" N, 19° 02" 46" E). Two parallel experiments were carried out.

Experiment 1: Temperature related growth in juvenile lumpfish at five different temperatures.

Between 11 April 2012 and 16 July 2012 a total number of n = 35 tagged fish per unit were reared at 4, 7, 10, 13 °C and ambient (5.8 °C, S.D. ± 0.3, TABLE I, Appendix II) seawater temperature in two replicate units per temperature group. Growth and weight were recorded at 3 week intervals.

Experiment 2: Temperature related feed consumption in juvenile lumpfish at two different temperatures.

Two groups of fish (n = 60) were reared at 13 °C and ambient temperature (5.8 °C, S.D. ± 0.1, TABLE I, Appendix II) during two experimental periods, each lasting for 17 days (Replicate A: 15 May - 1 June 2012 and replicate B: 22 June – 9 July 2012). Feed consumption was recorded as means of number of pellets eaten per day, and the trial was repeated with a second batch of fish one month later to produce two replicates.

From here onwards, the experimental groups are abbreviated as follows:

Experiment 1

- Constant temperature 4°C = T4
- Constant temperature 7°C = T7
- Constant temperature 10°C = T10
- Constant temperature 13°C = T13
- Ambient temperature = T-AMB

Experiment 2

- Constant temperature 13°C = S13
- Ambient temperature = S-AMB

TABLE 1 Experimental conditions. Means of temperatures and oxygen saturation (%) ± S.D. measured in the tank outlet. N is total number of fish per temperature group on 11 April 2012 (W1) for experiment 1. Initial biomass for experiment 1 is calculated based on initial weight of the fish prior to temperature adjustment, W1. Initial density for experiment 2 (S-AMB; 4.5 kg / m^3, S13; 6.7 kg / m^3) was recorded at W1 as the initial weight prior to first day of collection of excess feed. The temperature data for experiment 1 (T-AMB to T13) are based on measurements from day 6, after first temperature adjustment. For Experiment 2 (S-AMB to S13) temperature data is based on temperatures during feed collection.

Experimental conditions						
Treatment group	Fish (Total N)	Temperature (°C)		O_2 saturation (%)		Density (kg / m^3)
	W1	Means	± S.D.	Means	± S.D.	Initial / Final
T4	69	4.2	0.4	84	4.5	1.88 / 8.16
T-AMB	72	5.8	0.3	88	4.8	1.96 / 10.47
T7	70	7.1	0.2	89	7.5	1.95 / 12.68
T10	69	9.9	0.4	101	12.6	1.70 / 15.62
T13	68	12.9	0.7	92	6.4	1.65 / 17.47
Total:	348					
S-AMB	121	5.8	0.3	91	3.5	4.46 / 6.79
S13	120	13.1	0.2	95	4.1	6.81 / 13.11
Total:	241					

On 11 April 2012 fish were anaesthetized with FINQUEL (Scanvacc, Årnes, Norway (0.1 g / l^{-1}) before intra-peritoneal tagging with Trovan ® Passive Integrated Transponder (PIT) tags. Fish were subsequently weighed to the closest 0.2 g and length measured to the closest 1 mm before being randomly distributed in 10 rearing tanks. The fish were reared at ambient (5.8 °C S.D. ± 0.3) temperature and at constant light (LD 24:0). Fish were allowed to recover after tagging and adapt to new environmental conditions for four days prior to initial temperature adjustment 16 April 2012.

At the startup of experiment 1 each tank hosted an average of 69 fish including a subgroup of approximately 35 tagged individuals. Initial biomass in the tanks including both untagged and tagged individuals (n = 69) varied between 6.0 - 7.2 kg / m^3 (S.D. ± 0.3) at first sampling prior to temperature adjustment. Mean initial weight for all groups was 26.5 g (S.D. ± 10.4) (TABLE IV, Appendix II). Individual fish weights were recorded at approximately 3 week intervals. In experiment 1 the first sampling prior to temperature adjustment (W1) was conducted 11 April 2012. The fish were sampled at the following dates: 15 May 2012 (W2), 2 June 2012 (W3), 26 June 2012 (W4) and at experiment termination on the 16 July 2012 (W5).

Start up for experiment 2 replicate A was conducted simultaneously with start up for experiment 1 on 11 April (W0). Approximately 61 unmarked fish per tank (n = 2) with

average initial weights of 25 g (25.3 g, S.D. ± 5.6 g, TABLE III, Appendix II) were selected at random and anesthetized according to the same methods as stated above, before initial weighing to the closest 0.2 g. Only one replicate (A) of each temperature group (S-AMB and S13) was produced at this point. Fish were allowed to adapt to new environments and temperatures prior to initial weighing on 15 May (W1) and startup of feed collection. During the adaption period fish were fed to satiation and were offered a continuous feed supply and reared at continuous light. No excess feed was collected during this period. This procedure was repeated on the 22 June (W1) when experiment 2 replicate B was initiated. Average initial weight for the fish in the two replicate units was 25 g (25.1 g, S.D. ± 4.6, TABLE III, Appendix II) and n = 60 fish per tank.

In experiment 1 fish were fed to satiation and feed was available 24 h daily using automatic feeders. In experiment 2 fish were fed to satiation between 07:00 and 15:00, and excess feed pellets collected in a sieve and counted daily at 15:00. Excess feed in the tank was gently brushed into the outlet, flushed, collected in the sieve and counted. Meal size for the following day was then adjusted according to the amount of excess feed, and weighed out in grams.

During the experiments, fish were fed a commercial formulated feed, Amber Neptun ST (Skretting, Norway). Pellet size was adjusted from 1.5 mm to 3 mm during the trial period dependent on fish size with the first introduction of 2 mm pellets to all tanks on 7 May in experiment 1. Pellet size 3 mm was introduced to temperature groups T10 and T13 on 5 July.

At all sampling dates, fish were anaesthetized (FINQUEL, Scanvacc, Årnes, Norway (0.1 g / l^1). All fish were starved 24 hours prior to all samplings. For tagged fish, individual tag number was recorded prior to weight-measurements to the nearest 0.2 g and length measured to the nearest 0.1 cm. All untagged fish were individually weighed to the nearest 0.2 g. After measurements, fish were allowed to recover before being returned to their respective tank.

For experiment 2 the feed collection period lasted from 15 May (W1) to 1 June (W2) for replicate A. Feed collection for replicate B lasted from 22 June (W1) to 9 July (W2). Experiment 2 was terminated on 1 June (Replicate A) and 9 July 2012 (Replicate B) in relation to the final sampling (W2).

2.3 Growth and feed calculations

Specific growth rate (SGR) was calculated according to the following formula (Houde & Schekter 1981)

$SGR = (e^{g}-1) \times 100$

Where g is the instantaneous growth coefficient defined as:

$g = \frac{\ln(W_1)-\ln(W_2)}{t_2-t_1}$ where W_2 and W_1 are mean wet weights for individually tagged fish in grams at days t_2 and t_1, respectively.

Geometric mean (GM) weight were calculated using

$GM= \sqrt{(W1 * W}$, where W1 and W2 W_2 and W_1 are mean wet weights for individually tagged fish in grams at days t_2 and t_1, respectively.

Total feed consumption (C_T) was calculated as:

$C_T = F_s - F_c$

Where F_s is total feed supplied in grams (g) and F_c is total collected excess feed.

Daily feeding rate (F%) was calculated as:

$$F\ \% = 100\left[\frac{C}{\frac{B1+B2}{2}}\right] x\ (t_2 - t_1)^{-1}$$

Where C is total feed consumption per tank in the period, and B_1 and B_2 are fish biomass (g) in the tank at days t_2 and t_1, respectively.

Feed conversion rate (FCE) was calculated as:

$$FCE = \frac{(B_2 - B_1)}{C}$$

Where C is total feed consumption in the tank for the period, and B_1 and B_2 are fish biomass (g) in the tank at days t_2 and t_1.

Temperature effect on growth (Q_{10}) was calculated according to Houde and Schekter (1981) and Schmidt-Nielsen (1990):

$$Q_{10} = (SGR_2 / SGR_1)^{10/(T2-T1)}$$

Where SGR_2 and SGR_1 are specific growth rates for two temperature groups where T2 and T1 are temperatures for the two groups, respectively.

2.4 Statistical Methods

All statistical analyses in this study were done in STATISTICA 10.0 (Statsoft, Inc., 2012).

Normality of distributions was assessed using a Kolmogorov-Smirnov test (Zar 1984) and homogeneity of variances was tested using Levene's F-test. The effect of temperature on weight and SGR was tested using a two-way nested Analysis of Variance (ANOVA) (Zar 1984), where the replicates were nested in temperature treatment groups.

For FCE, F % and C_T where only group data existed, a one-way ANOVA was applied. Significant ANOVA's were followed by a Student-Newman-Keuls multiple comparison test (Zar 1984) in order to identify differences among treatments.

Size ranking (initial size rank versus final size rank) was tested using Spearman's rank correlation (Zar 1984), where a parabolic regression was used to analyze the relationship between SGR and temperature. The regression was made using the average growth rates of tagged fish in three size groups; 25 - 30, 100 - 110 and 160 - 200 g. An equal number of fish from the same size class at each temperature was selected in terms of individual wet weight in grams. The optimal temperature for growth (T_{opt} SGR) was calculated as the zero solution to the first derivate of the parabolic regression. Geometric mean weight versus daily specific

growth rate (SGR % day^{-1}) were analyzed using a two way Analysis of Covariance (ANCOVA).

A significance level of $\alpha = 0.05$ was used unless stated otherwise.

3. Results

3.1 Experiment 1

3.1.1 Mortality

No mortality occurred during the experimental period, and fish in all treatment groups looked healthy. No deformities or injuries were recorded amongst the individuals.

3.1.2 Effects of temperature on growth

The initial mean mass ranged between 24.3 (S.E. ± 1.0) to 27.9 (S.E. ± 1.4) g at the start of the experiment to 118.4 (S.E. ± 4.8) to 256.8 (S.E. ± 6.9) g at the end of the experiment on day 95 (FIGURE 3.1, TABLE IV, Appendix II). There was no significant difference in initial mean mass between treatment groups (Two-way nested ANOVA; $p = 0.27$, TABLE IX, Appendix II).

At sampling W2 most groups showed significantly different mean weights (T7 = 59.5, T10 = 71.8, T13 = 79.4 g, FIGURE 3.1, Two-way nested ANOVA; $p < 0.001$, TABLE X, Appendix II), with the exception of temperature groups T-AMB and T4 (48.2 and 48.9 g, FIGURE 3.1, TABLE IV, Appendix II). These two groups did not display a significant difference in mean weight until the final sampling on the 16th of July (W5). The significant difference between T13, T10 and T7 remained throughout the experiment (SNK-test; $p < 0.05$, TABLE XXX-XXXIV, Appendix II). Temperature group T13 had the highest mean mass from sampling W2 and onwards, followed by temperature groups T10 and T7 (FIGURE 3.1 and TABLE IV, Appendix II). All groups displayed significantly different weights at the final sampling W5 (SNK-test; $p < 0.05$, TABLE XXXIV, Appendix II), where mean weights of T13 and T10 (256.2 and 226.5 g) were respectively 53.8 and 47.7 % higher than group T4 which displayed the lowest mean weight (118.4 g, FIGURE 3.1 and TABLE IV, Appendix II).

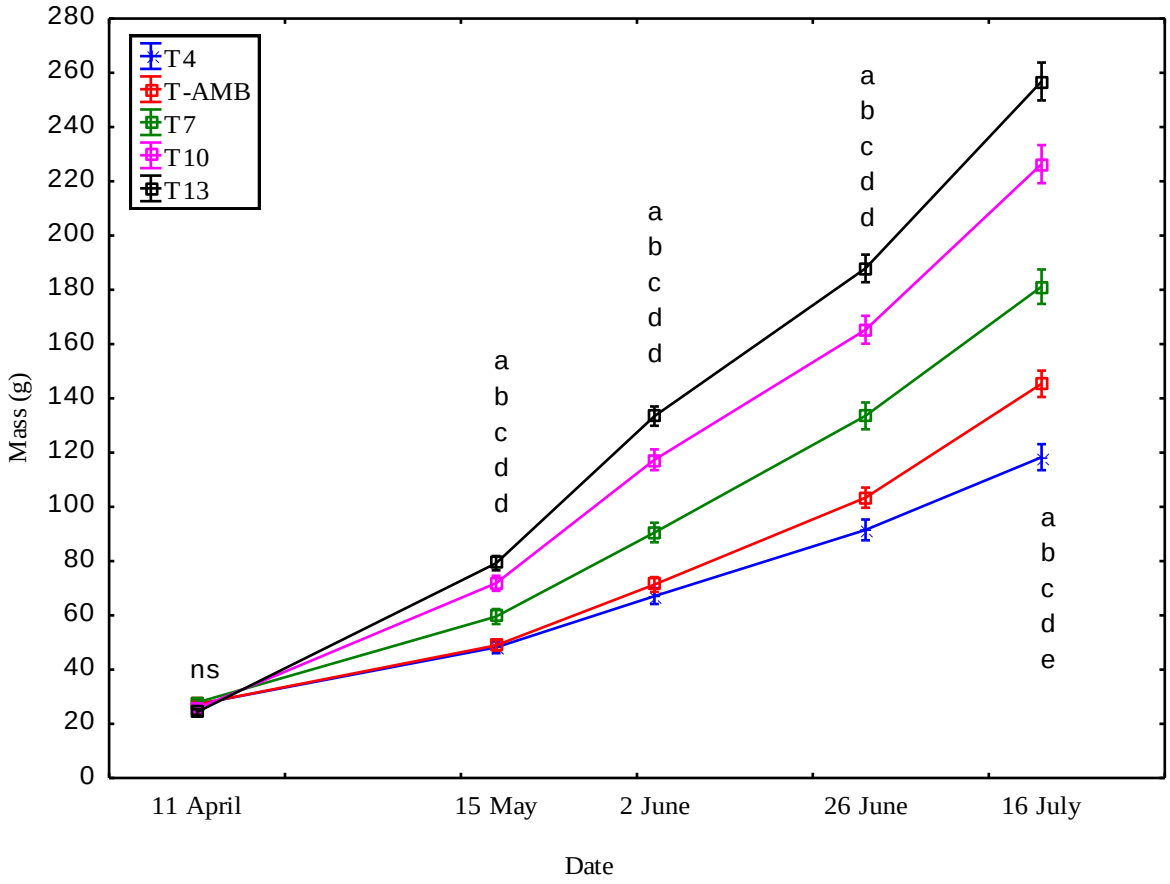

FIGURE 3.1. Weight development for juvenile lumpfish reared at five different temperatures (n = 68-72 in each experimental group).Vertical whiskers indicate standard error of mean (± S.E.) of individually tagged fish. Results for two replicates are combined. Different letters indicate statistical differences between groups, with "a" as the highest value. Vertical lines indicating S.E.. may be obscured by symbols.

The same temperature effect was visible for length measurements. Mean lengths ranged from 8.4 (S.E. ± 0.1) to 8.8 cm (S.E. ± 0.2) at the start of the experiment W1 on 11 April to 14.4 (S.E. 0.2) to 18.2 cm (S.E. ± 0.2) at the final sampling W5 at 16 July (FIGURE 3.2 and TABLE V, Appendix II). No significant difference was found in initial lengths between treatments (Two-way nested ANOVA; p = 0.47, TABLE XIV, Appendix II).

On 15 May (W2) no significant differences in length were found between temperature groups T4 and T-AMB (Both groups 10.6 cm (S.E. ± 0.2 / 0.2), FIGURE 3.2 and TABLE V, Appendix II, SNK-test; p = 0.92, TABLE XXXVI, Appendix II). Lengths for temperature groups T10 (11.9, S.E. ± 0.2) and T13 ((12.1 cm, S.E. ± 0.1), FIGURE 3.2, TABLE V, Appendix II) were also insignificantly different to each other (SNK-test; p = 0.27, TABLE XXXVI, Appendix II).

At W3 (2 June) no significant difference was found between mean lengths of temperature groups T4 and T-AMB (11.7 cm (S.E. ± 0.2) and 11.8 cm (S.E. ± 0.2), FIGURE 3.2, TABLE V, Appendix II). The two groups had a significantly lower mean length than all other temperature groups throughout the experimental period (SNK-test; TABLE XXXVI-XXXIX, Appendix II). After 2 June all groups showed significantly different mean lengths (T4 = 12.9 cm (S.E. ± 0.2), T-AMB = 13.5 cm (S.E. ± 0.2), T7 = 14.9 cm (S.E. ± 0.2), T10 = 15.9 cm (S.E. ± 0.2), T13 = 16.5 cm (S.E. ± 0.2), FIGURE 3.2, TABLE VI, SNK-test; TABLE XXXVII-XXXIX, Appendix II).

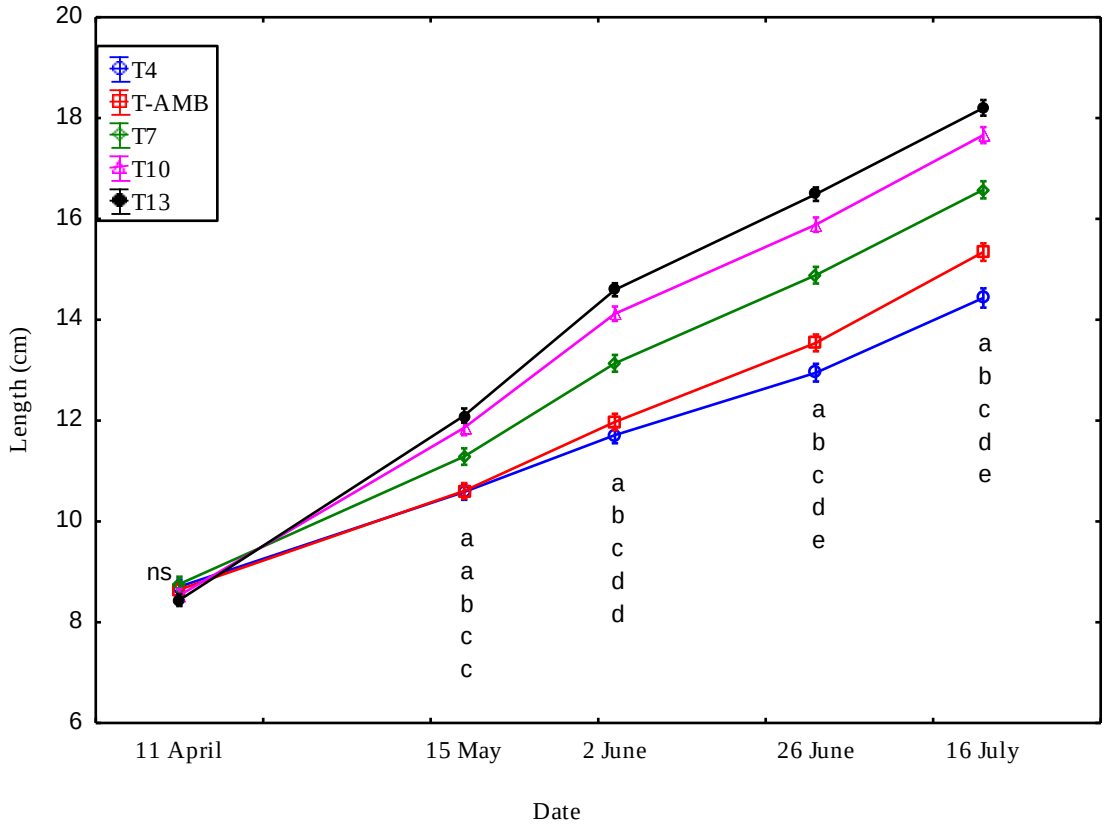

FIGURE 3.2. Length development for juvenile lumpfish reared at five different temperatures (n = 348).Vertical whiskers indicate standard error of mean (± S.E.) of individually tagged fish. Results for two replicates are combined. Different letters indicate statistical differences between groups, with "a" as the highest value. Vertical lines indicating S.E may be obscured by symbols.

Mean specific growth rates ranged from 1.30 to 3.54 % day^{-1} (FIGURE 3.3, TABLE VI, Appendix II). The temperature effect on SGR % day^{-1} was clearly observable in the first period 11 April – 15 May (W1 – W2), as most temperature groups were significantly different to each other with the exception of temperature groups T4 and T-AMB (1.71 and 1.78 % day^{-1}, FIGURE 3.3, TABLE VI, Appendix II, SNK-test; p = 0.19, TABLE XLI, Appendix II). Similarly, between 15 May and 2 June all groups displayed significantly different growth

rates (T4 = 1.85, T-AMB = 2.12, T7 = 2.43, T10 = 2.81, T13 = 2.97 % day^{-1}. FIGURE 3.3 and TABLE VI, SNK-test; $p < 0.05$, TABLE XLI, Appendix II).

An overall decline in specific growth rate was observed for all groups from 3 June – 26 June (W3 – W4).. In this period no significant differences were found between treatment groups T4, T10 and T13 (T4 = 1.31, T10 = 1.44, T13 = 1.42 % day^{-1}, FIGURE 3.3, TABLE VI, SNK-test; T4 - T10: $p = 0.09$, T4 - T13: $p = 0.06$ and T10 - T13 $p = 0.82$, TABLE XLII, Appendix II). Temperature groups T-AMB and T7 displayed similar mean growth rates in this period (1.58 and 1.65 % day^{-1}, FIGURE 3.3 and TABLE VI, SNK-test; $p = 0.22$, TABLE XLII, Appendix II).

A slight overall increase for all groups with the exception of T4 was observed for the last period 27 June – 16 July (W4 - W5) (FIGURE 3.3 and TABLE VI), and the lack of significant difference between T10 and T13 persisted from 27 June to 16 July (W1 – W2) (T10 = 1.57, T13 = 1.58 % day^{-1}, FIGURE 3.3, TABLE VI, SNK-test; $p = 0.81$, TABLE XLIII, Appendix II), when temperature group T7 also displayed an insignificantly different SGR % day^{-1} to the above mentioned groups (1.55 % day^{-1}, FIGURE 3.3, TABLE VI, SNK-test; $p = 0.88$ and $p = 0.81$, TABLE XLIII, Appendix II). Specific growth rate for T-AMB was significantly higher during this period than the other four temperature groups T4, T7, T10 and T13 (T-AMB = 1.74 % day^{-1}, FIGURE 3.3, TABLE VI, Appendix II). The average SGR % day^{-1} for the entire experimental period between 11 April – 16 July (W1 – W5) was significantly different between all temperature groups with an increase in SGR % day^{-1} for increasing temperatures (FIGURE 3.3 and SNK-test; $p<0.001$, TABLE XLIV, Appendix II).

Q_{10} of overall specific growth rate for tagged individuals was 3.16 between T4 and T-AMB, 1.48 between T-AMB and T7, 1.09 between T7 and T10 and 1.15 between T10 and T13 (TABLE LXI, Appendix II).

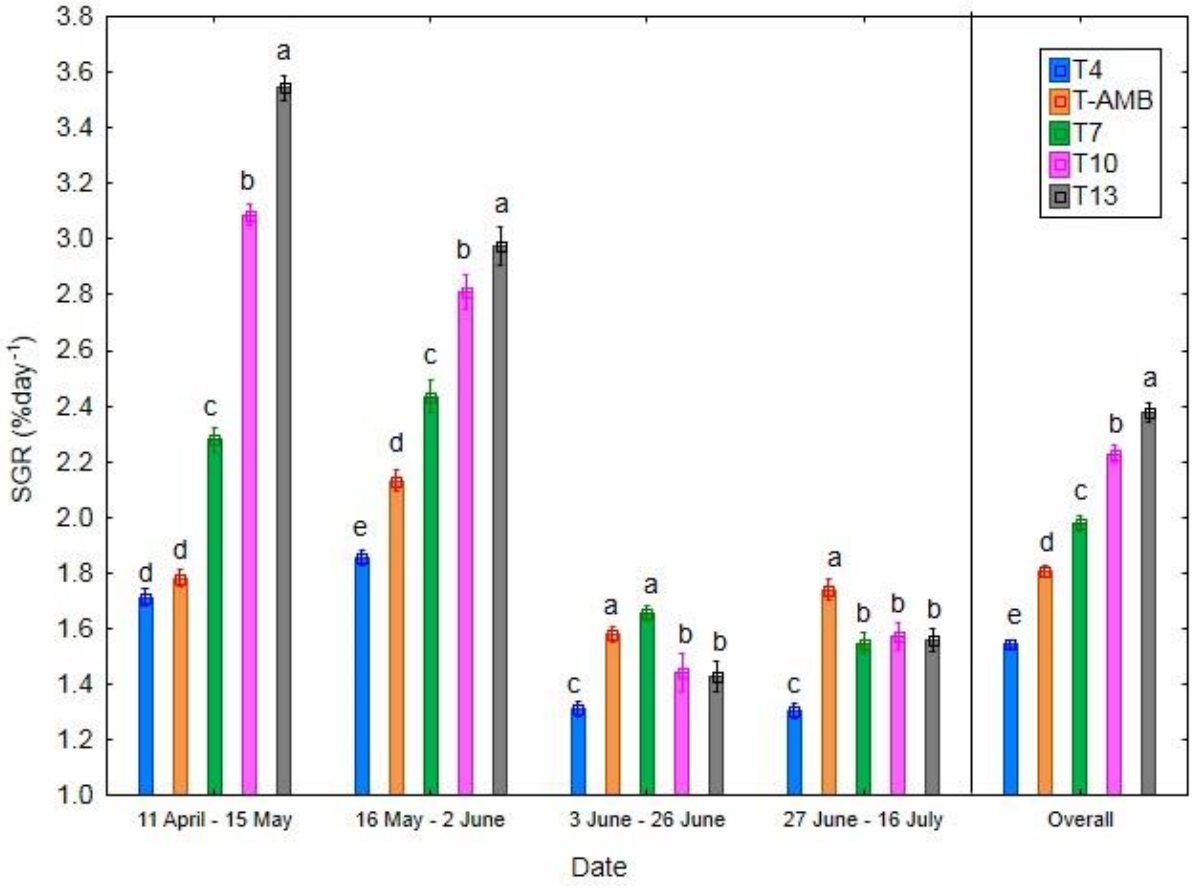

FIGURE 3.3. Specific growth rates (SGR % day^{-1} ± S.E., n = 68-72, see TABLE VI, Appendix VI) for individually tagged juvenile lumpfish during the experimental period. The five temperature regimes are separated by colors as presented. Different letters indicate significant differences between treatments (SNK-test; $p<0.05$, TABLE XL – XLIV, Appendix II).

3.1.3 Effect of fish size on growth

Regression lines for SGR was highly correlated to mass (two-way ANCOVA, $p<0.001$; TABLE LIII Appendix II) and growth rates declined with body weight for temperature groups T4, T7, T10 and T13 (linear regression; $p = 0.019$, $p = 0.006$, $p = 0.004$ and $p = 0.002$, FIGURE 3.4 (a), (c) and (d)), but was not significant for temperature group T-AMB (linear regression; $p = 0.346$, FIGURE 3.4 (b)). The negative effect of increasing weight on SGR % day^{-1} was especially pronounced for temperature groups T10 and T13 (FIGURE 3.4 (d) and (e)). ANCOVA comparisons of linear regressions for T10 and T13 displayed parallel lines for the two groups (two-way ANCOVA; $p = 0.132$, TABLE LVII, Appendix II), while regression lines for temperature groups T4, T-AMB and T7 were non-parallel (two-way ANCOVA, TABLE LIV – LVI, Appendix II).

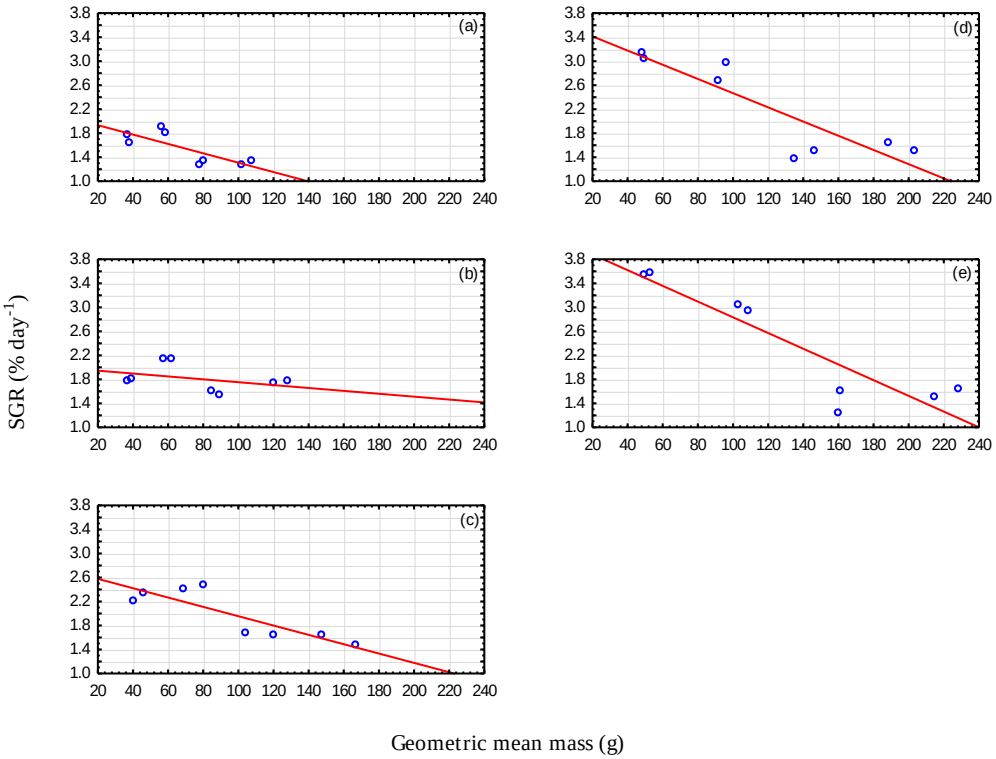

FIGURE 3.4. Specific growth rate (SGR % day^{-1}) plotted against geometric mean weight of juvenile lumpfish. Each data point consists of 32-37 individually tagged fish from each replicate. (a) T4: SGR = 2.089 - 0.0078X, p = 0.019, (b) T-AMB: SGR = 1.9943 – 0.0024X, p = 0.346, (c) T7: SGR = 2.7313 - 0.0078X, p = 0.006, (d) T10: SGR = 3.6491 - 0.0118X, p = 0.004, (e) T13: SGR = 4.1425 - 0.0131X, p = 0.002. Number of data points (n) = 8 for all groups.

Growth rates were plotted against temperature for three size classes of fish (25 - 30, 100 - 110 and 160 - 200 g: FIGURE 3.5 (a) – (c)) to produce the parabolic regressions: 25-30 g $Y = 0.472 + 0.3011x – 0.0049 x^2$, 100 - 110 g $Y = 0.4131 + 0.258x – 0.0101x^2$, 160 - 200 g $Y = 0.673 + 0.1669x – 0.0087 x^2$ (FIGURE 3.5 (a) – (c)). A trend in decreasing growth rate (SGR % day^{-1}) indicated that temperature optimums for maximum specific growth rate declined markedly with increasing body mass for all temperature groups.

Temperature optimums were calculated from the first order derivate of the parabolic regressions ($dSGR / dT = 0$). The T_{opt} SGR was estimated to be 9.6 °C for the 160 - 200 g group, while fish in size rank 100 - 120 g did not reach their temperature optimum during the study but the first order derivate of the parabolic regression demonstrates a calculated T_{opt} SGR at 12.8 °C. The smallest group (25 - 30 g) did not seem to have reached their temperature optimum for maximal growth at 13 °C (FIGURE 3.5 (a)).

3.1.4 Size and growth ranking

A significant size rank correlation (initial weight *v.* final weight) was maintained at all temperature regimes ($r_{Sp} > 0.50$, $p<0.05$, TABLE LX, Appendix II). Size rank correlation was highest for the coldest temperature groups T4 and T-AMB ($r_{Sp} = 0.95$, $r_{Sp} = 0.93$), and lowest for the warmest temperature group T13 ($r_{Sp} = 0.67$, TABLE LX, Appendix II). Initial vs. final growth rates were only correlated for T-AMB ($r_{Sp} = 0.25$)) and was negative for T4 and T13 ($r_{Sp} = -0.05$ and $r_{Sp} = -0.002$, TABLE LX, Appendix II).

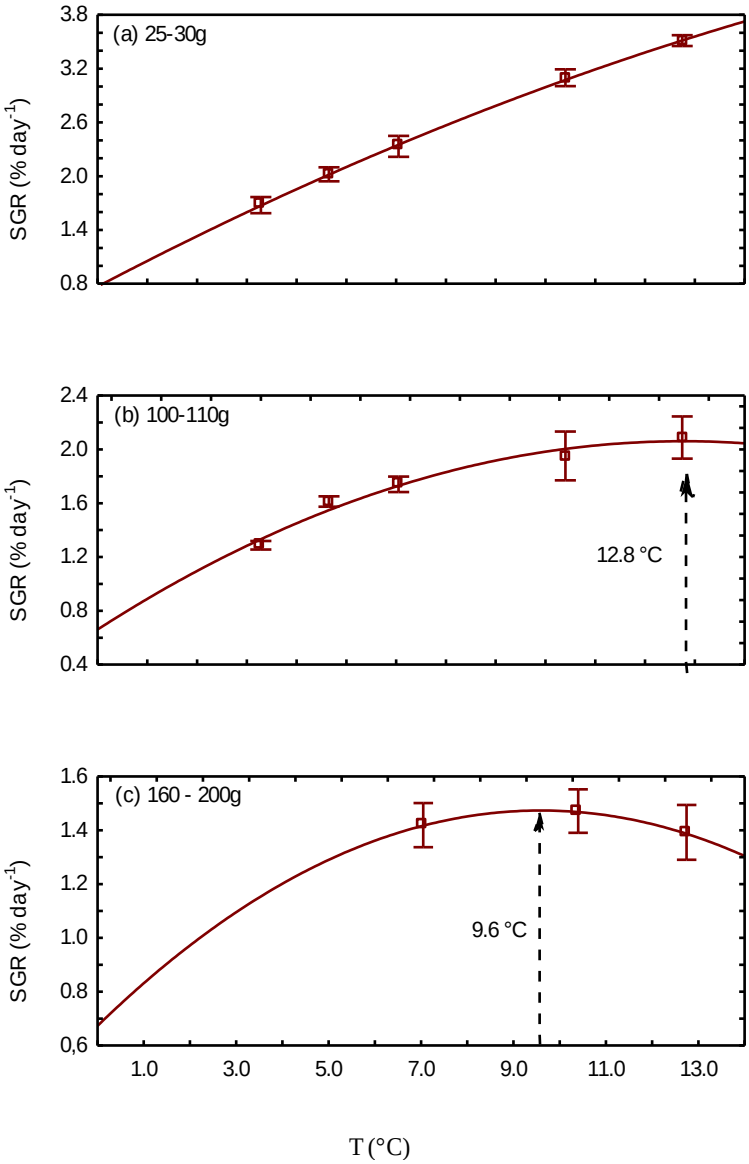

FIGURE 3.5. Changes in specific growth rate with temperature for three different size classes of juvenile lumpfish. The lines represent the least-squares second order polynomial fit to the data: $SGR = aT + bT^2 + c$ where SGR = Specific growth rate, T = temperature, and a, b, and c are constants determined by the regression. Whiskers indicate ± standard error of mean (S.E..). (a), 25 - 30 g; $Y = -0.0049x^2 + 0.3011x + 0.472$, n = 10 - 17 for each point. (b), 100 - 110 g; $Y = -0.0101x^2 + 0.258x + 0.4131$, n = 9 - 16 for each point. (c), 160 - 200 g; $Y = 0.0087x^2 + 0.1669x + 0.673$, n = 18 - 26 for each data point. Optimum temperature for specific growth rate was calculated from the first order derivate of the parabolic regressions. Note different scale on the Y-axis.

3.2 Experiment 2

3.2.1 Mortality

No mortality was recorded for experiment 2. All fish appeared healthy and without injuries throughout the experimental period.

3.2.2 Effect of temperature on feed utilization

Feed consumption (C_T) differed between temperatures with higher C_T observed at 13°C (TABLE 3.1 , One-way ANOVA; $p < 0.005$, TABLE XXVII and SNK-test; $p = 0.019$, TABLE L, Appendix II). No significant difference was found between groups for F% (daily feeding rate, TABLE 3.1, One-way ANOVA; $p = 0.169$, TABLE XXIX, Appendix II) or FCE (feed conversion efficiency, TABLE 3.1, One-way ANOVA; $p = 0.501$, TABLE XXVIII, Appendix II).

TABLE 3.1. Feed conversion efficiency (FCE), daily feeding rate (F%) and total feed consumption (C_T) for juvenile lumpfish reared at two different temperature regimes. Results are presented as mean (± S.D.), with n = 2 replicates for each temperature group (S-AMB and S13).

Treatment	FCE	F%	C_T (g)
S-AMB	2.68 (0.45)	0.93 (0.15)	438.9 (28.8)
S13	2.95 (0.18)	1.28 (0.17)	1062.7 (117.8)

4. Discussion

4.1 The effect of temperature on growth

The results of the present study show that mean weights, lengths and growth rates of juvenile lumpfish were significantly influenced by temperature and fish size. Mean weights and lengths showed a significant, stepwise increase with increasing temperature for most samplings. Mean weights and lengths were significantly lower for groups T4 and T-AMB compared to all other temperature groups, during and at the end of experiment 1. No other published studies of temperature related growth studies in the presented size range for juvenile is known at the time of writing.

Overall SGR % day^{-1} was found to have a significant increase with increasing temperature. Fish reared at 13 °C (T13) had the highest growth-rates for the two first periods of the experiment, 11 April – 15 May and 16 May – 2 June (3.09 and 2.91 % day^{-1} respectively) while juvenile lumpfish reared at ambient temperature (5.1 – 6.5 °C , T-AMB) and 7 °C (T7) had the highest growth rates for the third period 3 June – 26 June (1.65 % day^{-1}). Lumpfish reared at ambient temperature (T-AMB) displayed the highest growth rate during the final period 27 June – 16 July (1.74 % day^{-1}, FIGURE 3.3 and TABLE VI, Appendix II). The low overall SGR % day^{-1} for groups reared at the lowest temperatures (T4 and T-AMB) produced a fish with an overall mean weight which is 54 and 43 % lower compared to that of fish reared at 13 °C (T13). Fish reared at 4 °C (T4) displayed the overall lowest growth rate (TABLE VI, Appendix II). This is in accordance with findings from wild juveniles caught outside southwestern Iceland, as wild fish displayed little or no growth during the cold months of the first winter (Ingolfsson & Kristjansson 2002).

The decline in growth is consistent with findings for juvenile halibut in the same size range, displaying a 8 – 35 % increase in final body weight when increasing temperature from 10.0 to 13.0 °C (Hallaraker et al. 1995; Bjornsson & Tryggvadottir 1996), and a increase in final body weight by 46 and 49 % when increasing temperature from 6.0 and 7.0, respectively to 13.0 °C (Hallaraker et al. 1995; Jonassen et al. 1999). A further increase in temperature from that of T_{opt} SGR is found to decrease maximum weight (Björnsson & Steinarsson 2002). Despite consistency in the findings of increased final weight with increasing temperature, these comparisons of final weight between studies do not take into consideration possible

effects of different photoperiods (Jonassen et al. 2000b; Imsland & Jonassen 2001; Lohne et al. 2012), feeding regimes (Brown et al. 1997) and time scopes (Björnsson et al. 2007; Arnason et al. 2009).

In our study the SGR % day^{-1} decreases rapidly over time for groups reared at high temperatures, while growth rates in the group reared at ambient temperature (T-AMB) had a less rapid decrease and displayed the highest SGR % day^{-1} in the final period (TABLE VI, Appendix II, FIGURE 3.3). In the study by Arnason et al. (2009) juvenile turbot reared below suboptimal temperatures would over time catch up with initially faster growing individuals reared at higher temperatures. This may be a possible long-term effect in juvenile lumpfish as T_{opt} SGR changes with increasing size, but the time scope for our study may have been too short to display this effect. Brown et al. (1992) observed lumpfish reared at ambient temperatures to reach mean weights of above 500 g within two years, which suggests great growth potential in juvenile lumpfish.

4.2 Size-related growth and optimum temperatures for growth

Juvenile lumpfish display a large ontogenetic variation in optimum temperatures for growth (T_{opt} SGR) with increasing body weight, demonstrated by high growth rates over a large temperature interval (FIGURE 3.4 and 3.5). A change in T_{opt} SGR with increasing size could easily be distinguished as fish reared close to T_{opt} SGR at 13 °C displayed very high growth rates in the first period when body weight was low (3.54) and hence achieved high overall growth rates (2.38 % day^{-1}, FIGURE 3.3, TABLE VI, Appendix II). For periods W3-W4 and W4-W5 the growth rate of the fish reared at 13 °C displayed a rapid decrease, while slower growing fish reared at ambient temperature (T-AMB) displayed better growth than high temperature groups T10 and T13 in the final period. Despite this, the low temperature groups (T4 and T-AMB) could not compete in terms of overall weight gain and SGR % day^{-1} in comparison to groups reared at 10 and 13 °C.

An inverse relationship between the slopes for increasing body weight and growth could be observed for all temperature groups, but was significantly negative for temperatures 10 and 13 °C (FIGURE 3.4). This indicates a rapid downwards shift in the optimum temperature for growth with increasing body weight (FIGURE 3.4), which is in accordance with the findings

for plaice (Fonds et al. 1992), turbot (Imsland et al. 1996; Arnason et al. 2009), spotted wolffish (Imsland et al. 2006), juvenile cod (Björnsson & Steinarsson 2002; Imsland et al. 2005), halibut (Hallaraker et al. 1995; Aune et al. 1997; Jonassen et al. 1999) and the general pattern of fish growth with increasing body size (Brett & Groves 1979; Jobling 2010).

The parabolic regressions for growth (FIGURE 3.5) suggest a T_{opt} SGR of 9.6 - 12.8 °C for juvenile lumpfish in the size range 100 - 200 g, indicating an ontogenetic change in T_{opt} SGR. This decline in T_{opt} SGR with increasing weight is in accordance with growth studies for juvenile cod (Jobling 1988; Otterlei et al. 1999; Björnsson et al. 2007), juvenile turbot (Imsland et al. 1996; Arnason et al. 2009), plaice (Fonds et al. 1992), spotted wolffish (Imsland et al. 2006) and Atlantic halibut (Hallaraker et al. 1995; Bjornsson & Tryggvadottir 1996; Jonassen et al. 1999). The observed scope for T_{opt} SGR in this study is similar to that proposed for halibut by Hallaraker et al. (1995) (10.0 – 13.0 °C for 20 – 90 g) and Bjornsson and Tryggvadottir (1996) (14.0 and 11.4 for size groups 10 – 60 and 100 – 500 g respectively). Juvenile cod also display similar temperature preferences (14.7 – 12.6 for 5 – 50 g) (Imsland et al. 2005), while the suggested T_{opt} SGR for turbot is higher (16.0 – 23.0 °C at 7.5 – 75 g) (Imsland et al. 1996; Imsland et al. 2000). Juvenile wolfish display a significantly lower T_{opt} SGR for juveniles in the same size range (6.6 – 7.6 °C for 71 – 380 g) (Imsland et al. 2006). These differences reflect different life strategies of juvenile marine fish.

The observed change in T_{opt} SGR for lumpfish in this study is somewhat higher than the presumptions of a 1 - 2 °C decrease in temperature preference for fish weighing 10 - 500 g as presented by Cuenco et al. (1985). The fish in our study displayed a change in T_{opt} SGR of 3 °C between 100 - 110 g and 160 - 200 g (FIGURE 3.5). If we include the smallest group 25 - 30 g, who according to our parabolic regression had not reached their T_{opt} SGR at 13 °C, the window for temperature preference in 25 - 200 g juvenile lumpfish will become even broader, with a marked shift in preference between the 25 - 30, 100 - 110 and 160 - 200 g groups.

The rapid decrease in daily growth with increasing size for all groups except at ambient temperature is in in terms with findings for juvenile Atlantic cod (Imsland et al. 2005) and Atlantic halibut (Hallaraker et al. 1995). Juveniles in size group 25 – 30 g did not seem to have reached their growth potential at 13 °C, while juvenile cod in the same size group (5 – 50 g) reached their temperature optimum at 12.9 °C, and also had a lower temperature optimum for larger size intervals (40 - 50 g, 12.6 °C) (Imsland et al. 2005). This indicates that

lumpfish have a higher SGR % day^{-1} at T_{opt} SGR (2.4 % day^{-1}, TABLE VI, Appendix II) and a larger scope for somatic growth than that of juvenile cod (Imsland et al. 2005).

Large fish are seemingly less affected by temperature than smaller fish when reared close to optimal temperature (FIGURE 3.5). Our results show that the parabolic regression curve flattens out close to T_{opt} SGR with increasing fish weight. From this it can be deduced that despite a restricted temperature-range for optimal growth of small juvenile lumpfish (25 - 30 g, >13 °C, FIGURE 3.5), large juvenile lumpfish display a broader scope for growth close to the maximum at T_{opt} SGR with temperatures ranging from 7.0 to 13.0 °C (FIGURE 3.5). This trait in juvenile teleosts has been observed previously for Atlantic halibut (Bjornsson & Tryggvadottir 1996; Jonassen et al. 1999) and spotted wolffish (Imsland et al. 2006). Lumpfish hence appears to be eurythermal, expressed by high growth rates over a broad range of temperatures, which is in accordance with findings for wild specimens of lumpfish (Davenport 1985). To fully utilize the growth potential in lumpfish, the ontogenetic change in preference for T_{opt} SGR would propose a decrease in temperature with increasing body size (Temperature steps, T-steps) as presented for cod (Imsland et al. 2005), spotted wolffish (Imsland et al. 2006) and turbot (Imsland et al. 2008).

It is unlikely that juvenile lumpfish is able to exploit the full growth potential of T_{opt} SGR in the wild, mainly because juvenile stages are spent in the physically challenging intertidal zone. Despite this, larval and juvenile lumpfish weighing up to 0.84 g have been found to display explosive growth in terms of weight and length in the wild (Moring 2001), higher than that of cultured fish in the same size range (Benfey & Methven 1986). The study of cultured fish by Benfey and Methven (1986) was executed with live feeds, which may have a lower nutritional value than that of formulated start feed as used in our study (Hamre 2006), indicating that potential growth was not fully utilized due to nutritional requirements, which should be taken into consideration. Studies of both wild and cultured larval and juvenile lumpfish growth patterns show a rapid increase in growth-rate from mid-July to August, before decreasing in August-September (Benfey & Methven 1986; Moring 2001; Ingolfsson & Kristjansson 2002). This reflects life strategies of wild lumpfish and may be correlated to the T_{opt} SGR for age 0 – 2 years old lumpfish in captivity. The juvenile lumpfish spend their first 1 - 2 years in the intertidal zone, where it is reported to grow rapidly, and larger specimens are assumed to leave the temporal zone before smaller fish (Myrseth 1971). The

decreasing change in temperature preference with increasing body size may reflect the secondary effect of migration, as sea migrating juvenile lumpfish may better exploit the differences in temperature between the inter-tidal zone, which is in accordance with the findings of juvenile plaice, (Fonds 1979; Fonds et al. 1992), and juvenile turbot (Imsland et al. 1996; Imsland & Jonassen 2001).

It has been suggested that lumpfish return to spawn in the same area year after year (Thorsteinsson 1981; Mitamura et al. 2012). Species with large north-south distributions such as juvenile turbot and Atlantic cod have been shown to display differences in terms of T_{opt} SGR between latitudinal groups, indicating a genetic difference between populations which cannot be overcome during an acclimatization process (Imsland et al. 2000; Pörtner et al. 2001). This may very well be a pronounced effect in lumpfish culture as well, as this is a specie with a large geographic distribution and large size differences between populations (Davenport 1985), and large observed polymorphism in loci between populations (Skirnisdottir et al. 2013). It is however uncertain to what extent genes affect fitness. The differences in terms of life strategies of marine fish are found to produce little differentiation in genetic markers within a specie (Imsland et al. 2000), but temperature has been observed to have a greater effect on growth rate in cod than genetic influences (Björnsson & Steinarsson 2002). Genotype vs. environment interactions are difficult to predict as they depend on family (population), life stage of the fish, environmental factors and specie. Environmental factors such as temperature, salinity and available food sources have been observed to have great importance on family-effects in juvenile cod, accounting for up to 90 % of a trait such as growth (Imsland et al. 2011).

Increasing mortality with increasing weight at high temperatures as observed for Atlantic cod (Bjornsson et al. 2001) may also be a problem in lumpfish culture. Hence, more experiments to investigate the optimal temperatures with increasing growth should be undertaken to investigate possible long-term effects of this rapid growth at high temperatures, together with possible effects of the thermal history of the fish during juvenile stages, which may also have consequences for further growth potential (Aune et al. 1997).

4.3 Growth effects in terms of Q_{10}

Increasing the temperature from 4 (T4) to 5.8 °C (T-AMB) gave the highest overall increase in growth rate calculated as Q_{10} ratio (Q_{10} = 3.16, TABLE LXI, Appendix II), while increasing the temperature from 5.8 °C (T-AMB) to 7 °C (T7) increased the Q_{10} ratio by 1.48, indicating that an increase of temperature from ambient has a positive effect on specific growth rate. The effect on Q_{10} for increasing the temperature from 7 °C and 10 °C to 13°C was 1.12 and 1.15 respectively, suggesting little positive effect of further increasing the temperature for culture of juvenile lumpfish. These values for Q_{10} in juvenile lumpfish indicate a lower temperature optimum compared to that of juvenile halibut (Hallaraker et al. 1995; Jonassen et al. 1999). This indicates that the temperature was in overall not above optimum of the size groups investigated, though parabolic regression concluded otherwise in terms of the largest size groups from 7, 10 and 13 °C. The smallest effect in terms of Q_{10} was observed between 7 °C and 10 °C at Q_{10} =1.09. The effect on Q_{10} is reduced as temperature draws near to T_{opt} SGR, and is more pronounced when suboptimal and lower temperatures are compared. This observed effect is a common finding when using Q_{10} as a thermal coefficient in terms of growth (Hallaraker et al. 1995; Imsland et al. 1996; Jonassen et al. 1999; Imsland et al. 2005). Heating water from 4 °C to 5.8 °C in lumpfish aquaculture can therefore produce high growth at low costs for the size-group studied.

4.4 Size rank – correlation

An evident size rank correlation was found for all temperature groups, which is consistent with findings for juveniles of several marine species (Hallaraker et al. 1995; Imsland et al. 1996; Aune et al. 1997; Jonassen et al. 1999; Imsland et al. 2005; Imsland et al. 2006).The stabile size rank, especially for low-temperature groups (TABLE LX, Appendix II) indicates that individual fish remain their relative size position throughout the experimental period. Correlation for weight was lower for high temperature groups (T13 r_s = 0.65, T10 r_s = 0.71, TABLE LX, Appendix II) than for low temperature groups (T7 r_s = 0.87, T-AMB r_s = 0.93, T4 = 0.95). This is in accordance with findings for juvenile fish (Hallaraker et al. 1995; Jonassen et al. 1999), and may indicate that some individuals have a larger window for growth related temperature preference, which may be underlined by genetic effects. Our results indicate that individuals maintain their relative size position throughout the

experimental period, indicating what may be the early establishment of a stable size ranking, which is consistent with other findings for juvenile lumpfish (Haaland 1996) and other marine species (Imsland et al. 1998; Imsland et al. 2006).

Juvenile lumpfish are found to be highly territorial and group together within the same size groups (Haaland 1996). Haaland (1996) found that the impact of hierarchies in captive juvenile lumpfish had an increasing impact on growth rates with time. Larger, dominant individuals would spend more time swimming and hunting for prey, hence increasing their energy requirements. Smaller, subordinate individuals would on the other hand spend more time stationary at the bottom of the tank, limiting their access to feed. This prevented neither the dominant nor the subordinate fish to maximize their potential growth, which also may have been an effect in our study as individual growth rates varied greatly within all temperatures, displaying significant size correlations throughout the experimental periods.

4.5 Effect of temperature on feed related parameters

The morphology, physiology and behavior of a species is closely related to each other (Huey 1991) and the energy available for anabolic processes in ectotherms is restricted by reduced energy intake at low temperatures (Jobling 1994). Optimum temperatures for growth are often slightly higher than that of optimum temperatures for maximum feed conversion efficiency (Jobling 1994; Bjornsson & Tryggvadottir 1996; Jobling 1997). Our results indicated that there was no significant difference in feed conversion ratio between ambient temperature groups (S-AMB, 5.1 – 6.3 °C, TABLE I, Appendix II) and the groups reared at 13 °C (S13). The minimum feed conversion efficiency (FCE) was very high in all experiments (S-AMB = 2.68 and S13 = 2.96 , TABLE VIII, Appendix II), far superior to that of juvenile cod (1.18 – 1.35 %) (Imsland et al. 2005), turbot (0.82 – 1.21 %) (Imsland et al. 2000; Imsland et al. 2007a), spotted wolffish (1.11 – 1.37 %) (Imsland et al. 2006; Magnussen et al. 2008), Atlantic salmon (0.77 – 1.35 %) (Handeland et al. 2008; Björnsson & Jönsson 2012) when reared close to T_{opt} FCE in the same size range. This represents a very significant cost benefit for lumpfish farming, as feed costs are the greatest single cost factor in aquaculture.

The high FCE in juvenile lumpfish can be explained by several factors. Ingolfsson and Kristjansson (2002) found that only 28 % of 1482 juvenile lumpfish collected in June-

September had food in their stomachs, whereas 27 % of the juveniles in the same study had remains of food in their stomach. This may indicate a rapid degradation of prey in juvenile lumpfish, as a study by Vandendriessche et al. (2007) observed food in all stomachs, also in winter.

Juvenile lumpfish have a limited aerobic scope, which limits the possibility of active searching. The effect of temperature on metabolism may therefore be assumed to have a great impact on lumpfish. Lumpfish's foraging profile indicates minimizing its prey encounter rate on the behalf of not maximizing their net energy gain (Jobling 1997), and display a sit-and-wait foraging strategy, clinging to substrate before ambushing their prey (Brown 1986; Haaland 1996; Ingolfsson & Kristjansson 2002; Jobling et al. 2012). In comparison to most other fish larvae, lumpfish do not form the s-shaped posture before ambush (Brown 1986). This strategy is 6 - 12 % more efficient in terms of metabolic costs, compared to that of active searching, but only if the prey density supplies a sufficient net energy gain (Jobling et al. 2012). When prey density is low, actively searching for food is a more efficient foraging strategy than clinging, allowing lumpfish to yield energy at a faster rate, despite the increase in energy expenditure (Jobling et al. 2012) but also increases risk of predation (Killen et al. 2007).

Brown (1986) observed that juvenile lumpfish above 40 mm would utilize the suction disc to cling while feeding. This size range overlaps with the size group observed in our study, hence indicating that the juvenile lumpfish in our experiment may have utilized this foraging strategy. This underlines the possible importance of the adhesive disc for feeding strategies throughout juvenile stages. Despite this, there has been some doubt with regard to feeding strategies in captive lumpfish with increasing size, as Haaland (1996) and Killen et al. (2007) found that only smaller submissive fish would feed while clinging, and larger specimens would readily feed in the water column. Due to the significant size rank in our study one would assume that stable hierarchies as observed by Haaland (1996) do exist (TABLE LX, Appendix II), which may have caused differences in foraging strategy between size-rankings of fish.

Juvenile lumpfish feed throughout the year (Vandendriessche et al. 2007). Small juvenile lumpfish with yolk sacks are found to feed on small crustaceans (harpacticoid copepods) before shifting to amphipods (*Callopius laeciusculus* and *Parathemisto gaudichaudi*) with

increasing size (Daborn & Gregory 1983; Ingolfsson & Kristjansson 2002). This further indicates an ontogenetic change in terms of prey size in juvenile lumpfish, which further may reflect their change in habitat and hence temperature preference with increasing size (Daborn & Gregory 1983; Brown 1986; Moring 1989).

In our study, no difference in FCE between temperature groups was observed, which is similar to effects earlier observed for Atlantic cod (Imsland et al. 2005). FCE for juvenile turbot has been found to accelerate until maximum FCE at T_{opt} FCE, before decelerating again at suboptimal temperatures (Van Ham et al. 2003). T_{opt} FCE is generally somewhat lower than that of T_{opt} SGR (Jobling 1997). The two temperatures presented in experiment 2 may hence have been on each side of this scale, but results for T_{opt} SGR presented for the same size interval in experiment 1 (>13 °C for 25 – 30 g and 12.8 °C for 100 – 100 g, FIGURE 3.5) would indicate a T_{opt} FCE higher than the temperature in the highest temperature group (S13), as T_{opt} SGR was not reached for the group reared at 13 °C (T13) in experiment 1. This may have been a contributing factor to the lack of significant difference in FCE between the high and low temperature groups, as both temperatures may have been suboptimal for FCE. Temperature therefore had an effect on metabolism and total feed consumption, but did not have an effect on overall feed utilization. In terms of commercial lumpfish farming this indicates that lumpfish can reach optimal sizes in shorter time at optimal temperatures, but at the same feed cost as for fish reared at ambient temperatures. Another possible explanation for the insignificant difference in FCE between temperature groups S-AMB and S13 at satiation level in our study may be explained by the approximately same stomach size and same ability to digest. Despite these findings, feed consumption is to a large extent influenced by feed composition in terms of energy content, along with the feeding frequency (Brett & Groves 1979).

Imsland et al. (2000) suggested an inter-population difference in energy utilization, with increasing FCE at higher latitudes due to shorter growth periods in the north. The same study furthermore suggested that the energy allocation and utilization of a specie can vary according to differences in the environment. Lumpfish have been found to display high polymorphism in genetic loci between Icelandic and Canadian populations (Skirnisdottir et al. 2013). Due to the large geographic distribution one may hence be able to exploit differences in feed

utilization in culture of juvenile lumpfish for commercial production as well, if such genetic effects of growth exists in lumpfish.

Lumpfish juveniles are known to feed selectively, ignoring plentiful species of prey, especially slow moving or small particles (Haaland 1996; Ingolfsson & Kristjansson 2002) showing little interest for pellets in the bottom of the tank (Haaland 1996). In our study there was very often excess pellets at the bottom of the tank, which may indicate that despite having unlimited access to feeds in the time span offered, fish may not have fed to satiation, and may very well have exposed an effect of compensatory growth similar effect to that of juvenile turbot (Van Ham et al. 2003).

5. Conclusions

Increased temperatures had a positive effect on growth in terms of weight, length and growth rate, but had no significant effects on feed conversion efficiency. Temperatures of 10 - 13 ℃ led to 31- 35 % higher overall growth rates and 48-53 % higher final weights compared to that of juvenile lumpfish reared at 4 ℃.

Overall growth rate, weight and length was significantly higher for T13 (13 ℃) compared to that of all other temperature groups, and mean lengths and weights displayed a significant, stepwise increase with increasing temperature for most samplings. Temperature optimums for growth decreased with increasing body weight, suggesting an ontogenetic shift in temperature preferences for growth. For size groups 100 – 110 g and 160 – 200 g parabolic regressions suggested optimum temperatures for growth of 12.8 and 9.6 ℃ respectively. The temperature optimum for growth in the size group 25 – 30 g was not reached in the investigated temperature interval (4 – 13 °C), but an optimum temperature for growth above 13 ℃ is suggested. Growth rate declined with increasing size for all temperature groups, but temperature tolerance increased with increasing body size. In order to fully utilize the decrease in optimum temperature with increasing fish size, the utilization of "temperature steps", a reduction in temperature with increasing body size is suggested.

Increased temperature caused an increase in total consumption of feed (C_T), but had no effect on feed conversion efficiency (FCE) or daily feeding rate (F%). The limited effect of temperature seen on temperature on FCE and F% may be due to the limited duration of the experiment, and too coarse temperature ranges to be able to pinpoint a temperature optimum for feed conversion efficiency. This lacking sensitivity of FCE close to optimal temperature of growth may have important consequences for lumpfish farming, as this indicates that lumpfish can reach a large size in a short time, but at the same cost as slower growing individuals at low temperatures.

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Appendix I - Discussion of material and methods

Fish stock

The juvenile lumpfish in this study were collected from wild caught brood stock, and all egg batches were pooled. All batches of roe were fertilized with milt from the same male, hence all juveniles in experiment 1 and 2 were half siblings. Due to the mixed set-up of spawning groups, the maternal effects cannot be separated from other possible genetic stock effects. A genetic differences in terms of scope of growth between populations of lumpfish is also possible, as size differences between latitudinal populations have been observed (Davenport 1985). Observations by Imsland et al. (2000) indicate that juvenile turbot from high latitudes display greater growth capacity and a higher T_{opt} FCE & SGR than that of populations of a southern bound distribution. This rapid growth is assumed to be an effect of short growing season, which may also be an explanatory factor for the small size of lumpfish in the Bay of Fundy (Daborn & Gregory 1983; Davenport 1985) relative to other observations of counter specimens further north, though temperatures in shallow areas of the Bay of Fundy are observed to reach 0 – 4 °C in winter (Campbell & Stasko 1986). It is also possible that the individual differences in growth were due to sex-related differences, as lumpfish females generally are larger than male specimens of the same age (Davenport 1985; Moring 2001). No visual differences between males and females were observed throughout the experimental period, therefore it is not possible to relate the effects of sex to size or growth rate in this study.

Rearing conditions

Flow rates were increased from 16 l min^{-1} to 20 l min^{-1} during the adaption period of the experiment 1 in order to adjust the circulation and oxygen level in the rearing tanks. For experiment 2 the flow rate was set to 20 l min^{-1} for the entire experimental period. The measured oxygen saturation fell below 80% saturation in the high temperature groups on some occasions (TABLE II, Appendix II), but this was corrected by the addition of oxygen in the holding tanks for the groups reared at 7, 10 and 13 °C. The addition of oxygen to the high

– temperature groups caused slight differences in oxygen saturation between groups, but this is assumed to have little or no effects on growth, as all groups were reared at mean values above 80 % saturation (TABLE II, Appendix II), and drops in saturation were short term and corrected for by the means of manual adjustment on a daily basis.

The time span of experiment 1 was approximately 3 months, which is thought to be sufficient to evaluate the temperature effects on growth in juvenile marine fish (Imsland et al. 2005; Björnsson et al. 2007; Imsland et al. 2007b) and subsequently to provide evidence of differences in size due to temperature manipulation and hence the establishment of T_{opt} SGR for different size groups. In our study the indications of an ontogenetic shift were prominent from period 3 (3 June – 26 June, FIGURE 3.3 and TABLE VI, Appendix II). In order to evaluate whether the observed size differences between temperature groups were maintained over a longer period or if fish reared at ambient temperatures would catch up with high temperature groups over time as suggested by (Van Ham et al. 2003) our experimental period should ideally consist of additionally 1 – 2 growth periods. Our results do in fact indicate an incipient increase in SGR at ambient temperature over time (FIGURE 3.3, TABLE VI, Appendix II). In contrast, the time span proposed for experiment 2 is suggested to be too short to observe any effects of temperature on feeding regime (Björnsson et al. 2007), as no effects on FCE or F were observed in this study.

Size hierarchies

The fish used in both experiments were graded according to weight prior to the experiment (TABLE III, Appendix II) to reduce the effects of size hierarchies on feeding and growth, as observed in previous studies of juvenile lumpfish (Hallaraker et al. 1995; Killen et al. 2007) and other juvenile marine fish (Hallaraker et al. 1995; Imsland et al. 1996; Aune et al. 1997; Jonassen et al. 2000b; Imsland et al. 2005). Results from size ranking (initial versus final weight) indicate that size hierarchies in fact did form in all groups of experiment 1 (TABLE LX, Appendix II), but it is unknown to which extent this may have had an effect on growth and feeding behavior. Growth capacity in the fish may be a result of inherent growth capacity (Imsland et al. 2006), but this effect is questionable in the present study as all fish were half-siblings. No evident signs of physical damage on pectoral fins, tails or eyes were observed

throughout the experimental period, but this proposed effect of dominance has been observed in larger groups of lumpfish at higher densities (own observations).

Mortality

No mortality was observed during the experiment, but juvenile lumpfish reared at 16 °C have been observed to show negative effects of long term rearing at such high temperatures (own observations). Cataract leading to blindness, and in the long term decreased ability to forage and increased mortality as a consequence has been observed for lumpfish at approximately 150 g (own observations). Hence, the long-term effects of increased temperatures should be questioned, as rearing fish at 13 °C over long periods of time may cause undesirable effects. The utilization of T-steps to adapt T_{opt} SGR to increasing body size may be an efficient solution (Imsland et al. 2006; Imsland et al. 2008).

Possible secondary effects of photoperiod

Lumpfish in this study were reared under constant light. Photoperiod affects melatonin synthesizing pineal and retinal cells, hence producing an endocrine and neuroendocrine signal of darkness and light through the pituitary-thyroid-axis (Boeuf & Le Bail 1999). Possible secondary effects on photoperiod should not be out ruled, as temperature – growth studies for juvenile halibut have shown enhancing effects on continuous light periods for growth at low or sub-optimal temperatures and negative effects close to T_{opt} SGR (Jonassen et al. 2000b; Lohne et al. 2012), but this growth effect may be species specific (Boeuf & Le Bail 1999) and dependent on the changing optimum temperature for growth with increasing size (Imsland et al. 2007b). Despite this, Lohne et al. (2012) found no indications that photoperiod modulates T_{opt} for SGR and feeding in juvenile halibut, while (Jonassen et al. 2000a) found that growth in juvenile halibut would gradually increase with increasing natural day length, though not exceeding the growth of fish reared at constant photoperiod. Imsland and Jonassen (2001) suggested an increase in overall metabolism due to increased metabolic capacity at constant photoperiod at low temperatures, but the nocturnal activity of lumpfish is yet unknown. It has been suggested that the effect of photoperiod increases for species with a low standard metabolic rate (Boeuf & Le Bail 1999) which according to the findings of our study in terms

of growth and FCE may lead us to believe that photoperiod modulation most likely would have a great effect on juvenile lumpfish growth. It has also been questioned whether the positive effect of increased temperature on growth has been related to elevated FCE or C_T (Boeuf & Le Bail 1999). Further studies must be undertaken to undermine the possible effects of photoperiod on growth in juvenile lumpfish at different temperatures. Experiment 2 was carried out in two time periods, one in May – June (5.1 – 5.7 °C, TABLE I, Appendix II), the other in June – July (5.9 – 6.3 °C, TABLE I, Appendix II). For the group reared at ambient temperature, this may have affected the results of increasing water temperature between the two experiments, but no attempts at correcting for such effects were undertaken.

Feed-related parameters

The feed conversion efficiency (FCE) observed in experiment 2 was very high. Probable errors includes the underestimation of feed intake C_T, or the overestimation of growth in the time-scope studied. The underestimation of feed intake is highly unlikely, as pellets were counted manually every day. On the contrary, crushed pellet were not observed in the sieve used for collecting feed in experiment 2, but the long time-span between initial feeding pulse (07:00) and collecting and counting of pellets at the end of the day (15:00) may have caused some food particles to run through the sieve, though this is not likely. Upon feed collection all pellets present in the sieve appeared compact and firm, therefore it unlikely that this may have led to an overestimation of C_T causing the high FCE observed in experiment 2. The SGR % day^{-1} for both experiment 1 and 2 were calculated according to the formula presented in "materials and methods", and were conducted using the same personnel and equipment at each sampling. In the results for experiment 2 a significant difference in SGR was found, but no difference in FCE between temperature groups S-AMB and S13. Temperature therefore had an effect on metabolism and total feed consumption, but did not have an effect on overall feed utilization. There is a trend for higher FCE for fish reared at high temperatures, but the observed difference is insignificant (SNK-test; $p = 0.0506$, TABLE LI, Appendix II). The lack of difference in observed FCE and F % between S-AMB and S13 may be explained by only two replicates per group and a short sampling period (17 days) to observe an effect of increased temperature. The lack of difference in temperature effect has also been shown in experiments for juvenile Atlantic cod (Björnsson et al. 2007). Björnsson et al. (2007) stated

that adaption time for fish reared at high temperatures is longer than that of fish reared at low temperatures. Furthermore, fish were offered an unlimited amount of feed prior to start-up of the experiment, before being offered a feed during a restricted time interval per day during the experimental period. Positive effects of high feeding frequencies at high temperatures have been observed in juvenile cod (Imsland et al. 2007a). Pulse feeding of larval lumpfish has been observed to increased growth and feeding behavior, presumably due to mimicking the pattern of food abundance in tide pools (Brown et al. 1997). Van Ham et al. (2003) found that juvenile turbot exposed to restricted feeding would optimize their feeding regime in order to utilize the feed stuffs in a more efficient manner, hence increasing feed conversion efficiency. In experiment 2 of the present study, juveniles were subjected to unlimited feeding 24 hours per day in short pulses prior to experimental start up. During the experimental period feed was offered between 07:00 – 15:00, hence restricting the time scope in which feed was offered. This may have had an effect on the compensatory growth as displayed in turbot (Van Ham et al. 2003), but the time scope (here 17 days) is probably too short to establish the existence of such effect (Björnsson et al. 2007). Despite offering the same amount of feed as prior to the experimental period, one may argue that fish may have had an increased C_T if feed was offered in a longer time span per day. As lumpfish prefer foraging in the water column in contrast to at the bottom of the tank, it may be argued that the feeding pulses provided between 0700 – 1500 were too short to allow the fish to fully utilize the daily scope of C_T, as the feed would reach the bottom of the tank quite rapidly, allowing feed to accumulate at the tank bottom. Standard deviations for measurements of C_T were high due two only two replicate groups for each sample and as a result of increasing feed consumption per day with increasing body size, due to the rapid growth. Only one experimental period was used for observing temperature related feeding behavior in lumpfish, and the experimental period was probably too short to observe an effect on temperature on feeding parameters (Björnsson et al. 2007).

Maturing lumpfish do not appear to feed during the winter before or during the breeding season in spring – summer (Davenport et al. 1983; Davenport 1985). Females from present experiment did in fact begin to spawn batches of eggs in the following spring, aged 2 years (Personal observation). As present experiments were conducted in spring to summer one year in advance to mating behavior was observed, one would assume that the effect of maturation would not have any impact on the results of present study.

Appendix II - Tables

1. Descriptive statistics

1.1 Experimental conditions

Appendix II TABLE I. Descriptive statistics based on daily temperature measurements in all tanks. Means, total number of observations (n), standard deviation (S.D.), standard error (S.E.), minimum (Min.) and maximum (Max.) of temperatures (°C) are included in the table.

Temperature (°C)							
Treatment	Replicate	Means	n	S.D.	S.E.	Min.	Max.
T4	A	4.2	80	0.4	0.05	3.5	5.8
T4	B	4.2	80	0.3	0.04	3.5	5.0
T-AMB	A	5.8	80	0.3	0.03	5.1	6.2
T-AMB	B	5.8	80	0.3	0.04	5.2	6.5
T7	A	7.1	80	0.2	0.03	6.5	7.7
T7	B	7.1	80	0.2	0.03	6.4	7.7
T10	A	9.9	80	0.4	0.05	8.4	10.5
T10	B	10.0	80	0.5	0.06	7.9	10.6
T13	A	12.9	80	0.6	0.07	10.6	13.5
T13	B	12.8	80	0.8	0.10	9.5	13.4
S-AMB	A	5.5	17	0.1	0.03	5.1	5.7
S-AMB	B	6.1	17	0.1	0.03	5.9	6.3
S13	A	13.0	17	0.2	0.04	12.8	13.3
S13	B	13.1	17	0.2	0.04	12.9	13.4

Appendix II TABLE II. Descriptive statistics based on daily temperature measurements in all tanks. Means, total number of observations (n), standard deviation (S.D.), standard error (S.E.), minimum (Min.) and maximum (Max.) of temperatures (°C) are included in the table.

Oxygen saturation (%)							
Treatment	Replicate	Means	N	S.D.	S.E.	Min.	Max.
T4	A	83.3	80	4.6	0.58	67	96
T4	B	84.6	80	4.4	0.56	68	95
T-AMB	A	87.4	80	5.3	0.67	74	99
T-AMB	B	88.5	80	4.2	0.52	75	98
T7	A	89.3	80	7.5	0.94	72	106
T7	B	89.6	80	7.6	0.96	73	103
T10	A	100.1	80	12.8	1.61	73	119
T10	B	102.8	80	12.4	1.57	77	103
T13	A	92.5	80	6.4	0.81	76	105
T13	B	91.7	80	6.4	0.81	77	106
S-AMB	A	91.3	17	2.1	0.51	88	96
S-AMB	B	90.7	17	4.5	1.09	85	103
S13	A	93.7	17	4.8	1.15	80	101
S13	B	96.5	17	2.9	0.70	91	100

Appendix II TABLE III. Descriptive statistics based on measurements of weight for treatments and replicates at all samplings W1 (11 April), W2 (15 May), W3 (2 June), W4 (26 June), W5 (16 July). Weight means (g), total number of observations (n), standard deviations (± S.D.), minimum (Min.) and maximum (Max.) values for individual weights in each group are included in the table.

Total weight (g) – All groups								
Treatment	Replicate	Sampling Period	Weight Means	(n)	Weight Sum	± S.D.	Weight Min.	Weight Max.
T4	A	W1	28.3	33	932.4	9.8	13.8	44.4
T4	A	W2	49.1	33	1620.2	16.3	25.8	82.4
T4	A	W3	67.9	33	2239.3	22.2	36.4	108.4
T4	A	W4	93.4	33	3082.8	29.3	52.2	146.8
T4	A	W5	121.8	33	4018.0	35.9	66.0	180.2
T4	B	W1	26.3	36	943.8	11.6	11.0	54.4
T4	B	W2	47.3	36	2704.0	19.6	22.4	92.2
T4	B	W3	66.4	36	2389.6	26.5	29.8	126.6
T4	B	W4	89.7	36	3230.8	34.5	39.2	169.2
T4	B	W5	115.1	36	4144.6	43.6	51.0	216.2
T-AMB	A	W1	26.2	37	970.0	10.7	6.4	53.6
T-AMB	A	W2	47.2	37	1745.6	17.0	12.4	91.6
T-AMB	A	W3	69.0	37	2554.4	24.4	18.8	127.0
T-AMB	A	W4	100.5	37	3781.8	33.9	36.2	178.2
T-AMB	A	W5	140.7	37	5205.4	43.8	56.4	234.2
T-AMB	B	W1	28.2	35	987.6	10.7	13.2	51.4
T-AMB	B	W2	50.8	35	1776.6	16.5	25.0	93.2
T-AMB	B	W3	73.8	35	2583.4	22.0	38.6	135.6
T-AMB	B	W4	106.5	35	3725.8	29.4	59.8	180.4
T-AMB	B	W5	150.3	35	5060.6	38.6	86.6	86.8
T7	A	W1	36.7	35	933.6	11.6	12.6	49.8
T7	A	W2	55.5	35	1941.4	21.2	29.8	105.0
T7	A	W3	83.8	35	2933.4	27.5	51.8	150.2
T7	A	W4	124.3	35	4351.4	38.3	74.8	203.6
T7	A	W5	171.4	35	6000.6	49.8	109.4	276.0
T7	B	W1	29.1	35	1016.8	12.2	10.2	54.6
T7	B	W2	63.6	35	226.6	24.3	22.8	115.0
T7	B	W3	97.2	35	3402.6	31.7	41.4	167.4
T7	B	W4	142.8	35	4997.0	42.6	65.0	245.0
T7	B	W5	190.9	35	6680.8	55.4	86.2	323.2
T10	A	W1	26.3	34	895.0	11.6	12.2	59.4
T10	A	W2	72.4	34	2461.2	26.8	37.4	146.8
T10	A	W3	121.3	34	4125.2	37.9	70.8	223.0
T10	A	W4	172.8	34	5876.4	48.2	108.0	283.8
T10	A	W5	234.1	34	7959.6	67.2	141.6	382.6
T10	B	W1	25.0	35	874.8	66.9	12.0	47.6
T10	B	W2	71.3	35	2493.8	19.3	33.0	119.4
T10	B	W3	113.5	35	3971.0	24.7	62.0	168.6
T10	B	W4	157.9	35	5527.6	35.9	102.2	232.8
T10	B	W5	218.8	35	7658.6	48.2	148.6	315.6
T13	A	W1	23.6	36	848.2	6.9	7.6	36.8
T13	A	W2	76.64	36	2764.8	18.8	34.2	114.4
T13	A	W3	130.7	36	4705.4	26.6	77.8	178.8
T13	A	W4	191.8	36	6906.2	40.0	113.8	271.4
T13	A	W5	266.4	36	9591.2	59.0	158.2	367.6

T13	B	W1	25.1	32	804.4	10.3	10.0	56.6
T13	B	W2	82.0	32	2624.0	24.3	34.0	145.4
T13	B	W3	136.5	32	4368.2	32.4	73.8	228.4
T13	B	W4	183.5	32	5871.2	44.1	108.6	324.0
T13	B	W5	246.1	32	7873.8	54.9	167.4	430.4
S-AMB	A	W1	39.1	61	2385.0	8.9	23.6	62.6
S-AMB	A	W2	59.7	61	3649.9	13.2	35.2	97.8
S-AMB	B	W1	34.5	60	2070.6	6.9	21.2	50.2
S-AMB	B	W2	52.6	60	3158.8	10.2	34.4	79.4
S13	A	W1	68.2	60	4093.5	12.6	40.2	97.8
S13	A	W2	127.1	60	7623.8	23.3	84.0	176.0
S13	B	W1	45.2	60	2714.8	7.6	32.8	64.8
S13	B	W2	91.5	60	5488.0	14.5	57.6	131.8

2. Response variables

Experiment 1

Appendix II TABLE IV. Descriptive statistics for weight measurements at W1 (11 April), W2 (15 May), W3 (2 June), W4 (26 June) and W5 (16 July). Weight means (g), total number of observations (n), standard deviation (± S.D.) and standard error (± S.E.) for all groups are included in the table.

Treatment	**Mass (g) W1**				**Mass (g) W2**				**Mass (g) W3**				**Mass (g) W4**				**Mass (g) W5**			
	Means	(n)	S.D.	S.E.	Means	(n)	S.D.	S.E.	Means	(n)	S.D.	S.E.	Means	(n)	S.D.	S.E.	Means	(n)	S.D.	S.E.
T4	27.2	69	10.7	1.3	48.2	69	18.0	2.2	67.1	69	24.4	2.9	91.5	69	31.9	3.8	118.3	69	39.9	4.8
T-AMB	27.2	72	10.7	1.3	48.9	72	16.7	2.0	71.4	72	23.3	2.7	103.4	72	31.7	3.8	145.4	72	41.4	4.9
T7	27.9	70	11.8	1.4	59.5	70	23.0	2.8	90.5	70	30.2	3.6	133.5	70	41.3	4.9	181.2	70	53.2	6.4
T10	25.7	69	9.9	1.2	71.8	69	23.1	2.8	117.3	69	31.9	3.8	165.3	69	42.8	5.2	226.4	69	58.4	7.0
T13	24.3	68	8.6	1.1	79.2	68	21.6	2.2	133.4	68	29.4	3.6	187.9	68	41.9	5.1	256.8	68	57.6	7.0

Appendix II TABLE V. Descriptive statistics for length measurements at W1 (11 April), W2 (15 May), W3 (2 June), W4 (26 June) and W5 (16 July). Weight means (g), total number of observations (n), standard deviation (± S.D.) and standard error (± S.E.) are included in the table.

Treatment	**Length (cm) W1**				**Length (cm) W2**				**Length (cm) W3**				**Length (cm) W4**				**Length (cm) W5**			
	Means	(n)	S.D.	S.E.	Means	(n)	S.D.	S.E.	Means	(n)	S.D.	S.E.	Means	(n)	S.D.	S.E.	Means	(n)	S.D.	S.E.
T4	8.7	69	1.2	0.1	10.6	69	1.3	0.2	11.7	69	1.3	0.2	12.9	69	1.5	0.2	14.4	69	1.6	0.2
T-AMB	8.7	72	1.2	0.1	10.6	72	1.3	0.2	11.8	72	1.4	0.2	13.5	72	1.4	0.2	15.4	72	1.5	0.2
T7	8.8	70	1.2	0.2	11.3	70	1.4	0.2	13.1	70	1.4	0.2	14.9	70	1.4	0.2	16.6	70	1.4	0.2
T10	8.5	69	2.0	0.1	11.9	69	1.2	0.2	14.1	69	1.2	0.1	15.9	69	1.1	0.1	17.6	69	1.3	0.2
T13	8.4	68	1.0	0.1	12.1	68	1.2	0.1	14.6	68	1.1	0.1	16.5	69	1.1	0.1	18.2	68	1.3	0.2

Appendix II TABLE VI. Descriptive statistics for SGR (% day^{-1}) for W1-W2 (11 April – 15 May), W2-W3 (16 May – 2 June), W3-W4 (3 June – 26 June) and W4-W5 (27 June – 16 July) and overall (11 April – 16 July). Weight means (g), total number of observations (n), standard deviation (± S.D.) and standard error (± S.E.) are included in the table.

Treatment	SGR (% day^{-1}) W1-W2				SGR (% day^{-1}) W2-W3				SGR (% day^{-1}) W3-W4				SGR (% day^{-1}) W4-W5				SGR (% day^{-1}) Overall			
	Means	(n)	S.D.	S.E.	Means	(n)	S.D.	S.E.	Means	(n)	S.D.	S.E.	Means	(n)	S.D.	S.E.	Means	(n)	S.D.	S.E.
T4	1.71	69	0.26	0.03	1.85	69	0.23	0.03	1.31	69	0.21	0.03	1.30	69	0.24	0.03	1.54	69	0.13	0.02
T-AMB	1.78	72	0.27	0.03	2.13	72	0.31	0.04	1.58	72	0.26	0.03	1.74	72	0.32	0.04	1.81	72	0.18	0.02
T7	2.28	70	0.37	0.04	2.43	70	0.50	0.06	1.65	70	0.25	0.03	1.55	70	0.30	0.04	1.99	70	0.23	0.03
T10	3.09	69	0.30	0.03	2.81	69	0.53	0.06	1.44	69	0.56	0.07	1.57	69	0.40	0.05	2.23	69	0.28	0.03
T13	3.54	68	0.37	0.04	2.97	68	0.56	0.07	1.43	68	0.46	0.06	1.56	68	0.35	0.04	2.38	68	0.29	0.04

Experiment 2

Appendix II TABLE VII. Descriptive statistics for mass (g) and SGR % day^{-1} at samplings W0 , W1, and W2 for experiment 2. Weight means (g), total number of observations (n), standard deviation (± S.D.) and standard error (± S.E.) are included in the table. SGR is calculated from total weight per replicate group at each sampling.

Treatment	Mass (g) W0				Mass (g) W1				Mass (g) W2				SGR % W0-W1				SGR % W1-W2			
	Means	(n)	S.D.	S.E.	Means	(n)	S.D.	S.E.	Means	(n)	S.D.	S.E.	Means	(n)	S.D.	S.E.	Means	(n)	S.D.	S.E.
S-AMB	25.1	121	5.4	0.5	36.8	121	8.3	0.8	56.2	121	12.3	1.1	0.44	2	0.03	0.02	1.14	2	0.10	0.07
S13	25.3	120	4.8	0.4	56.7	120	15.5	1.4	109.3	120	26.3	2.4	0.99	2	0.36	0.26	2.68	2	1.11	0.78

Appendix II TABLE VIII. Descriptive statistics based on calculated C_T, FCE and F% for days 0-17 for temperature groups S0 and S13. Weight means (g), total number of observations (n), standard deviation (± S.D.) and standard error (± S.E.) are included in the table.

Treatment	C_T (g) W1 – W2				FCE W1 –W2				F% W1 – W2			
	Means	(n)	S.D.	S.E.	Means	(n)	S.D.	S.E.	Means	(n)	S.D.	S.E.
S-AMB	438.9	2	28.8	20.4	2.68	2	0.45	0.31	0.93	2	0.15	0.11
S13	1062.7	2	117.8	83.3	2.96	2	0.18	0.12	1.28	2	0.18	0.13

3. ANOVA

3.1 Two-way nested ANOVA

Experiment 1

Appendix II TABLE IX. Test results from two-way nested ANOVA for individual weight data at sampling W1. Replicate group is nested in treatment group.

Weight W1					
Two-way nested ANOVA					
	SS	DF	MS	F	p
Intercept	243328.10	1	243328.10	2224.829	<0.001
Treatment	556.40	4	141.60	1.295	0.272
Replicate (Treatment)	315.00	5	63.00	0.576	0.718
Error	36966.80	338	109.40		

Appendix II TABLE X. Test results from two-way nested ANOVA for individual weight data at sampling W2. Replicate group is nested in treatment group.

Weight W2					
Two-way nested ANOVA					
	SS	DF	MS	F	p
Intercept	1317628.00	1	1317628.00	3093.621	<0.001
Treatment	52709.00	4	13177.00	30.938	<0.001
Replicate (Treatment)	1936.00	5	387.00	0.909	0.475
Error	143960.00	338	426.00		

Appendix II TABLE XI. Test results from two-way nested ANOVA for individual weight data at sampling W3. Replicate group is nested in treatment group.

Weight W3					
Two-way nested ANOVA					
	SS	DF	MS	F	p
Intercept	3202600.00	1	3202600.00	4107.961	<0.001
Treatment	230374.00	4	57593.00	73.875	<0.001
Replicate (Treatment)	5231.00	4	1046.00	1.342	0.246
Error	263508.00	338	780.00		

Appendix II TABLE XII. Test results from two-way nested ANOVA for individual weight data at sampling W4. Replicate group is nested in treatment group.

Weight W4					
Two-way nested ANOVA					
	SS	DF	MS	F	p
Intercept	6457168.00	1	6457168.00	4471.505	<0.001
Treatment	452569.00	4	113192.00	78.384	<0.001
Replicate (Treatment)	11838.00	5	2368.00	1.640	0.149
Error	488096.00	338	1444.00		

Appendix II TABLE XIII. Test results from two-way nested ANOVA for individual weight data at sampling W5. Replicate group is nested in treatment group.

Weight W5					
Two-way nested ANOVA					
	SS	DF	MS	F	p
Intercept	11962691.00	1	11962691.00	4705.930	<0.001
Treatment	880894.00	4	220223.00	86.632	<0.001
Replicate (Treatment)	20088.00	5	4018.00	1.580	0.165
Error	859212.00	338	2542.00		

Appendix II TABLE XIV**.** Test results from two-way nested ANOVA for individual length data at sampling W1. Replicate group is nested in treatment group.

Length W1					
Two-way nested ANOVA					
	SS	DF	MS	F	p
Intercept	23809.56	1	258080.56	20222.41	<0.001
Treatment	4.58	4	1.14	0.90	0.466
Replicate (Treatment)	4.00	5	0.80	0.63	0.680
Error	431.38	338	1.28		

Appendix II TABLE XV. Test results from two-way nested ANOVA for individual length data at sampling W2. Replicate group is nested in treatment group.

Length W2					
Two-way nested ANOVA					
	SS	DF	MS	F	p
Intercept	44275.88	1	44375.88	27278.80	<0.001
Treatment	134.54	4	22.64	20.72	<0.001
Replicate (Treatment)	6.17	5	1.23	0.76	0.579
Error	548.60	338	1.62		

Appendix II TABLE XVI. Test results from two-way nested ANOVA for individual length data at sampling W3. Replicate group is nested in treatment group.

Length W3					
Two-way nested ANOVA					
	SS	DF	MS	F	p
Intercept	59700.01	1	59700.01	36473.11	<0.001
Treatment	446.73	4	111.68	68.23	<0.001
Replicate (Treatment)	6.12	5	1.22	0.75	0.588
Error	553.25	338	1.64		

Appendix II TABLE XVII. Test results from two-way nested ANOVA for individual length data at sampling W4. Replicate group is nested in treatment group.

Length W4					
Two-way nested ANOVA					
	SS	DF	MS	F	p
Intercept	75612.68	1	75612.68	43563.16	<0.001
Treatment	621.87	4	155.47	89.57	<0.001
Replicate (Treatment)	9.66	5	1.93	1.11	0.353
Error	586.67	338	1.74		

Appendix II TABLE XVIII. Test results from two-way nested ANOVA for individual length data at sampling W5. Replicate group is nested in treatment group.

Length W5					
Two-way nested ANOVA					
	SS	DF	MS	F	p
Intercept	93962.31	1	93962.31	46126.95	<0.001
Treatment	667.48	4	169.37	83.15	<0.001
Replicate (Treatment)	5.77	338	1.15	0.57	0.726
Error	688.52	338	2.04		

Appendix II TABLE XIX. Test results from two-way nested ANOVA for individual SGR data for W1-W2 (11 April – 15 May). Replicate group is nested in treatment group.

SGR % day^{-1} W1 – W2					
Two-way nested ANOVA					
	SS	DF	MS	F	p
Intercept	2136.421	1	2136.421	21438.68	<0.001
Treatment	181.344	4	45.336	454.94	<0.001
Replicate (Treatment)	0.766	5	0.153	1.54	0.177
Error	33.683	338	0.100		

Appendix II TABLE XX. Test results from two-way nested ANOVA for individual SGR data for W2-W3 (16 May – 2 June). Replicate group is nested in treatment group.

SGR % day^{-1} W2 – W3					
Two-way nested ANOVA					
	SS	DF	MS	F	P
Intercept	2067.727	1	2067.727	10570.78	<0.001
Treatment	59.305	4	14.826	75.80	<0.001
Replicate (Treatment)	2.061	5	0.412	22.11	0.041
Error	66.115	338	0.196		

Appendix II TABLE XXI. Test results from two-way nested ANOVA for individual SGR data for W3-W4 (3 June – 26 June). Replicate group is nested in treatment group.

SGR % day^{-1} W3-W4					
Two-way nested ANOVA					
	SS	DF	MS	F	P
Intercept	760.6371	1	760.6371	5721.232	<0.001
Treatment	5.1377	4	1.2844	9.661	<0.001
Replicate (Treatment)	2.6755	5	0.5351	4.025	<0.001
Error	44.9371	338	0.1329		

Appendix II TABLE XXII. Test results from two-way nested ANOVA for individual SGR data for W4-W5 (27 June – 16 July). Replicate group is nested in treatment group.

SGR % day^{-1} W4 – W5					
Two-way nested ANOVA					
	SS	DF	MS	F	P
Intercept	827.6687	1	827.6687	8029.407	<0.001
Treatment	6.7814	4	1.6954	16.447	<0.001
Replicate (Treatment)	1.3032	5	0.2606	2.529	<0.050
Error	32.8409	338	0.1031		

Appendix II TABLE XXIII. Test results from two-way nested ANOVA for overall average individual SGR data for W1-W5 (11 April – 16 July). Replicate group is nested in treatment group.

Overall SGR % day^{-1} W1-W5					
Two-way nested ANOVA					
	SS	DF	MS	F	P
Intercept	1377.003	1	1377.003	25365.87	<0.001
Treatment	30.245	4	7.561	144.78	<0.001
Replicate (Treatment)	0.404	5	0.081	1.55	0.174
Error	17.653	338	0.052		

Experiment 2

Appendix II TABLE XXIV. Test results from two-way nested ANOVA for individual weight data at sampling W0. Replicate group is nested in treatment group.

Weight W0					
Two-way nested ANOVA					
	SS	DF	MS	F	p
Intercept	153247.0	1	153247.0	5836.419	<0.001
Treatment	1.5	1	1.5	0.055	0.814
Replicate (Treatment)	24.1	2	12.1	0.460	0.632
Error	6222.9	237	26.3		

Appendix II TABLE XXV. Test results from two-way nested ANOVA for individual weight data at sampling W1. Replicate group is nested in treatment group.

Weight W1					
Two-way nested ANOVA					
	SS	DF	MS	F	p
Intercept	527146.4	1	527146.4	6167.761	<0.001
Treatment	23934.7	1	23934.7	280.043	<0.001
Replicate (Treatment)	16477.4	2	8238.7	96.395	<0.001
Error	20255.9	237	85.5		

Appendix II TABLE XXVI. Test results from two-way nested ANOVA for individual weight data at sampling W2. Replicate group is nested in treatment group.

Weight W2					
Two-way nested ANOVA					
	SS	DF	MS	F	p
Intercept	1648671	1	1648671	6399.989	<0.001
Treatment	169909	1	169909	659.570	<0.001
Replicate (Treatment)	39507	2	19753	76.681	<0.001
Error	61052	237	258		

3.2 One-way ANOVA

Experiment 2

Appendix II TABLE XXVII. Test results from one-way ANOVA for calculated C_T for day W1-W2 (0-17).

C_T Overall					
One-way ANOVA					
	SS	DF	MS	F	P
Intercept	2254706	1	2254706	306.7477	<0.005
Treatment	389137	1	389137	52.9413	<0.050
Error	14701	2	7350		

Appendix II TABLE XXVIII. Test results from one-way ANOVA for calculated FCE for W1-W2 (day 0-17).

FCE Overall					
One-way ANOVA					
	SS	DF	MS	F	p
Intercept	31.81191	1	31.81191	277.6550	<0.005
Treatment	0.07393	1	0.07393	0.6453	0.501
Error	0.22915	2	0.11457		

Appendix II TABLE XXIX. Test results from one-way ANOVA for calculated F % for W1-W2 (day 0-17).

F % Overall					
One-way ANOVA					
	SS	DF	MS	F	P
Intercept	4.852536	1	4.852536	174.4641	<0.01
Treatment	0.124224	1	0.124224	4.4663	0.169
Error	0.055628	2	0.027814		

4. Student-Newman-Keuls Test

Experiment 1

Appendix II TABLE XXX P-values from Student-Newman-Keuls test following ANOVA. Testing for differences in weight between treatment groups at W1 (11 April).

Weight W1					
Treatment	1	2	3	4	5
T4		0.999	0.705	0.659	0.362
T-AMB	0.999		0.923	0.385	0.234
T7	0.705	0.923		0.596	0.262
T10	0.659	0.385	0.596		0.448
T13	0.362	0.234	0.262	0.448	

Appendix II TABLE XXXI**.** P-values from Student-Newman-Keuls test following ANOVA. Testing for differences in weight between treatment groups at W2 (15 May).

Weight W2					
Treatment	1	2	3	4	5
T4		0.832	0.003	<0.001	<0.001
T-AMB	0.832		0.002	<0.001	<0.001
T7	0.003	0.002		<0.001	<0.001
T10	<0.001	<0.001	<0.001		0.034
T13	<0.001	<0.001	<0.001	0.034	

Appendix II TABLE XXXII. P-values from Student-Newman-Keuls test following ANOVA. Testing for differences in weight between treatment groups at W3 (2 June).

Weight W3					
Treatment	1	2	3	4	5
T4		0.367	<0.001	<0.001	<0.001
T-AMB	0.367		<0.001	<0.001	<0.001
T7	<0.001	<0.001		<0.001	<0.001
T10	<0.001	<0.001	<0.001		<0.001
T13	<0.001	<0.001	<0.001	<0.001	

Appendix II TABLE XXXIII. P-values from Student-Newman-Keuls test following ANOVA. Testing for differences in weight between treatment groups at W4 (26 June).

Weight W4					
Treatment	1	2	3	4	5
T4		0.065	<0.001	<0.001	<0.001
T-AMB	0.065		<0.001	<0.001	<0.001
T7	<0.001	<0.001		<0.001	<0.001
T10	<0.001	<0.001	<0.001		<0.001
T13	<0.001	<0.001	<0.001	<0.001	

Appendix II TABLE XXXIV. P-values from Student-Newman-Keuls test following ANOVA Testing for differences in weight between treatment groups at W5 (16 July).

Weight W5					
Treatment	1	2	3	4	5
T4		0.002	<0.001	<0.001	<0.001
T-AMB	0.002		<0.001	<0.001	<0.001
T7	<0.001	<0.001		<0.001	<0.001
T10	<0.001	<0.001	<0.001		<0.001
T13	<0.001	<0.001	<0.001	<0.001	

Appendix II TABLE XXXV. P-values from Student-Newman-Keuls test following ANOVA. Testing for differences in length between treatment groups at W1 (11 April).

Length W1					
Treatment	1	2	3	4	5
T4		0.754	0.789	0.635	0.511
T-AMB	0.754		0.830	0.552	0.534
T7	0.789	0.830		0.642	0.466
T10	0.635	0.552	0.642		0.636
T13	0.511	0.534	0.466	0.636	

Appendix II TABLE XXXVI. P-values from Student-Newman-Keuls test following ANOVA Testing for differences in length between treatment groups at W2 (15 May).

Length W2					
Treatment	1	2	3	4	5
T4		0.915	0.003	<0.001	<0.001
T-AMB	0.915		0.002	<0.001	<0.001
T7	0.003	0.002		0.008	<0.001
T10	<0.001	<0.001	0.008		0.265
T13	<0.001	<0.001	<0.001	0.265	

Appendix II TABLE XXXVII. P-values from Student-Newman-Keuls test following ANOVA Testing for differences in length between treatment groups at W3 (2 June).

Length W3					
Treatment	1	2	3	4	5
T4		0.230	<0.001	<0.001	<0.001
T-AMB	0.230			<0.001	<0.001
T7	<0.001	<0.001			<0.001
T10	<0.001	<0.001	<0.001		0.028
T13	<0.001	<0.001	<0.001	0.028	

Appendix II TABLE XXXVIII. P-values from Student-Newman-Keuls test following ANOVA. Testing for differences in length between treatment groups at W4 (26 June).

Length W4					
Treatment	1	2	3	4	5
T4		0.008	<0.001	<0.001	<0.001
T-AMB	0.008		<0.001	<0.001	<0.001
T7	<0.001	<0.001		<0.001	<0.001
T10	<0.001	<0.001	<0.001		0.007
T13	<0.001	<0.001	<0.001	0.007	

Appendix II TABLE XXXIX. P-values from Student-Newman-Keuls test following ANOVA Testing for differences in length between treatment groups at W5 (16 July).

Length W5					
Treatment	1	2	3	4	5
T4		<0.001	<0.001	<0.001	<0.001
T-AMB	<0.001		<0.001	<0.001	<0.001
T7	<0.001	<0.001		<0.001	<0.001
T10	<0.001	<0.001	<0.001		0.025
T13	<0.001	<0.001	<0.001	0.025	

Appendix II TABLE XL. P-values from Student-Newman-Keuls test following ANOVA. Testing for differences in SGR (% day^{-1}) between treatment groups for W1-W2 (11 April – 15 May).

SGR (% day^{-1}) W1-W2					
Treatment	1	2	3	4	5
T4		0.193	<0.001	<0.001	<0.001
T-AMB	0.193		<0.001	<0.001	<0.001
T7	<0.001	<0.001		<0.001	<0.001
T10	<0.001	<0.001	<0.001		<0.001
T13	<0.001	<0.001	<0.001	<0.001	

Appendix II TABLE XLI. P-values from Student-Newman-Keuls test following ANOVA Testing for differences in SGR (% day^{-1}) between treatment groups for W2-W3 (16 May – 2 June).

SGR (% day^{-1}) W2-W3					
Treatment	1	2	3	4	5
T4		<0.001	<0.001	<0.001	<0.001
T-AMB	<0.001		<0.001	<0.001	<0.001
T7	<0.001	<0.001		<0.001	<0.001
T10	<0.001	<0.001	<0.001		0.028
T13	<0.001	<0.001	<0.001	0.028	

Appendix II TABLE XLII. P-values from Student-Newman-Keuls test following ANOVA. Testing for differences in SGR (% day^{-1}) between treatment groups for W3-W4 (3 June – 26 June).

SGR (% day^{-1}) W3-W4					
Treatment	1	2	3	4	5
T4		<0.001	<0.001	0.085	0.058
T-AMB	<0.001		0.222	0.026	0.038
T7	<0.001	0.222		0.002	0.001
T10	0.085	0.026	0.002		0.817
T13	0.058	0.038	0.001	0.817	

Appendix II TABLE XLIII. P-values from Student-Newman-Keuls test following ANOVA. Testing for differences in SGR (% day^{-1}) between treatment groups for W4-W5 (27 June – 16 July).

SGR (% day^{-1}) W4-W5					
Treatment	1	2	3	4	5
T4		<0.001	<0.001	<0.001	<0.001
T-AMB	<0.001		0.003	0.003	0.003
T7	<0.001	0.003		0.881	0.808
T10	<0.001	0.003	0.881		0.814
T13	<0.001	0.003	0.808	0.814	

Appendix II TABLE XLIV. P-values from Student-Newman-Keuls test following ANOVA. Testing for differences in overall SGR (% day^{-1}) between treatment groups.

SGR (% day^{-1}) Overall					
Treatment	1	2	3	4	5
T4		<0.001	<0.001	<0.001	<0.001
T-AMB	<0.001		<0.001	<0.001	<0.001
T7	<0.001	<0.001		<0.001	<0.001
T10	<0.001	<0.001	<0.001		<0.001
T13	<0.001	<0.001	<0.001	<0.001	

Experiment 2

Appendix II TABLE XLVI. P-values from Student-Newman-Keuls test following ANOVA. Testing for differences in weight between treatment groups at W1 (Replicate A: 15 May, Replicate B: 22 June).

Weight W1		
Treatment	1	2
S-AMB		<0.001
S13	<0.001	

Appendix II TABLE XLVII. P-values from Student-Newman-Keuls test following ANOVA. Testing for differences in weight between treatment groups at W2 (Replicate A: 2 June, Replicate B: 10 July).

Weight W2		
Treatment	1	2
S-AMB		<0.001
S13	<0.001	

Appendix II TABLE XLVIII. P-values from Student-Newman-Keuls test following ANOVA. Testing for differences in overall SGR % day^{-1} between treatment groups for W0-W1 (Replicate A: 11 April – 15 May, Replicate B: 2 June – 22 June).

SGR W0-W1		
Treatment	1	2
S-AMB		0.163
S13	0.163	

Appendix II TABLE XLIX. P-values from Student-Newman-Keuls test following ANOVA.. Testing for differences in overall SGR % day^{-1} between treatment groups at W1-W2 (Replicate A: 15 May – 2 June, Replicate B; 22 June – 10 July).

SGR % day^{-1} W1-W2		
Treatment	1	2
S-AMB		0.190
S13	0.190	

Appendix II TABLE L. P-values from Student-Newman-Keuls test following ANOVA. Testing for differences in C_T between treatment groups for W1-W2 (Replicate A: 15 May – 2 June, Replicate B; 22 June – 10 July).

C_T W1-W2		
Treatment	1	2
S-AMB		0.019
S13	0.019	

Appendix II TABLE LI. P-values from Student-Newman-Keuls test following ANOVA. Testing for differences in FCE between treatment groups for W1-W2 (Replicate A: 15 May – 2 June, Replicate B; 22 June – 10 July).

FCE W1-W2		
Treatment	1	2
S-AMB		0.0506
S13	0.0506	

Appendix II TABLE LII. P-values from Student-Newman-Keuls test following ANOVA. Testing for differences in F% between treatment groups for W1-W2 (Replicate A: 15 May – 2 June, Replicate B; 22 June – 10 July).

F% W1-W2		
Treatment	1	2
S-AMB		0.169
S13	0.169	

5. ANCOVA

Experiment 1

Appendix II TABLE LIII. Two way ANCOVA (analysis of covariance) between all groups for W1-W5 (11 April – 16 July). Geometric mean (GM) is included as a co-varying factor in the analysis.

SGR and geometric mean weight (GM) W1-W5					
Two way ANCOVA – All groups					
	SS	DF	MS	F	p
Intercept	63.811	1	63.811	555.855	<0.001
GM Weight	9.150	1	9.150	79.707	<0.001
Treatment	9.148	4	2.355	20.510	<0.001
Error	3.903	34	0.115		

Appendix II TABLE LIV. Two way ANCOVA (analysis of covariance) between T-AMB and T4for W1-W5 (11 April – 16 July). Geometric mean (GM) is included as a co-varying factor in the analysis.

SGR and geometric mean weight (GM) W1-W5					
Two way ANCOVA – T-AMB and T7					
	SS	DF	MS	F	p
Intercept	8.566	1	8.566	201.550	<0.001
GM Weight	0.264	1	0.264	6.211	0.027
Treatment	0.348	1	0.348	8.190	0.013
Error	0.552	13	0.042		

Appendix II TABLE LV. Two way ANCOVA (analysis of covariance) between T-AMB and T7 for W1-W5 (11 April – 16 July). Geometric mean (GM) is included as a co-varying factor in the analysis.

SGR and geometric mean weight (GM) W1-W5					
Two way ANCOVA – T-AMB and T7					
	SS	DF	MS	F	p
Intercept	14.718	1	14.718	256.315	<0.001
GM Weight	0.787	1	0.787	13.700	0.003
Treatment	0.304	1	0.304	5.288	0.039
Error	0.746	13	0.057		

Appendix II TABLE LVI. Two way ANCOVA (analysis of covariance) between T7 and T10 for W1-W5 (11 April – 16 July). Geometric mean (GM) is included as a co-varying factor in the analysis.

SGR and geometric mean weight (GM) W1-W5					
Two way ANCOVA – T7 and T10					
	SS	DF	MS	F	p
Intercept	28.315	1	28.315	260.169	<0.001
GM Weight	4.109	1	4.109	38.751	<0.001
Treatment	0.902	1	0.902	8.292	0.013
Error	1.415	13	0.109		

Appendix II TABLE LVII. Two way ANCOVA (analysis of covariance) between T10 and T13 for W1-W5 (11 April – 16 July). Geometric mean (GM) is included as a co-varying factor in the analysis.

SGR and geometric mean weight (GM) W1-W5					
Two way ANCOVA – T10 and T13					
	SS	DF	MS	F	p
Intercept	43.113	1	43.113	254.856	<0.001
GM Weight	8.833	1	8.833	52.216	<0.001
Treatment	0.437	1	0.437	2.583	0.132
Error	2.199	13	0.169		

6. Levene's test for homogeneity of variance

Experiment 1

Appendix II TABLE LVIII. Test results from Levene's test, testing for homogeneity of variances. Performed on observations of all response variables for experiment 1.

Levene's test for homogeneity of variance				
Experiment 1				
Variable	MS Effect	MS Error	F	p
Weight W1	136.39	36.02	3.79	0.005
Weight W2	362.03	145.54	2.49	0.043
Weight W3	547.76	274.71	1.99	0.095
Weight W4	1327.47	470.18	2.82	0.025
Weight W5	3756.52	800.98	4.68	0.001
Length W1	0.98	0.41	2.37	0.052
Length W2	0.71	0.52	1.37	0.244
Length W3	1.28	0.51	2.50	0.043
Length W4	1.32	0.55	2.39	0.050
Length W5	0.84	0.61	1.37	0.242
SGR W1-W2	0.09	0.04	2.38	0.051
SGR W2-W3	0.75	0.09	8.84	<0.001
SGR W3-W4	0.76	0.06	11.80	<0.001
SGR W4-W5	0.10	0.05	2.08	0.084
SGR Overall	0.10	0.02	5.18	<0.001

Experiment 2

Appendix II TABLE LIX. Test results from Levene's test, testing for homogeneity of variances. Performed on observations of all response variables for experiment 2

Levene's test for homogeneity of variance				
Experiment 1				
Variable	MS Effect	MS Error	F	p
Weight W0	12.59	8.35	1.51	0.221
Weight W1	2490.66	48.80	51.03	<0.001
Weight W2	1382.98	85.64	16.15	<0.001

7. Spearman rank correlation

Experiment 1

Appendix II TABLE LX. Results from Spearman rank correlation (R_{sp}) analysis for mass (g) and size ranking of individually tagged juvenile lumpfish between treatment groups. Results are given for size ranking (initial mass W1 and final mass W5) and growth rank in adjacent periods (SGR % $day^{-1}period_n$ and SGR % $day^{-1}period_{n+1}$) together with initial and final growth rank (SGR1 and SGR4) for all treatment groups. Significant rankings are indicated by *.

Spearman rank correlation		
Treatment Group	Comparison factors	r_{sp}
T4	Mass (g) W1 & W5	0.95*
T4	SGR1 & SGR2	0.09
T4	SGR2 & SGR3	0.09
T4	SGR3 & SGR4	0.40*
T4	SGR1 & SGR4	-0.05
T-AMB	Mass (g) W1 & W5	0.93*
T-AMB	SGR1 & SGR2	0.30*
T-AMB	SGR2 & SGR3	0.17
T-AMB	SGR3 & SGR4	0.35*
T-AMB	SGR1 & SGR4	0.25*
T7	Mass (g) W1 & W5	0.87*
T7	SGR1 & SGR2	0.29*
T7	SGR2 & SGR3	0.60*
T7	SGR3 & SGR4	0.46*
T7	SGR1 & SGR4	0.16
T10	Mass (g) W1 & W5	0.71*
T10	SGR1 & SGR2	0.44*
T10	SGR2 & SGR3	0.51*
T10	SGR3 & SGR4	0.16
T10	SGR1 & SGR4	0.20
T13	Mass (g) W1 & W5	0.65*
T13	SGR1 & SGR2	0.47*
T13	SGR2 & SGR3	0.57*
T13	SGR3 & SGR4	0.06
T13	SGR1 & SGR4	-0.00

8. Q_{10} Temperature effect on specific growth rate

Experiment 1

Appendix II, TABLE LXI. Table of calculated values of temperature effect on growth rate, Q_{10}. Values for SGR % day^{-1} are calculated as overall group means for the period W1-W5 (11 April – 16 July).

Q_{10} of specific growth rate	
Comparison factor	Q_{10}
SGR T4 & SGR T-AMB	3.16
SGR T4 & SGR T7	2.25
SGR T4 & SGR T10	1.58
SGR T4 & SGR T13	1.41
SGR T-AMB & SGR T7	1.48
SGR T-AMB & SGR T10	1.20
SGR T-AMB & SGR T13	1.18
SGR T7 & SGR T10	1.09
SGR T7 & SGR T13	1.12
SGR T10 & SGR T13	1.15

9. Kolomgorov-Smirnov tests for normality

Experiment 1

Appendix II TABLE LXII. Test results from Kolmogorov-Smirnov test for normality. The analyzed weight distributions were measured at W1 (11 April), W2 (15 May), W3 (2 June), W4 (26 June) and W5 (16 July).

Treatment	Weight W1			Weight W2			Weight W3			Weight W4			Weight W5		
	N	d	P	N	D	P	N	d	P	N	d	P	N	d	P
T4	69	0.126	>0.20	69	0.293	<0.01	69	0.414	<0.01	69	0.434	<0.01	69	0.482	<0.01
T-AMB	72	0.063	>0.20	72	0.339	<0.01	72	0.405	<0.01	72	0.404	<0.01	72	0.380	<0.01
T7	70	0.119	>0.20	70	0.123	>0.20	70	0.166	<0.05	70	0.148	<0.10	70	0.157	<0.10
T10	69	0.138	<0.15	69	0.197	<0.01	69	0.280	<0.01	69	0.297	<0.01	69	0.296	<0.01
T13	68	0.214	<0.01	68	0.361	<0.01	68	0.472	<0.01	68	0.457	<0.01	68	0.445	<0.01

Appendix II TABLE LXIII. Test results from Kolmgorov-Smirnov test for normality. The analyzed length distributions were measured at W1 (11 April), W2 (15 May), W3 (2 June), W4 (26 June) and W5 (16 July).

Treatment	Length W1			Length W2			Length W3			Length W4			Length W5		
	N	d	P	N	D	P	N	d	P	N	d	P	N	d	P
T4	69	0.115	>0.20	69	0.256	<0.01	69	0.374	<0.01	69	0.421	<0.01	69	0.313	<0.01
T-AMB	72	0.050	>0.20	72	0.243	<0.01	72	0.341	<0.01	72	0.342	<0.01	72	0.419	<0.01
T7	70	0.109	>0.20	70	0.078	>0.20	70	0.090	>0.20	70	0.107	>0.20	70	0.114	>0.20
T10	69	0.114	>0.20	69	0.219	<0.01	69	0.337	<0.01	69	0.376	<0.01	69	0.338	<0.01
T13	68	0.156	<0.10	68	0.331	<0.01	68	0.499	<0.01	68	0.535	<0.01	68	0.471	<0.01

Appendix II TABLE LXIV. Test results from Kolmogorov-Smirnov test for normality. The analyzed distributions of calculated SGR were measured for periods W1-W2 (11 April-15 May), W2-W3 (16 May-2June), W3-W4 (3 June-26 June) and W4-W5 (27 June-16 July).

Treatment	SGR% W1-W2			SGR% W2-W3			SGR% W3-W4			SGR% W4-W5		
	N	d	P	N	d	P	N	d	P	N	d	P
T4		0.662	<0.01		0.568	<0.01		0.359	<0.01		0.416	<0.01
T-AMB		0.639	<0.01		0.361	<0.01		0.267	<0.01		0.284	<0.01
T7		0.316	<0.01		0.118	>0.20		0.295	<0.01		0.085	>0.20
T10		0.520	<0.01		0.322	<0.01		0.102	>0.20		0.086	>0.20
T13		0.698	<0.01		0.405	<0.01		0.119	>0.20		0.124	>0.20

Experiment 2

Appendix II TABLE LXV. Test results from Kolmogorov-Smirnov test for normality. The analyzed distributions of weights were measured on W0 (11 April / 2 June), W1 (15 May / 22 June) and W2 (2 June / 10 July).

Treatment	Weight W0			Weight W1			Weight W2		
	N	d	P	N	d	P	N	d	P
S-AMB	121	0.116	<0.10	121	0.396	<0.01	121	0.522	<0.01
S13	120	0.097	>0.20	120	0.237	<0.01	120	0.374	<0.01

Appendix III - The lumpfish

General lumpfish biology

The appearance of the lumpfish is highly characteristic (FIGURE 1, Appendix III). The head is thick and short with a blunted nose, and a high dorsal crest that covers the first dorsal fin entirely. The body is covered in scale-less skin, with rows of dark tubercles longitudinal of the body (Davenport 1985). The pelvic fins constitute a ventral suction disc, allowing fish to rest on rocky substrate, algae and vegetation. Lumpfish display a sexual dimorphism in size, skin color and blood plasma (Goulet et al. 1986). Mature females range from 35-45 cm and are often bluish gray or green in color, while males are 17-38 cm and display nuptial colors of pink, orange and deep red (Andrijasev 1964; Daborn & Gregory 1983). A decrease in average length of females from west to north due to gradual changes in habitat preference and decreasing water temperature has been observed (Rusyaev 2013). One year old juveniles are brown, dark purple red, brown spotted or red in colour (FIGURE 2, Appendix III), assumed to be a function of background colour and reflecting habitat choice (Moring, 1994). The predatory level of the lumpfish is assumed to be very low, limited to salmonids and sea mammals such as seals (Bigelow & Schroeder 1953; Holst 1993).

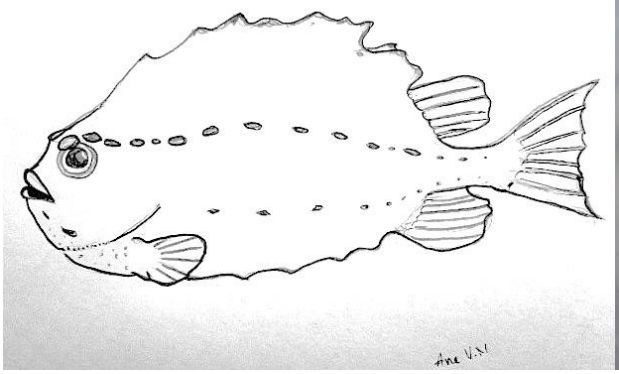

FIGURE 1. General appearance of juvenile lumpfish.

FIGURE 2. Colours of juvenile lumpfish.

Distribution

The species is commonly found in the Arctic margins of the North Atlantic, from Spitsbergen and Nova Zemlya in the north –to Portugal in the south. In west the species has been frequently found along the coasts of America from Cape Cod to Canada to 70° North of Greenland in the north- west (FIGURE 3, Appendix III) (Blacker 1983; Davenport 1985).

Despite the perception of the lumpfish as mainly a cold water fish, warmer waters as far as southern Galicia have been confirmed as important spawning grounds for the species, though spawning takes place somewhat earlier in spring (February-May) (Bañón et al. 2008) in comparison to that of specimens with a geographical distribution of higher latitudes (February - August) (Blacker 1983).

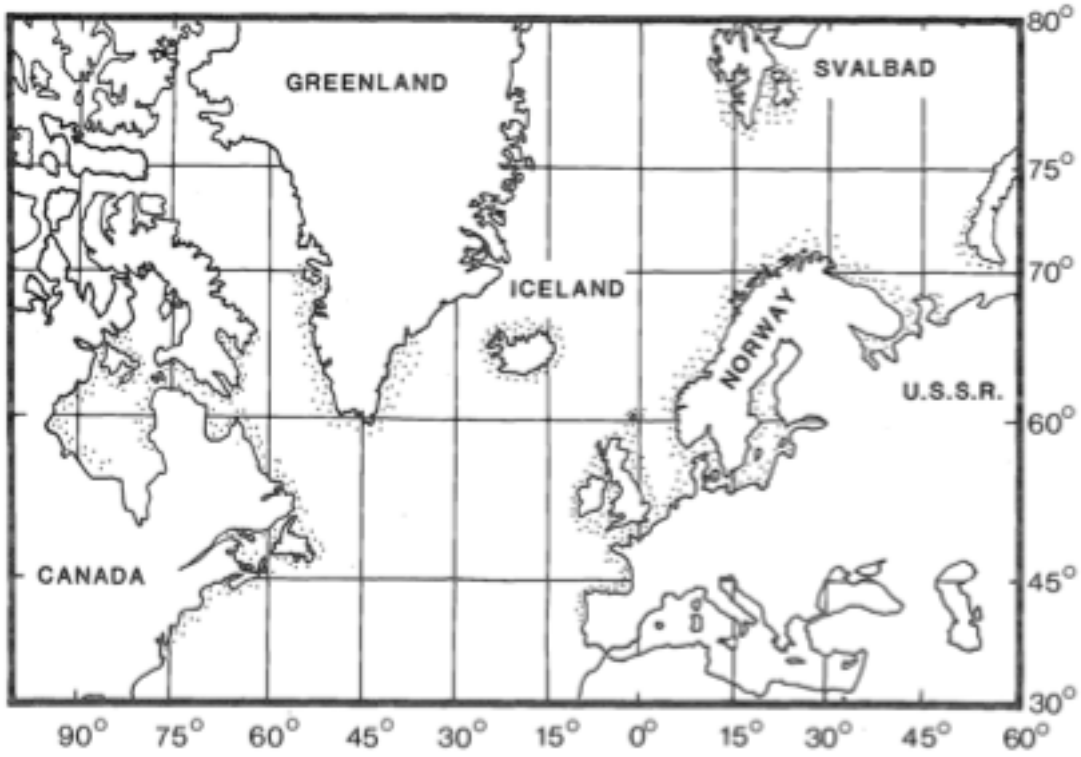

FIGURE 3. Distribution of lumpfish where dotted areas indicate spawning grounds for lumpfish (from Davenport 1985).

Spawning behavior

Spawning often takes place in shallow sub tidal waters when temperatures reach around 4 °C (Collins 1976; Daborn & Gregory 1983) and females spawn 1-3 batches of adhesive eggs

(Andrijasev 1964; Collins 1976; Daborn & Gregory 1983). Males enter fjords prior to females to establish nesting sites, usually depressions in the substrate in the near-shore tidal zone (Goulet et al. 1986). Each female produces approximately 80 000 – 159 000 eggs per spawning season (Andrijasev 1964; Collins 1976; Daborn & Gregory 1983). The sex-ratio of lumpfish catches is imbalanced, Bañón et al. (2008) found a 7 : 1 proportional balance favorable to female lumpfish, which is probably favorable to the spawning season as males are observed to take care of several batches at once (Mitamura et al. 2012). Eggs in nests can be the results of one or several female spawnings, as males can mate with several females, and females likewise with several males. Many batches of eggs can hence be deposited in the nest over time, allowing the male to guard from 10 000 to 200 000 eggs at once (Goulet et al. 1986). The male lumpfish guards the eggs until hatch at 190 – 220 day degrees, blowing and fanning fresh water over the eggs and chases away possible predators (Goulet & Green 1988), such as conger eel (Conger vulgaris) and common wolffish (*Anarhichas lupus*) and sea urchins (*Echinus esculentus*) (Davenport 1985; Goulet et al. 1986). The cunner (*Tautigikabrus adspersus*) often attacks nests in groups which causes the males to give up the fight (Goulet et al. 1986). Other potential predators include the red king crab (*Paralithides camtchaticus*), a new generalist predator in the Barents Sea (Michelsen 2011; Mikkelsen & Pedersen 2012). Temperatures below 5 °C are also observed to cause mortality in eggs (Goulet & Green 1988).

Eggs are approximately 2 mm in diameter and eggs of the same batch are uniform in color. Unripe eggs found in ovaries are rosy-pink, while spawned eggs vary from red, pink, green, purple, blue and yellow (FIGURE 4, Appendix III), gradually losing their color-intensity throughout egg development (Collins 1976; Davenport 1985). Larvae are 4 - 7.4 mm in length at hatch (Andrijasev 1964; Collins 1976; Daborn & Gregory 1983) and are well developed, already possessing the characteristic ventral adhesive disc (Benfey & Methven 1986).

FIGURE 4 .Colours of lumpfish roe

Courtship behavior in terms of nuptial coloration and body movements together with "parental care" such as fanning of eggs and nest cleaning is shown to increase the

reproductive success of males, on behalf of morphological traits such as body size (Goulet et al. 1986), but females are observed to have little or no preference in male body size or nest characteristics (Goulet & Green 1988).

Feeding, habitat preferences and migrations of larval- and juvenile lumpfish

Yolk sacks of larval lumpfish are spent within 10-15 days at 9.5 - 14 °C (Benfey & Methven 1986; Brown 1986) and larvae start feeding within one week of hatch (Benfey & Methven 1986). Both juvenile and larval lumpfish display the ventral suction disc, allowing them to cling to substrate from an early age. Larvae and small juveniles are frequently found in association to or clinging to algae (Daborn & Gregory 1983; Davenport 1985; Moring 1990), especially *Laminaria* (Moring & Moring 1991) or eelgrass (Moring 1989) providing shelter from predators, camouflage and allowing them to forage plankton in the pelagic zone (Moring & Moring 1991; Williams & Brown 1991). Juveniles mostly feed on phytal species with a preference for harpaticoids (genus *Harpacticus*) (Moring 1989).

Juveniles are selective feeders, eluding sessile and slow moving particles and prey (Ingolfsson & Kristjansson 2002). An ontogenetic change in prey size preference with increasing fish size has been observed (Ingolfsson & Kristjansson 2002) and small juveniles are mainly observed feeding while clinging to substrate, increasing swimming behavior with escalating body size (Brown 1986; Jobling et al. 2012).The density of prey organisms is also crucial for lumpfish behavior as juveniles at low prey densities are more active swimming and hunting for prey than that of fellow specimens at high prey densities. At high prey densities juveniles will to a great extent feed while clinging (Jobling et al. 2012).

Juvenile and larval lumpfish reside in the intertidal pool throughout their first summer and autumn, occasionally observed in the upper waters at open sea (Moring 1990), or at deep sea (Daborn & Gregory 1983; Davenport 1985). Tide pools and the inter-tidal zone provide important nurseries area for larval and juvenile lumpfish. Larval and juvenile lumpfish will selectively migrate between tide pools and sub tidal areas between tidal cycles, probably dependent of prey abundances and predators present (Moring & Moring 1991). In Maine, juveniles are found in tide pools between June and December (Moring 1990). With decreasing temperature the abundance of algal refugees decline and tide pools become less attractive habitats for juveniles (Moring & Moring 1991), and at 1-2 years of age the juveniles outgrow

the refugees of the tide pools, and are forced out to sea (Moring 2001).

Feeding, habitat preferences and migrations of adult lumpfish

Lumpfish do not school, but aggregate in spawning periods and occasionally when feeding (Andrijasev 1964; Bañón et al. 2008). They display similarities in migratory behavior to that of coastal benthic teleosts, but are also frequently found in mid-water in open oceans outside the spawning season (Davenport & Kjorsvik 1986). Lumpfish are semi-pelagic and will travel great distances to offshore feeding grounds, before returning to coastal spawning grounds between winter and early spring (Blacker 1983; Davenport 1985; Goulet et al. 1986; Holst 1993; Mitamura et al. 2012). They are often found mid-water as a frequent by-catch at 0-80 meters and occasionally feeding over abyssal waters up to 380 meters (Able & Irion 1985). Mitamura et al. (2012) observed females migrating between neighboring fjords in search of spawning males, before residing for approximately 1-3 weeks to spawn. Lumpfish are active swimmers, often observed swimming against the current when performing migratory movements away from shore (Mitamura et al. 2012), but can also exploit water currents facilitate rapid migrations to feeding grounds for lumpfish (Rusyaev 2013). Minimum speeds of female lumpfish leaving spawning grounds exceed 0.72 km per hour, making lumpfish females vulnerable to passive gears exploited in fisheries (Mitamura et al. 2012).

Temperature factors are crucial to lumpfish, as feeding grounds for the lumpfish spawning on the northern coasts of Norway and Russia are limited by the polar front (Rusyaev 2013). Adult fish mostly feed on macrozooplankton, euphasids, amphipods and isopods (Myrseth 1971; Blacker 1983; Daborn & Gregory 1983) and it is a common belief that lumpfish do not eat during the spawning period and in summer while performing spawning migrations (Andrijasev 1964; Myrseth 1971; Collins 1976; Daborn & Gregory 1983), though this has been a matter of discussion (Moring 1989A) as stomachs containing ctenophores or jellyfish during spawning season has occurred (Able & Irion 1985).

Stock size and fisheries

There is a considerable market for lumpfish roe, approximately 4 million kilos are sold annually. The eggs are dyed to imitate sturgeon caviar, and the product is often smoked, or salted and jarred to increase shelf-life for export (Johannesson 2006). Canada, Greenland, Iceland and Norway are the main exporting countries, but Sweden and Denmark also have minor fisheries for lumpfish roe. The world market for roe was decreasing until 2003 when production levels regained their average level of previous years. The world wide production of salted roe was 31 720 barrels in 2003 (Iceland, Canada, Norway, Denmark and Greenland: 41, 11, 17, 6, 25 % of the annual production respectively, FIGURE 5, Appendix III). The main markets for lumpfish roe are European countries, of which France, Germany, Spain and Italy are the greatest consumers (Johannesson 2006). Fishery is seasonal, with captures in spring when brood stock migrate to shore to spawn (Blacker 1983; Davenport 1985; Sunnanå 2007b). Fishing is to a great extent influenced by the price fluctuations for salted roe, but also environmental factors such as weather and governmental regulations (Johannesson 2006; Sunnanå 2009). Fisheries mainly commence at 5 - 40 meters in exposed coastal areas, but with the development of mid-water trawlers in the 1960's, lumpfish have been a frequent by-catch in mid-water in the upper 50-60 meters (Blacker 1983). They are often observed as a by-catch in the fisheries for horse mackerel (*Trachurus trachurus*), salmon (*Salmo salar*), herring (*Culpea harengus*) and mackerel (*Scomber scombrus*) (Holst 1993).

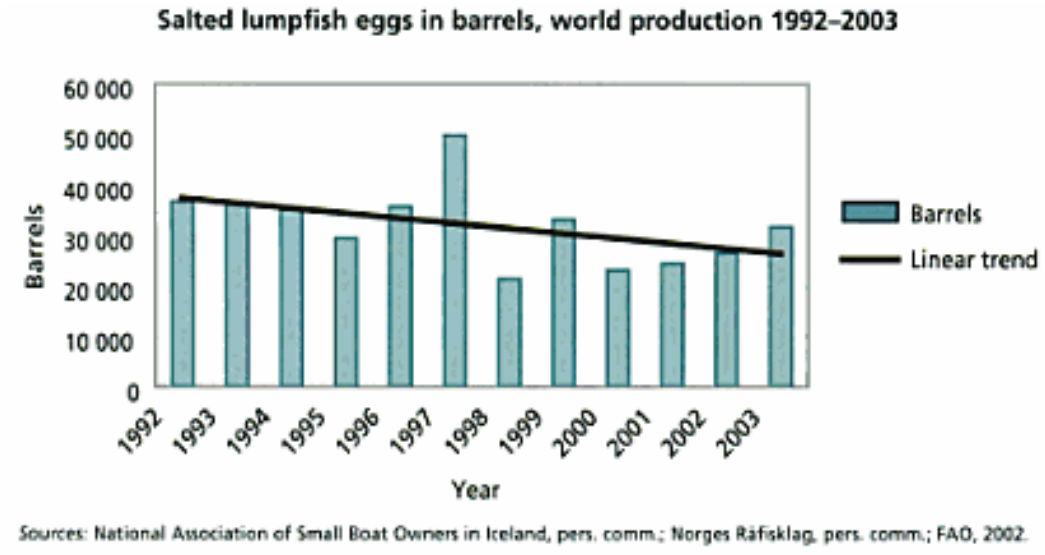

FIGURE 5. Total annual production of salted lumpfish eggs from 1992 to 2003 (Johannesson (2006).

Norwegian stocks have been reduced due to overfishing in the period 1980-1990 (Albert 2000; Sunnanå 2007b), but catches have been increasing in the last decades and the stock seems to be stabilizing (Torstensen 1998; Sunnanå 2007b). In 2009 Norway's institute of marine research suggested restrictions on the fisheries of the species due to low recruitment to the spawning stocks in the past few years, though no specific threats to the stock is known (Sunnanå 2009). East of the North Cape fisheries for lumpfish has been a premise for the assignment of fishery quotas for king crabs. This together with the restrictions of cod fisheries has led to an increase in the number of vessels participating in the annual lumpfish fishery (Sunnanå 2007b) which may be part of the explanation of the poor recruitment. Norway fished a total of approximately 3200 tonnes of lumpfish in 2008 (FIGURE 6, Appendix III), which produces approximately 675 tonnes of roe (Sunnanå 2009).

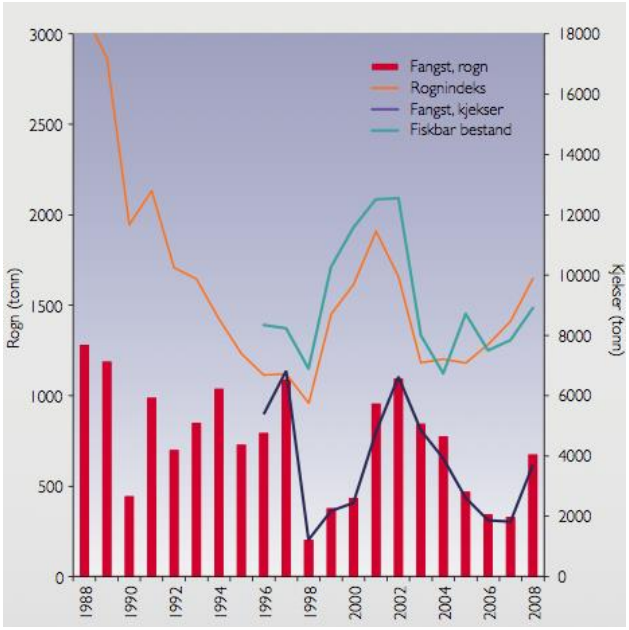

FIGURE 6. Annual landings of lumpfish roe in Norway, described in tonnes (red bars) and calculated content of roe in stock (index of roe in tonnes – orange curve) in the left axis, catch of females in tonnes (dark blue curve) and estimated catchable stock in tonnes (turquoise curve) on the right axis. From Sunnanå (2009).

Appendix IV – Mean weights – Experiment 2

Mean weights ranged from 21.2 (± S.E.) to 97.8 g (± S.E.) at the start of the experiment (W1) to 34.4 (± S.E.) to 176 g (± S.E.) after at experiment termination (W2) after 17 days (FIGURE 1, Appendix IV, TABLE III, Appendix II). A significant difference was found in initial mean weighs between group S-AMB (36.8 g) and S13 (56.7 g, TABLE III, Appendix II) at experiment start-up (W1) (Two-way nested ANVOVA; $p<0.001$, TABLE XXV, Appendix 2), together with a significant heterogeneity in normal distribution between groups (Levene's test; $p<0.001$, TABLE LIX, Appendix II). At experiment termination (W2) replicate group S-AMB had a significantly lower mean weight compared to that of S13 (56.2 vs. 109.3 g, TABLE VII, SNK-test; $p<0.001$, TABLE XLVII, Appendix II).

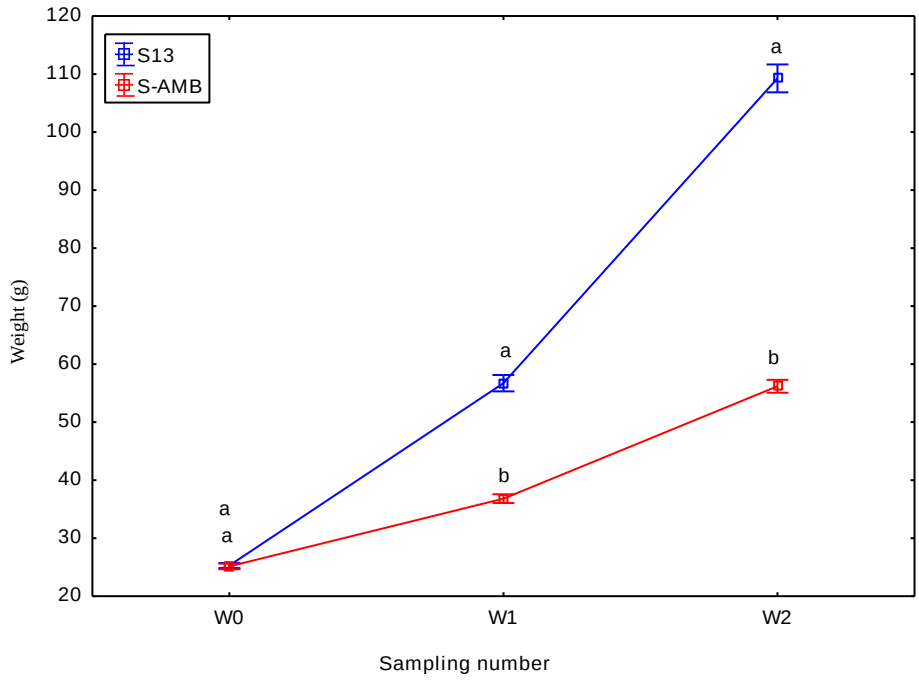

FIGURE 1. Weight development for juvenile lumpfish reared at two different temperatures (n = 241). Vertical whiskers indicate standard error of mean ± S.E. of total weights of two replicates of fish. Different letters indicate statistical differences between groups with "a" as the highest value.