Effects of Photoperiod Manipulation (24L:0D) on Somatic Growth and Endocrine Responses in Juvenile Barramundi (*Lates Calcarifer*) (Bloch)

By Kristen Lee Perks BSC (Zoo.) BAqua (Hons.)

The University of Tasmania,

Australian Maritime College,

National Centre for Marine Conservation and Resource Sustainability,

April, 2013

A thesis submitted in fulfilment of the requirement for the Degree of Doctor of Philosophy

TABLE OF CONTENTS

ABSTRACT

Photoperiod manipulation is a widely used technique in commercial aquaculture to influence endogenous rhythms in fish, which enables industry to control events such as reproductive timing, maturation and growth. The ability to use photoperiod manipulation to influence these events has benefited commercial industry by improving production of fish, thereby generating increased economic benefits. The majority of photoperiod manipulation research has being applied to temperate species, whereas recently these techniques have also been successful in sub-tropical and tropical species. Photoperiod manipulation research on barramundi has demonstrated conflicting results, and clarification is required as to whether using extended day length is effective in enhancing growth of juvenile barramundi. This thesis confirms photoperiod manipulation to be effective at enhancing growth of juvenile barramundi. In the majority of experiments, continuous light (24L:0D) significantly increased wet weight, total length and SGR weight and length of juvenile barramundi when compared to rearing fish under 12L:12D. In addition, growth increases observed in fish reared under 24L:0D occurred without significant increases in feed intake, demonstrating higher feed conversion efficiencies compared to fish reared under 12L:12D.

As the Australian barramundi industry currently only uses photoperiod manipulation to manipulate spawning events, this research enables commercial farmers to use this technique to improve growth as well. To confirm photoperiod manipulation techniques were applicable and effective in commercial conditions, a commercial scale experiment was undertaken, with juvenile barramundi demonstrating significant increases in growth when reared under 24L:0D supplied by artificial lighting.

To achieve maximal growth benefits to barramundi by using photoperiod manipulation, a better understanding of how extended day length improves growth is needed. This thesis further investigated the effects of continuous light in barramundi when reared under a range of water temperatures as well as varied feeding regimes – feeding to satiation, fed a ration of 3% body weight per day as well as increasing feeding frequency by feeding fish during what would normally be the scotophase (dark phase). These experiments demonstrated that growth performance of juvenile barramundi significantly increased with increased water temperature from 20°C to 30°C, irrespective of photoperiod. Importantly for commercial applications, photoperiod manipulation was observed to be ineffective at low water temperature between 20°C - 25°C, only at 29°C and 30°C did photoperiod manipulation become effective. Investigations into photoperiod and feeding regimes suggested a synergistic interaction as 24L:0D did not significantly affect growth when fed a low ration, only when fed to satiation did 24L:0D enhance somatic growth. Additionally, further significant growth increases in barramundi were observed with increased feeding frequency over what would normally be the scotophase when exposed to continuous light, albeit with lowered feed conversion efficiency suggesting fish are being overfed and cannot assimilate feed as efficiently during the "scotophase".

Growth and metabolism in fish is under the influence of many hormonal interactions. Among the many endocrine factors involved, the growth hormone (GH) / insulin-like growth factor (IGF) axis plays a central role as well as the photo-receptive hormone, melatonin. Measuring concentrations of these hormones following exposure to 24L:0D compared to fish reared under "normal" day length periods of 12L:12D, will ascertain if photoperiod is altering growth at an endocrine level. It is proposed in this thesis that

24L:0D affected endocrine parameters involved with photoperiod perception (melatonin) and fish growth (insulin-like growth factor I; IGF-I) which impacts on fish's ability to utilize feed more efficiently, ultimately influencing growth. An observed depressed amplitude of melatonin, during the scotophase in fish exposed to 24L:0D, may directly or indirectly alter fish growth via influencing the growth hormone (GH) / IGF-I axis. In the current study no clear effect of photoperiod on circulating IGF-I levels was observed, although after 56 days, increased growth in fish reared under 24L:0D was concomitant with depressed amplitude of melatonin concentrations during the scotophase as well alterations in circulating peaks of IGF-I peaks at feeding times. This could suggest photoperiod influences growth within a threshold, with increased growth not occurring until production of melatonin is suppressed below a certain point (as achieved in the current study after 56 days). Altogether, this suggests melatonin has indirect influence on the GH/IGF-I growth axis although investigating the IGF axis to its full extent (including GH, IGF-II, IGF binding proteins' and IGF receptors) will enable a better understanding of endocrine mechanisms involved with photoperiod perception and growth in barramundi.

Overall, this thesis has confirmed photoperiod manipulation techniques of extended day length of 24L:0D allow juvenile barramundi to utilize feed more efficiently and thereby increase growth without increasing feed intake. In addition, it is proposed growth increases observed under 24L:0D are due to an involvement of endocrine mechanisms associated with melatonin and IGF-I. This will prove economically beneficial for the Australian barramundi industry as this low-cost and relatively easy to install technique can improve commercial production with minimal outlay to farmers. Ultimately this

knowledge will enable the development of optimal artificial lighting regime to improve farming techniques for barramundi.

DECLARATION

"I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that, to the best of my knowledge and belief, it does not contain any material previously published or written by another person except where due reference is made in the text:

.....

Kristen Lee Perks

April, 2013

AUTHORITY OF ACCESS

This thesis may be made available for loan and limited copying and communication in accordance with the Copyright Act 1968.

.....

Kristen Lee Perks

April, 2013

STATEMENT OF ETHICAL CONDUCT

"The research associated with this thesis abides by the international and Australian codes on human and animal experimentation, the guidelines by the Australian Government's Office of the Gene Technology Regulator and the rulings of the Safety, Ethics and Institutional Biosafety Committees of the University."

.....

Kristen Lee Perks

April, 2013

ABBREVIATIONS

~	approximately
<	less than
>	greater than
Abs	absorbance
ANOVA	analysis of variance
cm	centimetre
cpm	counts per minute
EDTA	ethylenediaminetetra-acetic acid disodium salt
g	acceleration of gravity
g, µg, ng	gram, microgram, nanogram
GH	growth hormone
hrs	hours
IGF	Insulin-like growth factor (IGF-I and IGF-II)
IGFBP	insulin-like growth factor binding protein
IGF-IR	insulin-like growth factor type-I receptor
IgG	immuno-gamma globulin
K	condition factor
L, ml, µl	litre, millilitre, microlitre
min	minutes
n	sample size
ºC	degrees celcius
P	probability
PCR	polymerase chain reaction

RIA	radio-immunoassay
RNA, mRNA, tRNA	ribonucleic acid, messenger RNA, transcription RNA
rpm	revolutions per minute
SD	standard deviation
sec	second
SEM	standard error of the mean
% $bw.d^{-1}$	percent body weight per day
% $lt.d^{-1}$	percent total length per day

THESIS FORMAT

With the exception of Chapter 1 (Introduction) and Chapter 6 (General Discussion) this thesis has been prepared in manuscript form so that completed chapters could be submitted for publication prior to completion of the final thesis. As a result, some information may appear repetitive. All chapters were written by myself, but are co-authored to recognise the significant contribution of my supervisors, Dr Ryan Wilkinson, Professor Chris Carter and Dr Mark Porter.

Chapter 1 aims to provide the reader with a general introduction and overview of the biology and commercial aquaculture of barramundi in Australia as well as photoperiod manipulation techniques used in aquaculture industry and the biological mechanisms involved with photoperiod manipulation.

Chapter 2 investigates the use of photoperiod manipulation techniques over a range of water temperatures. A commercial trial involving juvenile barramundi reared in saltwater race-ways, demonstrated extended day length of 18L:6D did not improve growth of juvenile barramundi during winter temperatures of ~ 19°C. Therefore this experiment aimed to test whether photoperiod influenced growth of barramundi at low temperatures in a controlled experimental setting as well as investigating the efficiency of photoperiod manipulation at a medium and high water temperature. Tested temperatures are reflective of those commonly experienced under commercial conditions. The outcome of this experiment demonstrated 24L:0D only enhanced growth at higher temperatures of 29°C with growth increases observed without increases in feed intake. This led to Chapter 3, which investigated whether growth increases were occurring due to improved

feed utilization by looking at effects of feed ration and frequency when reared under 24L:0D.

Chapter 3 details the involvement of potential synergistic effects of photoperiod and feed intake as well as confirming 24L:0D enhanced growth of juvenile barramundi via an improved utilization of feed rather than increased feed intake. Confirmation of 24L:0D enhancing growth of juvenile barramundi in small scale recirculation systems, led to investigating the transference to commercial conditions in Chapter 4.

Chapter 4 demonstrated photoperiod manipulation techniques used in indoor recirculation systems can be transferred to commercial inland freshwater pond farms. Confidence this technique works in a range of farming settings, leads to optimising photoperiod regimes to best improve growth. This involves gaining a better understanding of biological mechanisms altering growth when fish are reared under 24L:0D. This subsequently led to Chapter 5 which investigated diurnal profiles of the hormone fish use to perceive the ambient photoperiod, melatonin, as well as one of the central hormones involved with fish growth, insulin-like growth factor (IGF-I).

Chapter 6 summarises the results of the previous chapters and the potential areas for future research and application of this knowledge to commercial industry.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

The effects of photoperiod manipulation and artificial illumination on reproductive timing, growth and developmental processes are well documented in a range of temperate aquaculture species (Oppedal et al., 1999; Endal et al., 2000; Hansen et al., 2001). Photoperiod manipulation techniques are used to improve commercial production of fish, generating increased economic benefits to industry. The majority of photoperiod manipulation research has been applied to temperate species, whereas recently these techniques have also been successful in sub-tropical and tropical species (Biswas et al., 2005, 2008 El-sayed and Kawanna, 2007). When the present research commenced, the Australian barramundi (*Lates calcarifer*) (Bloch) industry predominately used photoperiod manipulation to manipulate spawning events, however the commercial application of manipulating photoperiod to improve growth of barramundi was not commonly practiced.

Photoperiod manipulation of fry (2-10 days old) and juvenile (11 – 12 mm total length) barramundi was investigated by Barlow et al., (1993, 1995), observing significant increases in growth of fry but not juveniles when reared under extended photoperiods of 18L:6D and 24L:0D and fed live feeds. Hovette (2005) concurred with this result, observing no significant growth benefits when rearing juvenile barramundi under 18L:6D, however this experiment was carried out in suboptimal water temperatures of around 19°C. The effectiveness of photoperiod manipulation can vary with size, stage and rearing conditions (Falcon et al., 2010). In contrast to the previous findings Worrall et al., (2004), observed significant increases in growth of juvenile barramundi (4 – 12 cm total length) when reared under 24L:0D at 30°C and fed a commercial pelleted diet. As

far as I am aware this is the only research investigating growth effects of extended photoperiod on barramundi under varied rearing conditions. Clearly knowledge remains incomplete regarding the effectiveness of applying photoperiod manipulation techniques, such as 24L:0D, on juvenile barramundi of varying size/stages and rearing conditions. Additionally, gaps in knowledge regarding the mechanisms/pathways involved with enhanced growth under extended day length currently exist. The potential application of photoperiod manipulation techniques on barramundi may have beneficial implications to aquaculture in Australia. The ability to use artificial lighting to increase growth rates of barramundi would benefit industry by reducing time to harvest, which in turn would provide increased financial gains for Australian barramundi farmers.

This chapter will summarise the biology of barramundi and introduce photoperiod with reference to the importance and influence of photoperiod in endogenous rhythms of fish. Additionally, information on how fish perceive and how their endocrine system responds to photoperiod will be provided. Early and current studies into the application and effects of photoperiod manipulation on commercially cultured temperate and tropical species will be discussed and the commercial production of Australian barramundi outlined.

1.2 Barramundi

Barramundi, commonly called Asian sea bass, is a large predatory fish belonging to the family *Latidae* (Otero, 2004) (Figure 1.1). *Lates calcarifer* are distributed in tropical and sub-tropical areas of Western Central Pacific, East Indian Ocean, Japanese Sea and Torres Strait, where average sea surface temperatures range from 23°C to 32°C (FAO,

2012) (Figure 1.2). This euryhaline species moves between fresh and saltwater during its lifecycle (MacKinnon, 1995, Carter et al., 2010). Barramundi are protandrous hermaphrodites, maturing as males after 2-4 years (>80 cm) in fresh water then becoming females after 3-7 years (<100 + cm) in coastal waters (Carter et al., 2010). Barramundi migrates as adults, being a catadromous species, towards saltwater to spawn, which occurs on the incoming tide in the evening and for several days following the new and full moon (Carter et al., 2010). Areas such as mangrove swamps and low lying land that becomes flooded during spring tides and monsoonal rains provide ideal habitats for the growth of juvenile barramundi as the species is highly aggressive and opportunistic carnivore that will take a wide range of aquatic, avian and terrestrial prey, including other barramundi (MacKinnon, 1995; Carter et al., 2010).

Figure 1.1. Juvenile barramundi (~ 60 days old) (Photo taken at PEJO Enterprises, Innisfail).

Figure 1.2. Geographical distribution of *Lates calcarifer* (FAO, 2012).

1.3 Photoperiod and Fish

1.3.1 Photoperiod and Endogenous Circadian Rhythms in Fish

The photoperiod entrainment of fish is highly divergent with species being entrained to signals that are most relevant to their surrounding environment. In this way, different fish species respond differently to the ambient light environment. In response to their photo-environment, fish have evolved varied endogenous circadian rhythms between species, determining different fish behaviours and physiological functions. These functions, such as the timing of maturation and reproduction, growth, locomotor activity and metabolic rates are regulated by daily and annual variations of external environmental cues (Boeuf and Le Bail, 1999; Biswas et al., 2002). Photoperiod, the 24

hour alternation of light and dark cycle, is the most prominent and reliable 'zeitgeber' or cue in generating circadian rhythms, however, others such as temperature, food availability, rainfall or water salinity, may also shape these rhythms (Bromage et al., 2001; Hurd & Cahill, 2002; Falcon et al., 2010). Fish equipped with such time measurement systems, such as circadian rhythms, are able to synchronize upcoming predicted changes in environment conditions.

Diversity in response to photoperiod among fish species is likely to reflect specific adaptations to their environment, where light may vary in terms of intensity, spectral content and duration (Sumpter, 1992; Boeuf and Le Bail, 1999). For example, Bayarri et al., (2002) demonstrated the sub-tropical species, European sea bass was strongly affected by blue wavelengths (434-477nm) and required a minimum light intensity (6.0uW/cm^2) before artificial lighting became effective (by supressing the hormone melatonin). It has been hypothesized that strong endogenous rhythms in tropical species may reflect an adaptation to the steady photic environment they inhabit, as compared to the strong seasonal variations experienced by temperate species (Martinez-Chavez et al., 2008).

1.3.2 Organs and Endocrine Mechanisms involved in Perceiving Photoperiod

A circadian system involves photoperiod being perceived by fish and in turn producing timed rhythmic outputs in the form of endocrine signals. Fish perceive photoperiod using photoreceptive organs, which transduce this information to the specific target centres. The primary organ responsible for perceiving photoperiod in fish is the pineal gland (Figure 1.3). However, the retina of the lateral eyes and deep diencephalic

photoreceptors (DEP) of the brain also play a role (Ekstrom & Meissl, 1997; Boeuf and Falcon, 2001).

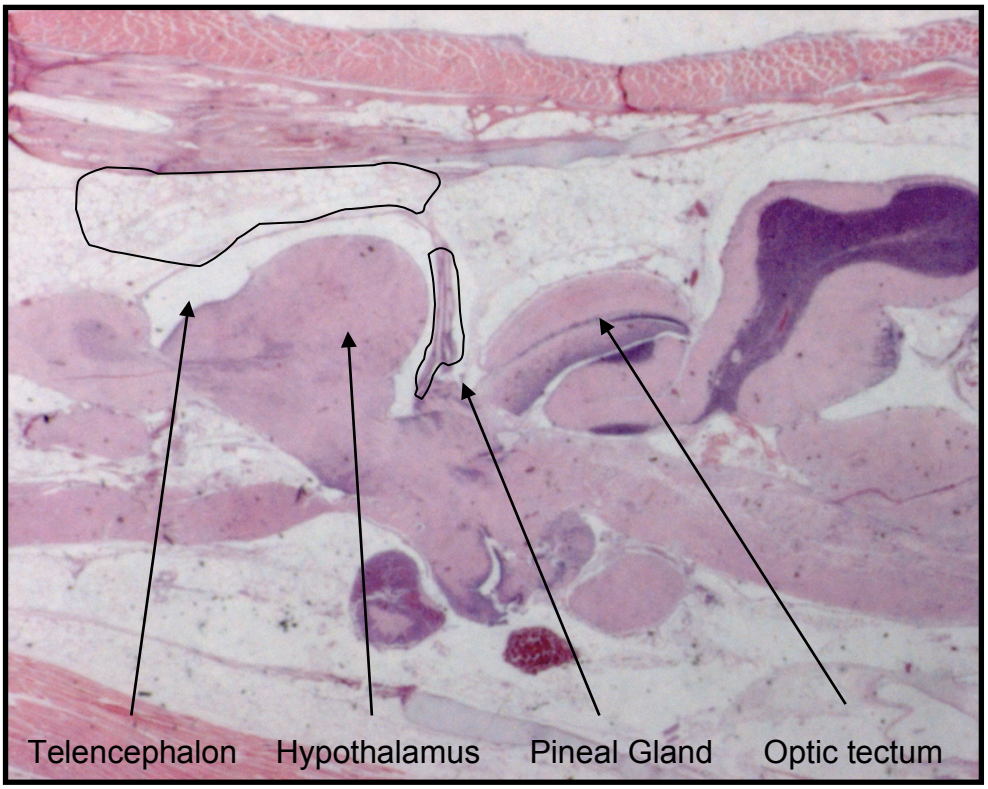


Figure 1.3. Position of the pineal gland within juvenile barramundi brain (Worrall et al., 2004) (x 25, anterior to posterior sagittal section of a juvenile barramundi brain).

Photoreceptors within the pineal and retina relay photoperiod information through neural and hormonal signals to the brain (Ekstrom and Meissl, 1997). These photoreceptor cells synthesize and secrete the hormone melatonin in a light dependent manner (Yanez & Meissl 1996). Within photoreceptor cells, intra-pineal oscillators drive molecular feed-back loops consisting of a repressor and activator which is synchronized to the prevailing 24 hour light dark cycle, therefore the clocks drive the production of rhythmic output signals (Falcon et al., 2010). The main rhythmic output is the hormone melatonin.

Synthesis and release of melatonin is synchronised to the 24 hour light / dark cycle with base levels occurring during the day (photophase) while highest levels occur at night (scotophase) (Porter et al., 2001). For the pineal gland to synthesise melatonin, tryptophan is required: tryptophan is converted to 5-hydroxytryptophane by means of the tryptophane hydroxylase (TPOH);5 – hydroxytryptophane is decarboxylated by the aromatic amino acid decarboxylase to produce serotonin; serotonin is converted to N-acetylserotonin by two enzymes: arylalkylamine N-acetyltransferase (AANAT) and hydroxyindole O-methyltransferase (HIOMT) which methylate N-acetyserotonin to produce melatonin (Klein et al., 1997).

The circadian rhythms of melatonin allow fish to perceive the time of day and season, because changes in day length and intensity are reflected by the duration and amplitude of plasma melatonin rhythms (Randall et al., 1995). In addition to melatonin being a "time keeping" hormone, melatonin rhythms have been found to act on neuroendocrine regulation of physiological processes such as growth and development processes (Falcon et al., 2003; Danilova et al., 2004).

1.3.3 Effects of Photoperiod and Associated Endocrine Pathways Involved with Fish Growth

Fish growth is regulated by various environmental factors, such as photoperiod, temperature, salinity, by biotic factors such as sex and genotype and by nutritional status (Boeuf and Falcon, 2001). Generally, fish follow a seasonal pattern of growth which varies as a function of day-length (Boeuf and Falcon, 2001). In conjunction with this seasonal pattern, changes in food intake, digestion and reproduction are observed, all of

which are related to specific behavioural rhythms which is thought to be controlled by the light perceiving hormone; melatonin (Volkoff et al., 2005).

Melatonin has been suggested to participate in the control of fish growth by controlling specific behavioural rhythms which ultimately affect growth, food intake and digestion, although the direct and/or indirect pathways are currently unknown (Zachmann et al., 1992; Ekstrom and Meissl, 1997; Porter et al., 1998). Research by Falcon et al., 2003 suggests the effects of melatonin on growth may thus result from the differential impact the hormone has on growth hormone (GH), prolactin (PRL) or other pituitary hormones. In addition to a direct effect on the pituitary, melatonin levels may influence the hypothalamus – pituitary axis and/or peripheral tissues involved in energy supply and food intake by altering the fishes perception of season (Boeuf and Falcon, 2001; Falcon et al., 2003; 2010; Vera and Brown, 2009). For example, Rubio et al., (2004) observed orally administered melatonin to affect both the amount of food consumed and the pattern of macronutrients selected in European sea bass (*Dicentrarchus labrax*). Melatonin also altered carbohydrate and protein intake, resulting in increased body fatty acids (Rubio et al., (2004) as well as affecting glucose and body lipid content (Ekstrom and Meissl, 1997).

Melatonin may also indirectly influence fish growth via the growth hormone/insulin-like growth factor – I (GH/IGF-I) axis. GH and IGF-I are a central step in the endocrine pathway involved with fish growth (Peter and Marchant, 1995; Le Bail et al., 1998, Moriyama et al., 2000). As melatonin stimulates production of GH from the anterior

pituitary, GH in turn, is the primary stimulus for the synthesis and release of plasma IGF-I from the liver (Reineke et al., 2010).

IGF-I is a 70 amino acid protein structurally related to proinsulin that acts through endocrine (long distance chemical signalling to target organs), paracrine (signalling to nearby, local cells) and autocrine (signally within the cell) modes to induce growth-related cellular processes like cell proliferation and differentiation that ultimately results in overall somatic and skeletal growth of fish (Duan et al., 1997; Duan, 1998). Endocrine IGF-I is mainly produced by the liver through secretion of GH from the pituitary although IGFs have been found in a number of other tissues and appears to travel in the blood bound to specific binding proteins called insulin-like binding proteins (IGFBP's) (Butler & Roith 2001). These high-affinity binding proteins (IGFBPs) act as carrier proteins that transport IGFs to the target tissues and protect IGFs from proteolytic degradation (Lee and Cohen, 2002). In addition to their roles in the circulation, most target tissues also express IGFBPs, which regulate the local action of IGFs (Rechler, 1993). IGF-I has also been found to respond positively to increases in nutritional quantity or quality (Duan et al., 1995; Perez-Sanchez et al., 1995; Pierce et al., 2001), as well as positive correlations being observed between extended photoperiod, higher plasma IGF-I levels and increased growth (Beckman et al., 1998, 2004; Mingarro et al., 2002; Taylor et al., 2005, 2008)

The detection of IGFs in barramundi is relatively recent, with the liver being identified, as in other fish, the major site of IGF-I mRNA synthesis (Kinhult et al., 1999). Matthews et al., 1997 found different ration sizes fed to juvenile barramundi significantly affected

the condition factor and hepatic IGF-I mRNA expression. In the same study, fasting barramundi had reduced hepatic IGF-I mRNA, suggesting food availability and nutritional status regulate IGF-I production at the mRNA level. Matthews et al., (1997) suggests systemic IGF-I of hepatic origin is the most important for somatic growth in barramundi.

As a result of these previous studies it has been suggested that IGF-I concentrations may serve as a useful biomarker to detect and possibly predict subtle changes in growth or growth status in barramundi (Matthews et al., 1997; Dyer et al., 2004)

1.4 Photoperiod Manipulation in Aquaculture

Many fish rely on light cues to time certain developmental processes during its lifespan. The mechanism that controls this process is endogenous, an entrained endogenous rhythm that varies greatly from one species to another and within the same species – from one developmental stage to another. In this regard, each fish species would respond in a differential manner to photoperiod manipulation.

For example, fish could show paradoxical effects with parallel groups demonstrating different responses to the same daylength when administered to different times of the year. Previously it was suggested that there were threshold or critical daylengths above and below in which fish may not respond to altered daylengths. However it is now evident, as further explained below, that daylengths can be considered long or short providing it is followed by a shorter or longer daylength respectively. It is the direction of change of photoperiod which is all important in the entrainment of the internal clock

and in determining the rate and timing of developmental processes (Bromage et al., 1993). Therefore photoperiodic history is of critical importance in photoperiod manipulation in fish.

The concept of using photoperiod manipulation in aquaculture is based on the aim of suppressing rhythmic melatonin signals, thereby compressing or extending cycles such as spawning, smoltification and maturation to the benefit of commercial industry. Manipulation of these parameters can alter flesh quality, spawning events and/or cause fish to attain market sizes in as short a time period as possible and hence improve farming efficiency (Porter et al., 1999, 2003; Hansen et al., 2001; Handeland and Stefansson, 2001; Taranger et al., 2006). Porter et al. (1999) demonstrated that the effectiveness of photoperiod manipulation could be ascertained by determining melatonin levels in fish as melatonin responds differently to variations in day length and light intensity. Hence, photoperiod manipulation using artificial lighting is now a commonly used tool within the aquaculture industry.

Early studies have predominately involved temperate species such as turbot (*Scophthalmus maximus*) and rainbow trout (*Oncorhynchus mykiss*) (Whitehead et al., 1980; Bromage et al., 1982). Hoover and Hubbard (1937) exposed brook trout (*Salvelinus fontinalis*) to compressed light cycles, finding fish spawned earlier than control fish. Since then, numerous studies have provided unequivocal evidence that day length can alter physiological events in fish.

Early commercial photoperiod manipulation was aimed at spreading the production of eggs over many months. Whitehead and Bromage's (1980) research demonstrated that increasing and decreasing components of the seasonally changing daylength could be replaced by period of constant long and short daylength. In the effect, the amount of light received per day and where it appears in the 24 hour light – dark cycle is all important and not its seasonal rate of change Whitehead and Bromage (1980) spawned rainbow trout 3-4 months earlier than the control group by exposing fish to the light/dark regime of 18 h and 6 h (LD 18:6) during January to May. This was followed by a square wave reduction in the photoperiod regime to LD 6:18. This research was applicable to covered tank systems where lighting parameters can be controlled, whereas it would be virtually impossible to use these techniques in sea or freshwater grow out cage systems which are exposed to ambient light conditions (Bromage et al., 2001). Further information was provided by Randall et al. (1991), who studied fish's response to light in a partially open building with uncovered tanks. This provided intermediate information on the application of artificial lighting being transferred from land locked tank systems to fresh/sea water cage systems.

The efficacy of artificial lighting is also found to vary between species and their stage of development. For example, Hallaraker et al. (1995) and Imsland et al. (1995) both demonstrated growth and metabolic rates of two flatfish, juvenile Atlantic halibut (*Hippoglossus hippoglossus*) and turbot (bottom dwelling species), were not significantly affected by photoperiod. In comparison, Barlow et al. (1995) found the tropical species, barramundi larvae (2-10 days old & 8 – 20 days old) grew progressively faster under conditions of 8, 16 and 24 hours light whereas his study showed that there was no growth

advantages in rearing juvenile barramundi (11-12mm total length) under extended light regimes.

Initially it was thought the length of the light period was of critical importance, whereas it was subsequently noted that in a number of fish photoperiod history was of equal significance. For example, spawning can occur under varied day lengths depending on the previous length of the light period experienced (Bromage et al., 1993). Similarly, Bjornsson et al. (1998) exposed Atlantic halibut to four month advanced and four month delayed annual photoperiod cycles to study sexual maturation. This manipulation significantly altered the timing of spawning, with the advanced group commencing spawning 114 days before the control group and the delayed group commencing 130 after the control group. This demonstrates the response of fish to light stimuli is not dictated solely by their exposure to a specific day length (Bromage et al., 2001). Additionally, the light period may be perceived as a long or short day length depending on the previous photoperiod to which the fish has been exposed (unless exposed to constant light) (Bromage et al., 2001). Randall et al. (1991) demonstrated how a fish perceives a day length of 13L: 5D is dependent on the length of the light/dark cycle preceding it i.e. a light period of greater than 16 hours would mean 13L: 5D was considered a short day. This demonstrates that photoperiodic history and direction of change of day lengths are important and why similar photoperiods can induce different effects if administered at different times of the year (Bromage et al., 2001).

A by-product of researching delayed maturation, had led to additional benefits such as increased growth rates in fish (Boeuf and Falcon, 2001). Kadmon et al. (1985) observed

Gilthead seabream (*Sparus aurata*) exposed to 16L: 8D photoperiod, significantly gained weight instead of the winter spawning weight loss experienced under natural conditions. Similarly, artificial lighting used in sea cage farming of Atlantic salmon (*Salmo salar*) during winter and spring was found to enhance growth and reduce the incidence of sexual maturation (Hansen et al., 1992; Taranger et al. 1995; Oppedal et al. 1997; Porter et al., 1999). Applying artificial lighting to Gilthead sea bream significantly increased growth and food conversion efficiencies, due to a delay in sexual maturation (Gines et al., 2003). Kissil et al., (2001) established long day-lengths postponed gonadal development, which resulted in continued somatic growth in Gilthead sea bream.

As increased day length was found to substantially improve growth the concept and hence introduction of continuous, 24 hour, light developed. El-Sayed and Kawanna (2004) found the optimum weight gain, specific growth rate, feed efficiency and fish survival in Nile tilapia (*Oreochromis niloticus*) fry was achieved at 24L: 0D and 18L: 6D photoperiod regimes. Berg et al. (1992) found juvenile Atlantic salmon held under constant light experienced increased growth compared to 12, 18 and 21 hours light. Similarly, Porter et al. (1999, 2001) used artificial photoperiods to inhibit maturation whilst increasing growth rates in a range of species such as Atlantic salmon and Atlantic cod (*Gadus morhua*).

In addition to duration of light, intensity of light also influences the efficiency of photoperiod manipulation. Hansen et al (1992), demonstrated continuous light altered maturation of sea cage farmed Atlantic salmon, providing the artificial lights were of sufficient brightness. In addition, a combination of light intensity and photoperiod was

further demonstrated to influence responses, such as growth, when applied at specific times (Stefansson et al., 1990; Oppedal et al., 1997; Porter et al., 1999). Trippel and Neil (2003) showed an 11% improvement in body mass of juvenile haddock (*Melanogrammus aeglefinus*) with reduced light intensity from 100 to 30 lux under 24 hours light. Porter et al. (2001) suggests synthesis of the photoreceptive hormone, melatonin responds in a differential manner to variations in light intensity and duration. Varying levels of light intensity has also been found to suppression of melatonin, depending on the fish species. For example, the lowest light intensity to supress melatonin production in vivo in European sea bass was 6.0uW/cm^2 (Bayarri et al., 2002), Senegal sole – 5.3uW/cm^2 (Oliveira et al., 2007) and tench – 3.3uW/cm^2 (Vera et al., 2005). Intensity of light is therefore an important consideration when applying artificial illumination to fish.

1.5 Commercial Production of Barramundi

Barramundi are a fast growing, hardy species that can be cultured using intensive and extensive methods, either indoor and outdoor as well as being cultivated across a full range of salinity (from fresh to salt water), including inland brackish bore water (Carter et al., 2010). The Australian barramundi industry can be categorised into: open freshwater ponds, cage culture in freshwater ponds, land based saltwater raceway/cages, sea cage culture in offshore or estuarine waters and intensive production in indoor facilities (Wingfield, 2002). Pond culture is the most common and currently accounts for the majority of barramundi production in north Queensland (Love & Langenkamp, 2003). As optimal growth for small barramundi occurs around 26 - 36°C (Katersky and Carter, 2005), commercial production at lower latitudes (South East Queensland, New South

Wales, Victoria and South Australia) commonly use intensive farming operations using heated indoor recirculation systems (Love and Langenkamp, 2003).

Barramundi have rapid growth rates, displaying feed conversion ratios (total weight of fish produced per total dry weight of feed consumed) of 1.5:1 to 2:1 in well managed operations (Rimmer, 1995; Schipp et al., 2007; Carter et al., 2010). Like many tropical species, wild barramundi exhibit strong seasonal growth. Generally, growth is maximal at the start of autumn, thereafter slowing down until a reduction and sometimes cessation of growth during the winter period (Xiao, 2000). During spring, barramundi resume positive growth and so the cycle continues (Xiao, 2000). This strong seasonality in the growth rate is related to the seasonal changes in water temperature and has important implications for barramundi aquaculture (MacKinnon, 1995; Rimmer, 1995).

Current Australian practices and technology have been adapted and improved from the culture of barramundi in Thailand, which began in the 1970's (Wongsomnuk and Manevonk, 1973). The culture of barramundi typically goes through several phases; spawning, hatchery, weaning and grow out phases (Schipp et al., 2007). Farming techniques still rely mainly on broodstock being collected from the wild, however a small proportion are maintained in captivity and spawn naturally or are induced to ovulate with hormone treatment (Schipp et al., 2007; Carter et al., 2010). Hatcheries use indoor recirculation tank systems which allow easy application of artificial lighting, enabling hatcheries to spawn barramundi all year round with controlled lighting and temperature (Schipp et al., 2007). Larval barramundi require live feeds and are reared on rotifers and brine shrimp before being weaned onto artificial diets. Cannibalism is common during

this fry and juvenile periods, causing massive losses under commercial conditions unless they are size graded every fourteen days (Rimmer, 1995; Schipp et al., 2007).

The Australia domestic market demands either a small plate sized fish of 400 – 500g or larger fish (1 – 3 kg) being used for fillets or banquet style. Currently farmers are opting to hold fish over the second summer thereby increasing size to 2-3 kg, which is suitable for the fillet market (ABFA, 2006). This provides an alternative product for consumers, having access to easily packaged fish, without bones, which requires minimal preparation and effort to eat. To produce a plate size fish typically take 6-9 months, a banquet fish takes 8-12 months to grow and a 3kg fillet size fish takes 18-24 months. All production time periods are heavily dependent on environmental and management influences such as water temperature, feed type, feeding management and fish health (Carter et al., 2010).

Australian aquaculture production of barramundi increased from 898 mt (2000-01) to 3361 mt in 2007- 08, an 80% increase in production (ABARE, 2008). Similar to its rapid expansion, barramundi aquaculture farming techniques and research into nutritional and physiological knowledge of barramundi is rapidly developing which is important in continually improving commercial production of Australian barramundi.

1.5.1 Potential Applications of Photoperiod Manipulation in Australian Barramundi Aquaculture

The ability to use photoperiod manipulation techniques to enhance barramundi growth has the potential to benefit industry by reducing time taken to reach market size and increase the number of possible harvests throughout the year. This could be

accomplished during two phases; during the fish's seasonal period of reduced growth (throughout winter) or while fish are experiencing periods of growth. Small increases in juvenile growth, during either the winter phase or periods of seasonal growth, may significantly alter long term growth trajectories (Jobling, 2010).

Additionally, manipulating growth rates in barramundi may enable farmers to manipulate harvest times to correspond when markets may show higher demand with increased wholesale prices. For example wild barramundi are caught from February until November with fisheries closing around October through to January, possibly giving rise to increased market prices due to lowered supply of wild fisheries.

At present, photoperiod manipulation is used in barramundi aquaculture to manipulate spawning but it is not known whether this technique can influence somatic growth in barramundi. It is suggested that photoperiod manipulation is not being used to its full potential, despite the fact numerous studies have demonstrated increased growth rates using artificial illumination (Oppedal et al., 1997; Endal et al., 2000; Hansen et al., 2001). Greater understanding of endocrine responses to light regimes potentially would provide a useful tool in quantifying barramundi's response to artificial illumination.

1.6 General Aims and Objectives

The ability to effectively use photoperiod manipulation on temperate species and more recently, tropical species, allows the potential of this technique to be introduced to barramundi. Currently in commercial barramundi aquaculture, photoperiod manipulation is only used to compress spawning cycles, allowing for increased and on-demand

spawning periods. Strong seasonality in growth of barramundi indicates an area where the application of artificial lighting may be commercially economically viable by increasing somatic growth during a period of minimal or no growth. A gap in knowledge regarding photoperiod manipulation on barramundi remains, with the only available research contradicting whether extended photoperiod enhances growth of juvenile barramundi.

The aims of this PhD research were to investigate the effects of photoperiod manipulation on the growth of juvenile barramundi when reared under varied situations, including on-farm. More specifically;

- To determine whether continuous light (24L:0D) significantly increases somatic growth of juvenile barramundi by comparing growth and endocrine parameters related to photoperiod and growth of fish when reared under 12L:12D and 24L:0D. (Chapters 2, 3, 4 and 5).

- To investigate the effectiveness of photoperiod manipulation methods of continuous light (24L:0D) when transferred from small scale indoor re-circulation systems to a commercial scale inland freshwater pond scenario (Chapter 4).

- To gain a better understanding how continuous light (24L:0D) influences somatic growth of juvenile barramundi at different temperatures by comparing growth and endocrine parameters of fish reared under 12L:12D and 24L:0D while being held at a low (20°C), medium (25°C) and high (30°C) water temperature (Chapter 2).

- To determine mechanisms for increased growth in juvenile barramundi reared under 24L:0D by feeding fish reared under 12L:12D and 24L:0D a set ration and to satiation, ascertaining if increased growth is due to increased feed intake or rather improved feed utilization (Chapter 3).

- To ascertain whether barramundi will continue to feed over a 24 hour period when reared under continuous light, determining any growth advantages conferred by synergistic effects of photoperiod and feeding regimes (Chapter 3).

- To ascertain endocrine responses involved with perception of photoperiod and growth stimulation in fish by investigating and comparing diurnal profiles of melatonin and IGF-I over time, in fish reared under 12L:12D and 24L:0D (Chapter 5).

This research will contribute towards understanding the effects on photoperiod manipulation in barramundi and tropical fish. This will equip the Australian barramundi industry, as well as other tropical finfish aquaculture industries, with knowledge regarding any beneficial application in using photoperiod manipulation to enhance somatic growth. Further, this research provides a better understanding how a tropical species such as barramundi perceives photoperiod and subsequent effects of photoperiod manipulation under varied rearing conditions commonly experienced/manipulated in commercial situations. Artificial lighting is a potential method to increase fish growth, facilitating economic gains to commercial aquaculture, depending on cost/benefit analyses. Overall this research aimed to improve farming practices associated with

temperate and tropical finfish aquaculture, assisting aquaculture to develop into a better managed sustainable resource.

1.7 REFERENCES

ABARE, 2008. Australian Bureau of Agricultural & Resource Economics Management. 2008, Australian Fisheries Statistics, Aquaculture, [Online], Available: http://www.abare.gov.au. [11 June 2010]

ABFA, Australian Barramundi Farming Association. 2001, Situation Analysis of Farmed Barramundi Marketing, [Online], Available: http://www.abfa.com.au [11 June 2010].

Barlow, C.G., Rodgers, L.J., Palmer, P.J., Longhurst, C.J., 1993. Feeding-Habits of Hatchery-Reared Barramundi *Lates-Calcarifer* (Bloch) Fry. Aquaculture 109, 131-144

Barlow, C.G., Pearce, M.G., Rodgers, L.J., Clayton, P., 1995. Effects of photoperiod on growth, survival and feeding periodicity of larval and juvenile barramundi *Lates calcarifer* (Bloch). Aquaculture 138, 159-168.

Beckman, B. R. and W. W. Dickhoff, 1998. Plasticity of smolting in spring chinook salmon: relation to growth and insulin-like growth factor-I. Journal of Fish Biology 53: 808-826.

Beckman, B. R., M. Shimizu, 2004. The effect of temperature change on the relations among plasma IGF-1, 41-kDa IGFBP, and growth rate in postsmolt coho salmon. Aquaculture 241(1-4): 601-619.

Berg, A., Hansen, T., Stefansson, S., 1992. First feeding of Atlantic salmon (*Salmo salar L.*) under different photoperiods. Journal of Applied Ichthyology 8, 251-256.

Biswas, A.K., Endo, M., Takeuchi, T., 2002. Effect of different photoperiod cycles on metabolic rate and energy loss of both fed and unfed young tilapia *Oreochromis niloticus*: Part I. Fisheries Science 68, 465-477.

Biswas, A. K., Seoka, M., Inove, Y., Takii, K., Kumai, H. 2005. Photoperiod influences the growth, food intake, feed efficiency and digestibility of red sea bream (*Pagrus major*) Aquaculture 250, 666-673.

Biswas, A. K., Seoka, M., Ueno, K., Takii, K., Kumai, H. 2008. Stimulation of growth performance without causing stress response in young red sea bream, *Pagrus major*, (Temminck and Schlegel), by photoperiod. Aquaculture Research 39, 457- 463.

Bjornsson, B.T., Stefansson, G.V., Berge, A.I., Hansen, T., Stefansson, S.O. 1998. Circulating growth hormone levels in Atlantic salmon smolts following seawater transfer: effects of photoperiod regime, salinity, duration of exposure and season. Aquaculture 168 (1-4), 121 - 137.

Boeuf, G., Le Bail, P.Y., 1999. Does light have an influence on fish growth? Aquaculture 177, 129-152.

Boeuf, G., Falcon, J., 2001. Photoperiod and Growth in Fish. Vie Milieu 51, 247-266.

Bromage, N.R., Whitehead, C., Breton, B. 1982. Relationships between serum levels of gonadotropin, oestradiol - 17B and vitellogenin in the control of ovarian development in the rainbow trout. II. Effects of alterations in environmental photoperiod. General Comparative Endocrinology 47, 366 - 376

Bromage, N.R., Randall, C.R., Thrush, M., Duston, J. 1993. The control of spawning in salmonids. In: Roberts, R.J., Muir, J. (Eds.), recent Advances in Aquaculture, vol. 4, Blackwell, Oxford, pp. 55-65.

Bromage, N., Porter, M., Randall, C., 2001. The environmental regulation of maturation in farmed finfish with special reference to the role of photoperiod and melatonin. Aquaculture 197, 63-98.

Butler, A. A. and Roith, D. L., 2001. Control of Growth by the Somatropic Axis: Growth Hormone and the Insulin-Like Growth Factors Have Related and Independent Roles. Annual Review of Physiology 63(1): 141-164.

Carter, C., Glencross, B., Katersky, R.S., Bermudes, M. 2010. The Snooks (Family: Centropomidae) In: Finfish Aquaculture: Species Selection for Diversification. (Ed Le Francois, N.R., Jobling, M., Carter, C., Bljer, P.U) CAB International, Walingford, UK. Chapter 14 pp 323 - 336

Danilova, N., Krupnik, V. E., Sugden, D., Zhadanova, I.V. 2004. Melatonin stimulates cell proliferation in zebrafish embryo and accelerates its development. Faseb Journal 18 (2), 75.

Duan, C. 1997 The Insulin-like Growth Factor System and Its Biological Actions in Fish. American Zoology 37, 491-503.

Duan, C., 1998. Nutritional and Developmental regulation of insulin-like growth factors in fish. American Society for Nutritional Sciences 128: 306S-314S.

Dyer, A.R., Barlow, C.G, Bransden, M.P., Carter, C.G., Glencross, B.D., Richardson, N., Thomas, P.M., Williams, K.C., Carragher, J.F. 2004. Correlation of plasma IGF-I concentrations and growth rate in aquacultured finfish: a tool for assessing the potential of new diets. Aquaculture 236, 583-592.

Ekstrom, P., Meissl, H., 1997. The pineal organ of teleost fishes. Reviews in Fish Biology and Fisheries 7, 199-284.

El-Sayed, A.M., Kawanna, M., 2004. Effects of photoperiod on the performance of farmed Nile tilapia *Oreochromis niloticus*: I. Growth, feed utilization efficiency and survival of fry and fingerlings. Aquaculture 231, 393-402.

Endal, H.P., Taranger, G.L., Stefansson, S.O., Hansen, T., 2000. Effects of continuous additional light on growth and sexual maturity in Atlantic salmon, *Salmo salar*, reared in sea cages. Aquaculture 191, 337-349.

Falcon, J., Besseau, L., Fazzari, D., Attia, J., Gaildrat, P., Beauchaud, M., Boeuf, G. 2003. Melatonin modulates secretion of growth hormone and prolactin by trout pituitary glands and cells in culture. Endocrinology 144 (10), 4648-4658.

Falcon, J., Migaud, H., Munoz-Cueto, J.A., Carrillo, M. 2010. Current knowledge on the melatonin system in teleosts fish. General and Comparative Endocrinology 165, 469-482.

Fisheries and Agricultural Organisation of the United Nations, Fisheries and Aquaculture Department, Species Fact Sheet, *Lates calcarifer*. [Online] Available http://www.fao.org/fishery/species/3068/en, 4 July 2012.

Gines, R., Arguello, A., Zamorano M.J., Lopez J.L. (2003). Growth in adult gilthead sea bream (*Sparus aurata L*) as a result of interference in sexual maturation by different photoperiod regimes. Aquaculture Research 34, 73-83.

Hallaraker, H., Folkvord, A., Stefansson, S.O. 1995. Growth of juvenile halibut (*hippoglossus hippoglossus*) related to temperature, day length and feeding regime. Netherlands Journal of Sea Research 34 (1-3), 139 - 147.

Handelland, S.O., Stefansson,S.O. 2001. Photoperiod control and influence of body size on off-season parr-smolt transformation and post-smolt growth. Aquaculture 192, 291-307.

Hansen, T., Stefansson, S., Taranger, G.L., 1992. Growth and sexual maturation in Atlantic salmon, *Salmo salar* L., reared in sea cages at two different light regimes. Aquaculture Fish Management 23, 275-280.

Hansen, T., Karlsen, O., Taranger, G.L., Hemre, G.I., Holm, J.C., Kjesbu, O.S., 2001. Growth, gonadal development and spawning time of Atlantic cod (*Gadus morhua*) reared under different photoperiods. Aquaculture 203, 51-67.

Hoover, E.E.and Hubbard, H.E., 1937. Modification of the sexual cycle in trout by control of light. Copea 4.

Hovette, E., 2005. Influence of photoperiod and light intensity on growth and survival of *Lates calcarifer*. Honours Thesis, Good Fortune Bay, Queensland.

Hurd, M.W. and Cahill, G.M. 2002. Entraining signals initiate behavioural circadian rhythmicity in larval zebrafish. Journal of Biological Rhythms, 17 307-314.

Imsland, A.K., Folkvord A., Stefansson S.O. 1995. Growth, oxygen consumption and activity of juvenile turbot (*Scophthalmus maximus L*) reared under different temperatures and photoperiods. Netherlands Journal of Sea Research 34 (1-3), 149 - 159.

Jobling, M. 2010. Are compensatory growth and catch-up growth two sides of the same coin? Aquaculture International 18, 501-510.

Kadmon, G., Gordin, H., Yaron, Z., 1985. Breeding-related growth of captive *Sparus aurata* (*Teleostei, Perciformes*). Aquaculture 46, 299-305.

Katersky, R.S. & Carter, C.G. 2005. Growth efficiency of juvenile barramundi, *Lates calcarifer*, at high temperatures. Aquaculture 250, 775-780.

Kinhult-Stahlbom, A., Sara, V.R., Hoeben, P. 1999. Insulin-like grwoth factor mRNA in barramundi (*Lates calcarifer*): alternative splicing and non-responsiveness to growth hormone. Biochemical Genetics 37, 69 - 93.

Kissil, G.W., Lupatsch I., Elizur A., Zohar Y., 2001. Long photoperiod delayed spawning and increased somatic growth in gilthead seabream (*Sparus aurata*). Aquaculture 200, 363-379.

Klein, D.C., Coon, S.L., Roseboom, P.H., Weller, J.L., Bernard, M., Gastel, J.A., Zatz, M., Iuvone, P.M., Rodriguez, I.R., Begay, V., Falcon, J., Cahill, G.M., Cassone, V.M., Baler, R., 1997. The melatonin rhythm-generating enzyme: Molecular regulation of serotonin N-acetyltransferase in the pineal gland, Recent Progress in Hormone Research, Proceedings of the 1996 Conference, Vol 52, pp. 307-358.

Lee, K.W, Cohen, P. 2002. Nuclear effects: unexpected intra cellular actions of insulin like growth factor binding protein - 3. Endocrinology 175, 33-40.

Le Bail, P.Y., Gentil, V., Noel, O., Gomez, J.M., Carre, F., Le Goff, P., Weil, C. 1998. Trends in comparative endocrinology and neurobiology - From molecular to integrative biology. Annuals of the New York Academy of Sciences 839, 157 - 161.

Love, G., Langenkamp, D., 2003. Australian aquaculture industry profile for selected species. ABARE Report 03.8. Australian Bureau of Agricultural & Resource Economics Management. [Online], Available: http://www.abare.gov.au. [11 June 2010]

Martinez-Chavez, C.C., Al-Khamees, S., Campos-Mendoza,A., Penman, D.J., Migaud, H. 2008. Clock controlled endogenous melatonin rhythms in Nile tilapia (*Oreochromis niloticus niloticus*) and African catfish (*Clarias gariepinus*). Chronobiological International 25, 31-49.

Matthews, S. J., Kinhult, A. K. K., Hoeben, P., Sara, V.R., Anderson, T.A. 1997. Nutritional regulation of insulin-like growth factor-I mRNA expression in barramundi, *Lates calcarifer*. Journal of Molecular Endocrinology 18, 273-276.

MacKinnon, M.R., 1995. Barramundi breeding and culture in Thailand: held at Songkhla, Thailand 1 to 21 June 1982., Dept. of Primary Industries, Queensland.

Mingarro, M., S. V. R. de Celis, 2002. Endocrine mediators of seasonal growth in Gilthead Sea bream (*Sparus aurata*): the growth hormone and somatolactin paradigm. General and Comparative Endocrinology 128(2): 102-111.

Moriyama, S., Ayson, F.G., Kawauchi, H., 2000. Growth regulation by insulin-like growth factor I in fish. Bioscience, Biotechnology and Biochemistry. 64 (8): 1553-1562

Oppedal, F., Taranger, G.L., Juell, J.E., Fosseidengen, E., Hansen, T., 1997. Light intensity affects growth and sexual maturation of Atlantic salmon (*Salmo salar*) postsmolts in sea cages. Aquatic Living Resources 10, 351-357.

Oppedal, F., Taranger, G.L., Juell, J.E., Hansen, T., 1999. Growth, osmoregulation and sexual maturation of underyearling Atlantic salmon smolt *Salmo salar* L. exposed to different intensities of continuous light in sea cages. Aquaculture Research 30, 491-499.

Otero, O. 2004. Anatomy, systematic and phylogeny of both recent and fossil latid fishes (*Teleostei, Perciformes, Latidae*). Zoological Journal of the Linnean Society 141, 81 – 133.

Perez-Sanchez, J., Marti-Palanca, H., Kaushik, S.J. 1995. Ration size and protein intake affect circulating growth hormone concentrations, hepatic growth hormone binding and plasma insulin-like growth factor-I immunoreactivity in a marine teleost, the gilthead sea bream (*Sparus aurata*). Journal of Nutrition 125, 546-552.

Peter, R.E., and Marchant, T.A. 1995. The endocrinology of growth in carp and related species. Aquaculture 129, 299-321.

Pierce, A.L., Beckman, B.R., Shearer, K.D., Larsen, D.A., Dickhoff, W.W. 2001. Effects of ration on somatotrophic hormones and growth in coho salmon. Comparative Biochemistry and Physiology B 128, 255-264.

Porter, M.J.R., Randall, C.F., Bromage, N.R., Thorpe, J.E. 1998. The role of melatonin and the pineal gland on development and smoltification of Atlantic salmon (*Salmo salar*) parr. Aquaculture 168 (1-4), 139 - 155.

Porter, M.J.R., Duncan, N.J., Mitchell, D., Bromage, N.R., 1999. The use of cage lighting to reduce plasma melatonin in Atlantic salmon (*Salmo salar*) and its effects on the inhibition of grilsing. Aquaculture 176, 237-244.

Porter, M.J.R., Duncan, N., Handeland, S.O., Stefansson, S.O., Bromage, N.R., 2001. Temperature, light intensity and plasma melatonin levels in juvenile Atlantic salmon. Journal of Fish Biology 58, 431-438.

Porter, M.J.R, Woolcott, H.M, Pankhurst, N.W. 2003. The use of additional lighting and artificial photoperiods to recondition early maturing Atlantic salmon (*Salmo salar*) in Tasmania. Fish Physiology and Biochemistry 28, 391-393

Randall, C., Bromage, N., Symes, J. 1991. Short periods of continuous light can both advance and delay spawning in the rainbow trout. In: Scott, A.P., Sumpter, J., Kime, D., Roffe, M. (Eds.), Proceedings of IV International Symposium on Reproductive Physiology of Fish. Fish Symp. 91, p. 171.

Randall, C.F., Bromage, N.R., Thorpe, J.E., Miles, M.S., Muir, J.S., 1995. Melatonin Rhythms in Atlantic Salmon (*Salmo-Salar*) Maintained under Natural and out-of-Phase Photoperiods. General and Comparative Endocrinology 98, 73-86.

Rimmer, M.A. 1995. Barramundi Farming - An Introduction. Department of Primary Industries, Queensland.

Reinecke, M. 2010. Influences of the environment on the endocrine and paracrine fish growth hormone – insulin-like growth factor-I system. Journal of Fish Biology 76, 1233 – 1254.

Rubio, V.C., Sanchez-Vazquez, J., Madrid, J.A. 2004. Oral administration of melatonin reduces food intake and modified macronutrients selection in European sea bass (*Dicentrarchus labrax L.*). Journal of Pineal Research 37, 42-47.

Schipp, G., Bosmans, J., Humphre, J. 2007. Barramundi Farming Handbook. Northern Territory Government, Department of Primary Industry, Fisheries and Mines. 3rd Edn.

Stefansson, S.O., Hansen, T., Taranger, G.L., 1990. The influence of light on growth and smolting of Atlantic salmon (*Salmo salar*): effects of spectral composition, intensity and

photoperiod. Proceedings of Canada-Norway finfish aquaculture workshop, September 11 - 14, 1989, 79-84.

Sumpter, J.P., 1992. Control of Growth of Rainbow-Trout (*Oncorhynchus-Mykiss*). Aquaculture 100, 299-320.

Taranger, G.L., Daae, H., Joegensen, K.O., Hansen, T., 1995. Effects of continuous light on growth and sexual maturation in sea water reared Atlantic salmon. In: Goetz, F., Thomas, P. (Eds), Reproductive Physiology of Fish V symposium. University of Texas Publications, Austin, USA. p 200.

Taranger, G.L., Aardal, L., Hansen, T., Kjesbu, O.S. 2006. Continuous light delays sexual maturation and increases growth of Atlantic cod (*Gadus morhua L.*) in sea cages. ICES Journal of Marine Science - Gadoid mariculture: development and future challenges 63 (2), 365-375.

Taylor, J.F., Migaud, H., Porter, M.J.R., Bromage, N.R. 2005. Photoperiod influences growth rate and plasma insulin-like growth factor-I levels in juvenile rainbow trout, *Oncorhynchus mykiss*. General and Comparative Endocrinology 142, 169 – 185.

Taylor, J.F., Porter, M.J.R., Bromage, N.R., Migaud, H. 2008. Relationships between environmental changes, maturity, growth rate and plasma insulin-like growth factor-I (IGF-I) in female rainbow trout. General and Comparative Endocrinology 155 (2) 257 - 270.

Trippel, E.A., Neil, S.R.E. 2003. Effects of photoperiod and light intensity on growth and activity of juvenile haddock (*Melanogrammus aeglefinus*). Aquaculture 217, 633-645.

Rechler, M.M. 1993. Insulin-like growth factor binding proteins. Vitamins and Hormones 47, 1 - 114.

Vera, E.M. and Brown, C.L. 2009. Influence of the photoperiod on growth rate and insulin-like growth factor-I gene expression in Nile tilapia *Oreochromis niloticus*. Journal of Fish Biology 75, 130-141.

Volkoff, H., Canosa, L.F., Unniappan, S., Cerda-Reverter, J.M., Bernier, N.J., Kelly, S.P., Peter, R.E. 2005. Neuropeptides and the control of food intake in fish. General and Comparative Endocrinology 142, 3-19

Wingfield, M., 2002. The Australian barramundi industry, in Australian Aquaculture Yearbook 2002, National Aquaculture Council, Executive Media, Melbourne, p. 29.

Wongsomnuk, S. and Manevonk, S., 1973. Results of experiments on artificial breeding and larval rearing of the sea-bass *Lates calcarifer* (Bloch), Contributions of Songkhla Marine Fisheries Station, 5.

Worrall, K., Carter, C., Porter, M.J.R. 2004. Effects of Photoperiod on the Growth of Juvenile Barramundi (*Lates calcarifer*). Hons Thesis, University of Tasmania.

Whitehead, C., and Bromage, N.R. 1980. Effects of constant long and short day

photoperiods on the reproductive physiology and spawning of the rainbow trout. Journal of Endocrinology 87, 6-7.

Xiao, Y.S., 2000. Use of the original von Bertalanffy growth model to describe the growth of barramundi, *Lates calcarifer* (Bloch). Fishery Bulletin 98, 835-841.

Yanez, J., Meissl, H. 1996. Secretion of the methoxyindoles melatonin, 5-methoxytryptophol, 5-methoxyindoleactic acid, and 5-methoxytryptamine from trout pineal organs in superfusion culture: Effects of light intensity. General and Comparative Endocrinology 101 (2), 165-172.

Zachmann, A., Knijff, S.C.M, Ali, M.A, Anctil, M., 1992. Effects of photoperiod and different intensities of light exposure on melatonin levels in the blood, pineal organ, and retina of the brook trout (*Salvelinus fontinalis Mitchell*). Canadian Journal of Zoology 70 (1), 25 – 29.

CHAPTER 2

Effects of Photoperiod and Temperature on Growth of Juvenile Barramundi (*Lates calcarifer*) (Bloch)

K. L. Worrall[a], C. G. Carter[b], M. J.R. Porter[c], R. J. Wilkinson[a]

[a] National Centre for Marine Conservation and Resource Sustainability, University of Tasmania, Locked Bay 1370, Launceston, TAS, 7250, Australia

[b] Tasmanian Aquaculture and Fisheries Institute, University of Tasmania, Private Bag 49, Hobart, TAS 7001, Australia

[c] Ridley Aquafeed Pty Ltd, 12 Neon Street, Narangba Brisbane, QLD, 4504, Australia

Corresponding author. Tel.: +61 3 6324 3800; fax: +61 3 6324 3804

E-mail address: kworrall@utas.edu.au

Keywords: Asian sea bass; Barramundi; Growth; *Lates calcarifer*; Photoperiod; Temperature

2.1 Abstract

Two replicated experiments, conducted within the Aquaculture Centre at the University of Tasmania, investigated the effects of photoperiod and temperature on somatic growth of juvenile barramundi. Experiment 1 reared juvenile barramundi (2.77 ± 0.01 g) under three temperatures (20°C, 25°C, 30°C) and exposed fish to two photoperiods, twelve hours light and twelve hours dark (12L:12D) and continuous light (24L:0D). Experiment 2 reared juvenile barramundi (3.28 ± 0.01 g) under three temperatures (22°C, 24°C, 29°C) and exposed fish to either 12L:12D or 24L:0D. A constant light intensity of 700 lux (9.87 μmol s^{-1} m^{-2}) was maintained in both experiments. Fish in both experiments were fed a commercial pelleted diet to apparent satiation for 40 days. Final wet weight, total length and specific growth rate of barramundi significantly increased with increased water temperature. Photoperiod manipulation was ineffective at low water temperatures between 20°C - 25°C, only at 29/30°C did photoperiod become effective in manipulating growth.

However, contradicting results between experiments were observed in 24L:0D and 12L:12D treatments held at 29°C/30°C. In Experiment 1, juveniles exposed to 24L:0D significantly increased wet weight (19.38 ± 0.01 g), total length (11.77 ± 0.01 cm) and specific growth rate (6.98 ± 0.24 % bw.d^{-1}) compared to 12L:12D (13.48 ± 0.47 g; 10.38 ± 0.01 cm; 5.72 ± 0.11 % bw.d^{-1}). Growth increases observed under 24L:0D at 30°C occurred without significant increases in feed intake (9.57 ± 0.33 g.d^{-1}) compared to 12L:12D (10.65 ± 0.15 g.d^{-1}). Significantly different feed conversion efficiencies (FCE) were observed in fish reared under 24L:0D (156.19 ± 8.66 %) compared 12L:12D (112.40 ± 1.40 %). In contrast, in Experiment 2 there were significant increases in growth

in fish exposed to 12L:12D compared to 24L:0D. These were explained by increased feed intake with no significant difference in FCE. Juveniles exposed to 12L:12D in experiment 2 significantly increased wet weight (28.80 ± 0.67 g), total length (13.53 ± 0.21 cm), specific growth rate (5.39 ± 0.06 % $bw.d^{-1}$) and feed intake (17.66 ± 0.75) compared to 24L:0D (18.31 ± 0.68 g; 8.72 ± 0.09 cm; 4.25 ± 0.09 % $bw.d^{-1}$, 11.56 ± 0.59).

In both Experiment 1 and 2, higher plasma IGF-I concentrations were observed in fish demonstrating higher growth rates. Plasma IGF-I concentrations were significantly higher in fish exposed to 24L:0D held at 30°C (8.34 ± 0.23 $ng.mL^{-1}$) compared to 12L:12D (4.09 ± 1.07 $ng.mL^{-1}$) in experiment 1. In experiment 2, plasma IGF-I concentrations were significantly higher in fish exposed to 24L:0D held at 29°C (8.34 ± 0.23 $ng.mL^{-1}$) compared to 12L:12D (4.09 ± 1.07 $ng.mL^{-1}$).

Knowledge regarding effects of water temperature on the effectiveness of photoperiod manipulation will allow commercial barramundi farmers to determine suitable artificial lighting to enhance fish growth and potentially increase economic gain. At temperatures above 29°C the use of photoperiod may be effective at enhancing somatic growth of juvenile barramundi whereas appears to be ineffective a lower water temperatures.

Due to the contradicting results in the current experiments, further studies are needed to confirm whether 24L:0D enhances growth of barramundi and if so, investigate mechanisms involved with growth enhancement as well as possible stress effects of continuous light.

2.2 Introduction

Barramundi (*Lates calcarifer)* (Bloch) is a warm water fish distributed around the tropical regions of northern Australia and South East Asia, where the species moves between fresh and saltwater during developmental stages of its lifecycle (Carter et al., 2010). Barramundi aquaculture is an important and rapidly expanding industry in Australia increasing by 80% in production value from 2005 ($18.8M) to 2008 ($33.98M) (ABARE, 2008). Barramundi exhibit strong seasonal growth, with maximal growth during the warmer months, thereafter slowing down until a reduction and sometimes cessation of growth occurs during the winter period (Xiao, 2000). This strong seasonality in growth rate is related to seasonal changes in water temperature and has important implications for barramundi aquaculture in Australia (Carter et al., 2010; Katersky and Carter, 2005).

In many of today's intensively farmed aquaculture species it is commonplace to use environmental manipulation (such as photoperiod) to alter maturation, reproduction, spawning and growth (Biswas et al., 2005; Volkoff et al., 2010). Photoperiod manipulation is used to entrain fish to a different day length and therefore different time of year in order to advance or delay biological rhythms (Bolliet et al., 1996; Ekstrom and Meissl, 1997). This technique has been used successfully to improve growth in a number of fish species (Boeuf and Le Bail 1999; Porter et al., 1999, 2000, 2001; Biswas et al., 2008). Photoperiod manipulation has been of economic value to several temperate aquaculture industries (Kadri, 2003; Quigley, 2003), although information regarding the application of this technique on tropical aquaculture species is limited. As tropical

regions exhibit little seasonal variation in terms of day length it is suggested photoperiod manipulation techniques used on temperate species, may not be as effective on tropical species such as barramundi (Jobling, 1995; Barlow et al., 2005). However, extended photoperiods have been observed to increase growth performances in sub-tropical and tropical fish such as red sea bream (*Pagrus major*), striped knifejaw (*Oplegnathus fasciatus*) and Nile tilapia (*Oreochromis niloticus*) (El-sayed and Kawanna, 2007; Biswas et al., 2008)

Australian barramundi farmers are commonly holding fish for more than one year in response to market demand for fish of 1-3kg size (Carter et al., 2010). Barramundi farms situated at lower latitudes in Australia are forced to cope with low water temperatures around 20°C during winter and early spring. During this period, barramundi growth is greatly reduced due to reduced feeding. Farm profitability would be improved if low seasonal growth during winter could be increased through photoperiod manipulation. Currently the Australian barramundi industry only uses photoperiod manipulation to alter spawning. Increasing growth of barramundi would benefit industry by reducing time to harvest; and/or juveniles attaining larger sizes before winter.

Recent research investigating photoperiod manipulation in juvenile barramundi has suggested an important role for water temperature in determining the effectiveness of extended photoperiod (Hovette, 2005). Extended photoperiod of 18L:6D did not significantly increase growth rates of juvenile barramundi when reared in winter water temperatures of 19°C (Hovette, 2005) This suggests low water temperature may override the effect photoperiod has on growth, possibly due to a delayed or dampened response of

endocrine hormones (McCormick et al., 2000). This study investigated the effects of water temperature on photoperiod manipulation in juvenile barramundi. This was achieved by subjecting juvenile barramundi to two photoperiod regimes, (12L:12D and 24L:0D) at three water temperatures (between 20°C and 30°C) commonly experienced on Australian barramundi farms. Key growth parameters (wet weight, total length, feed intake, feed conversion efficiency, specific growth rate and hepatosomatic index) were measured. Circulating levels of the growth related hormone, insulin-like growth hormone (IGF-I) was measured to determine a possible endocrine mechanism for barramundi response to photoperiod and water temperature. Establishing which temperatures support photoperiod manipulation in being an effective growth enhancing technique will increase knowledge regarding appropriate timing for artificial lighting techniques in the commercial culture of barramundi. This experiment is separated into two parts; experiment 1 and experiment 2. The occurrence of disease in experimental tanks prevented the successful completion of experiment 1, however data concerning treatments reared at 30°C were included in the analysis as no disease symptoms were visible and growth rates were similar to previous experiments. Experiment 2 therefore is a repeat of experiment 1.

2.3 Materials and Methods

2.3.1. Experiment 1

Juvenile barramundi (fingerlings approximately 30 days old) from WBA Hatcheries, Adelaide (South Australia) were acclimated for a period of 2 weeks in three 180 L aquaria at 25^{o}C in 30 ‰ seawater. During acclimation, temperatures were adjusted 1°C.d^{-1} over the course of 5 days toward the desired experimental temperatures of 20^{o}C,

25°C and 30°C. Fish were not fed during the acclimation. Following acclimation, 40 fish (20°C; 2.82 ± 0.02 g, 25°C; 2.76 ± 0.02 g, 30°C 2.74 ± 0.02 g) were randomly allocated into 20 L tanks filled with 30 ‰ seawater (initial mean stocking density 5.64 kg/m^3).

Six experimental treatments consisted of:- three temperatures (20°C, 25°C and 30°C) were tested together with two photoperiod regimes (12L:12D and 24L:0D) although the 25 °C treatment was excluded from further analysis due to an unidentified disease and high mortality. Each experimental treatment consisted a recirculation system with three 20 L conical tanks attached to a 100 L reservoir and biological filter and partitioned using thick black plastic to exclude light interference from 24L:0D photoperiod. Water was delivered at a rate of 2.8 $L.min^{-1}$ with oxygen levels being maintained above 90% saturation. Water parameters (Appendix 1) were monitored daily and water changes performed as necessary to keep water quality within the limits for barramundi (Tucker et al., 2002). Particulate dacron filters were cleaned every day and water exchange was less than 10% per day (to replace water discarded during cleaning and siphoning uneaten feed).

Control over the water temperature was achieved by lowering the ambient temperature in the room to 20°C and heating the water in the reservoirs to either 20°C, 25°C or 30°C using submersible heaters each controlled with an individual thermostat. Temperature was recorded every half hour with StowAway Tidbit Temperature Loggers (Onset Computer Company, Bourne, MA, USA) as well as each system being manually checked

daily. Diurnal variation in water temperature in each re-circulation system was ± 0.5°C of the intended temperature.

Fish were hand fed a commercial high protein diet ranging from 1 mm initially to 4 mm by the end of the trial (Marine Start 1-3mm, Marine Float 54/10 4mm, crude protein 52%; crude fat 16%. Ridley Aquafeed, Australia). Feeding was conducted twice daily (0900 and 1700) to satiation; feed intake was recorded for each tank after uneaten pellets were siphoned out and counted after each feed. An average pellet weight was used to calculate feed intake per day and data summed over the entire experiment.

Artificial lighting positioned above tanks, was supplied by 500 W Fluorescent lights, with timers set to turn on lights at 0700 and turn off at 1900 for the 12L:12D treatments. Average light intensity throughout the experimental tank was 700 lux (9.87 μmol s^{-1} m^{-2}) which was measured from several points within the experimental tank using a Li-COR Underwater Quantum sensor (LI-192SA).

2.3.2. Experiment 2

The experimental design for Experiment 2 was replicated from Experiment 1, although initial fish size and water temperature varied slightly (temperature treatments of 22 ± 0.05°C, 24 ± 0.06°C and 29 ± 0.19°C). Following acclimation, 40 fish (22°C – 3.27 ± 0.02 g; 24°C – 3.23 ± 0.02 g; 29°C – 3.34g ± 0.03 g) were randomly allocated into each of six 20 L recirculating tanks systems with 30‰ seawater (mean initial stocking density 6.54 kg/m^3).

The following experimental details were applied for both experiments 1 and 2.

2.3.3. Sampling Procedures

In both experiment 1 and 2, fish were sampled on days 0, 20 and 40. On each sampling day, all fish were removed from the tank, anaesthetised using iso-eugenol at 40 $mg.L^{-1}$ (AQUI-S, New Zealand Ltd). All fish were measured for wet weight (to nearest 0.1 g) and total length (mm) whilst at day 20 and 40, 20 fish were blood sampled for circulating plasma IGF-I. Blood (approximately 300 µL) was drawn from the caudal vein of fish using heparinised (ammonium heparin, Sigma; 4mg/ml) syringes (1ml Terumo syringes, 25G Terumo hypodermic needles) then centrifuged at 3500 rpm at 4^{o}C, for 15 min and stored at -20°C until assayed for IGF-I.

2.3.4 IGF-I Radioimmunoassay (RIA)

Plasma levels of IGF-I were analysed by a heterologous radioimmunoassay validated for barramundi (Dyer et al., 2004). Anti-barramundi IGF-1, ^{125}I-labelled barramundi IGF-I tracer and recombinant barramundi IGF-I standard were purchased from GroPep Ltd (Adelaide, Australia). Recombinant barramundi IGF-I standards and samples (in 250 µl), and anti-barramundi IGF-I polyclonal antiserum (50 µl diluted 1:30,000) were all diluted in RIA buffer (30 mM NaH_2PO_4, 0.02 % protamine sulphate, 10 mM EDTA, 0.025 % NaN_3, 0.05% (v/v) Tween 20, pH 7.5). Duplicate tubes were incubated overnight at 4°C. Bound and free tracer were separated by adding 10 µL rabbit gamma globulin (1:200 dilution) and 50 µL sheep anti-rabbit c-globulin (1:20), and after incubating for 30 min at 4°C a 1 mL volume of cold 5% polyethylene glycol was added. The precipitate was then centrifuged at 4000 g for 30 min at 4°C. The supernatant was removed by decanting and

bound radioactivity determined using a gamma counter. Serial dilutions of acid/ethanol extracted barramundi plasma were parallel to the standard curve. The minimum detectable limit of the assay was 0.15 ng/ml. Inter-assay variation was 16% and intra-assay variation was 3%. Samples were assayed in duplicate.

2.3.5. Calculations

The following equations were used to calculate feed intake (FI), feed conversion efficiency (FCE), specific growth rate of weight and length (weight and SGR length), condition factor (K) and hepatosomatic index (HSI) for each replicate tank (n = 3) on day 20 and 40.

FI ($g.d^{-1}$) = total dry feed intake/time (days)

FCE (%) = 100 x (wet weight gain/total dry feed intake).

SGR weight ($\%.bw.d^{-1}$) = $100x (\ln W_2 - \ln W_1)$/time (days)

Where, W_1 and W_2 indicate the initial and final wet weight (g) respectively.

SGR length ($\%.tl.d^{-1}$) = 100 x (TL_2-TL_1)/time (days)

Where, TL_1 and TL_2 indicate the initial and final total length (cm) respectively.

$K = 100 \times (W / L^3)$

Where, W = wet body weight (g) and L = total body length (cm)

HSI (%) = 100 x (wet weight of liver (g)/wet body weight (g))

2.3.6. Statistical Analysis

Statistical analysis for both experiment 1 and 2 were carried out using SPSS 15.0 for windows (SPSS Inc.). The overall mean for growth data and feed intake of each replicate tank was analysed using one-way ANOVA, where there were significant differences a Tukeys post hoc test was performed to identify differences between pairs of treatment groups. A two-way nested ANOVA was also applied in order to calculate the overall effects of time, temperature and photoperiod on growth data with tanks nested within temperature and photoperiod. Differences were considered to be significant if $P<0.05$. Values are presented as means ± standard error (SEM).

2.4 Results

2.4.1 Growth – Experiment 1

Feed intake ($g.day^{-1}$) was significantly higher at 30°C compared to 20°C and 25°C (one-way ANOVA; $P<0.01$; F = 156.40; df = 5), which were not significantly different from one another (Figure 2.1A). At 20°C, significantly higher feed intake was observed for fish exposed to 24L:0D compared to 12L:12D (one-way ANOVA; $P<0.01$; F = 156.40; df = 5) (Figure 2.1A). At 30°C, significantly higher FCE was observed compared to 20°C or 25°C. Additionally, at 30°C, significantly higher FCE was observed in fish exposed to 24L:0D compared to 12L:12D (one-way ANOVA; $P< 0.01$; F = 49.38; df = 4) (Figure 2.1B).

Initial weights and lengths of fish were not significantly different between treatments (2.77 ± 0.04 g and 6.20 ± 0.04 cm (one-way ANOVA weight; $P>0.05$; F = 0.598; df = 17) (one-way ANOVA length $P>0.05$; F = 1.108; df = 17). Throughout the trial, wet

weight and total length significantly increased with increasing temperature regardless of photoperiod (two-way ANOVA weight; $P<0.01$; F = 64.76; df = 8) (two-way ANOVA length; $P<0.01$; F = 119.66; df = 8) (Figure 2.1A and B). Photoperiod only impacted on growth at an elevated temperature of 30°C. At 30°C, wet weight and total length significantly increased in fish exposed to 24L:0D compared to 12L:12D (t-test; $P<0.01$; F = 58.44; df = 1) (t-test; $P< 0.01$; F = 67.53; df = 1) (Figure 2.2A and B). SGR (% $bw.d^{-1}$) increased significantly with increasing temperature (one-way ANOVA; $P<0.01$; F = 120.53; df = 2) (Figure 2.3A and B). At 20°C and 25°C, SGR weight and length did not significantly differ between photoperiod treatments. At 30°C, a significantly greater SGR weight and length was observed in fish exposed to 24L:0D to 12L:12D (SGR weight = one-way ANOVA; $P <0.01$; F = 266.75; df = 4; SGR length = one-way ANOVA; $P <0.01$; F = 156.16; df = 4) (Figure 2.3A and B).

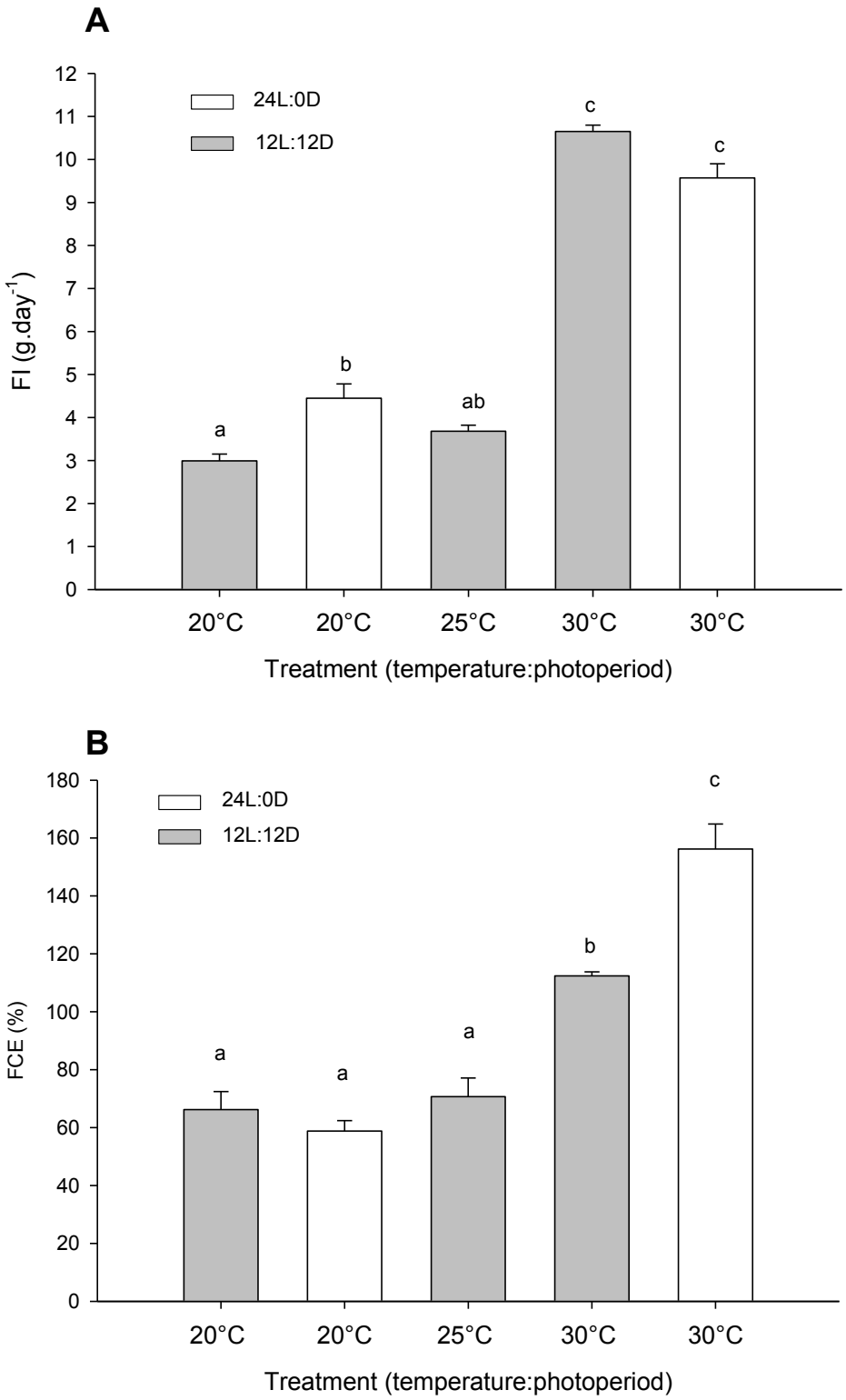


Figure 2.1. Experiment 1 - Feed intake (FI) (g.day-1 ± SEM) (A) and feed conversion efficiency (FCE % ± SEM) (B) of juvenile barramundi held at 20°C, 25°C and 30°C under 12L:12D and 24L:0D at Day 40. Different letters denote significant differences (P<0.05).

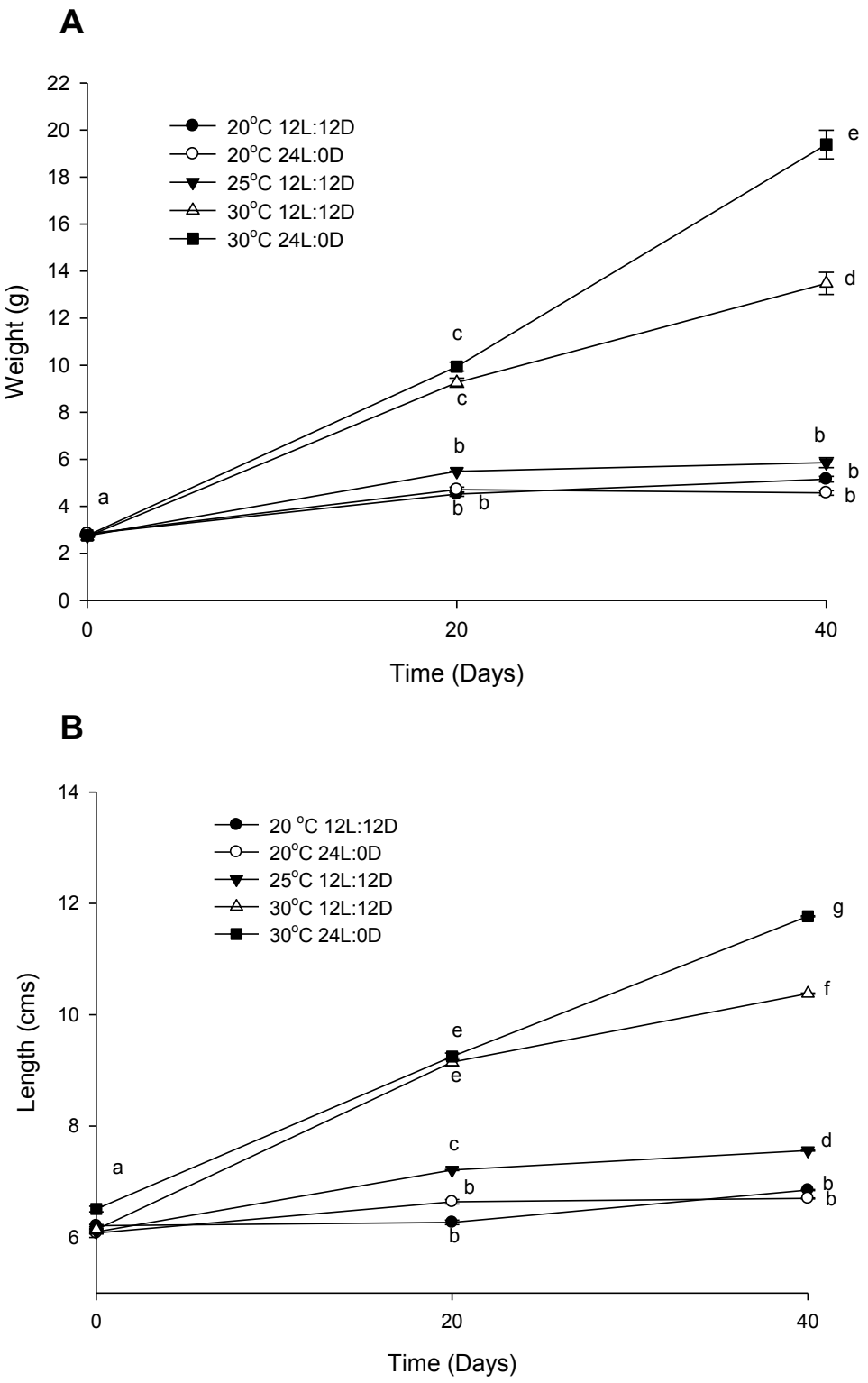


Figure 2.2. Experiment 1 - Mean wet weight (A) (g ± SEM) and mean total length (B) (cm ± SEM) of juvenile barramundi held at 20°C, 25°C and 30°C under 12L:12D and 24L:0D. Different letters denote significant differences (P<0.05).

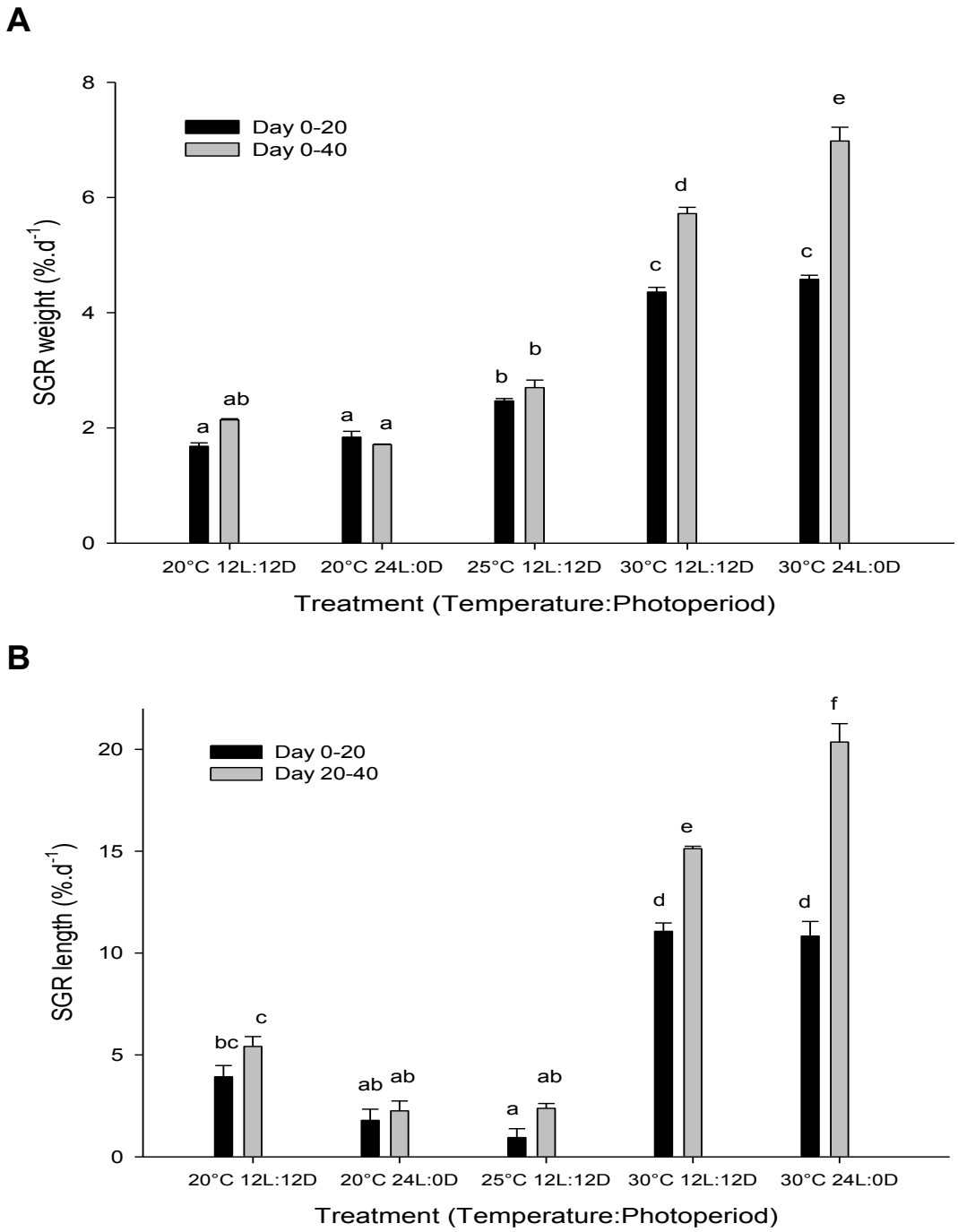


Figure 2.3. Experiment 1 - Mean specific growth rate for weight (SGR % bw.d^{-1} ± SEM) (A) and length (SGR % lt.d^{-1} ± SEM$^{)}$ (B) of juvenile barramundi, at Day 20 and Day 40, held at 20^{o}C, 25^{o}Cand 30^{o}C under 24L:0D and 12L:12D. Different letters denote significant differences (P<0.05).

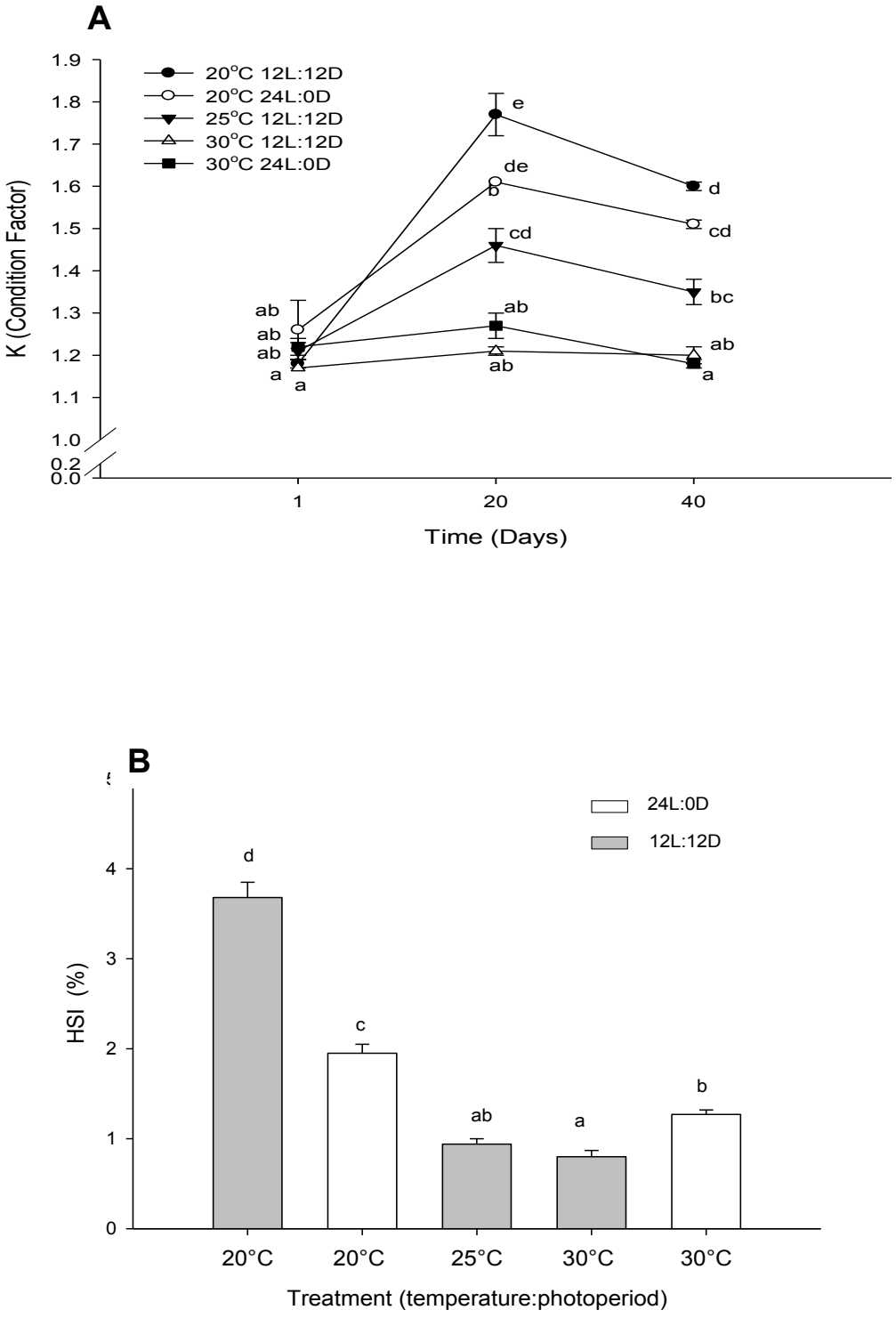


Figure 2.4. Experiment 1 - Mean condition factor (K ± SEM) of juvenile barramundi taken at Day 1, 20 and 40, held at 20°C, 25°C and 30°C under 12L:12D and 24L:0D (A) and hepatosomatic index (% ± SEM) of juvenile barramundi taken at Day 40 (B). Different letters denote significant differences (P<0.05).

Initial Condition factor (K) did not significantly differ and by day 40, condition factor in fish held at 30°C did not significantly differ from initial K (two-way ANOVA; $P< 0.01$; F = 34.62; df = 14). By day 20, significant increases in condition levels were observed with decreased water temperatures, while photoperiod did not significantly affect condition factor between treatments (two-way ANOVA; $P< 0.01$; F = 34.62; df = 14) (Figure 2.4A).

A significantly greater HSI was observed in fish at 20°C compared to fish held at 25°C and 30°C (one-way ANOVA; $P< 0.01$; F = 99.45; df = 2) (Figure 2.4B). At 20°C, significantly increased HSI was observed in fish exposed to 12L:12D compared to 24L:0D (one-way ANOVA; $P<0.01$; F = 128.12; df = 4) (Figure 2.4B). At 30°C, a significantly lowered HSI was observed in fish exposed to 12L:12D compared to 24L:0D (one-way ANOVA; $P<0.05$; F = 128.12; df = 4) (Figure 2.4B).

Plasma IGF-I concentration was highly variable within treatments on day 20 and day 40 (Figure 2.5A and B). At day 40, significant increases in IGF-I levels were observed in fish exposed to 24L:0D at 30°C compared to all other treatments (one-way ANOVA; $P<0.05$; F = 9.08; df = 4) (Figure 2.5B).

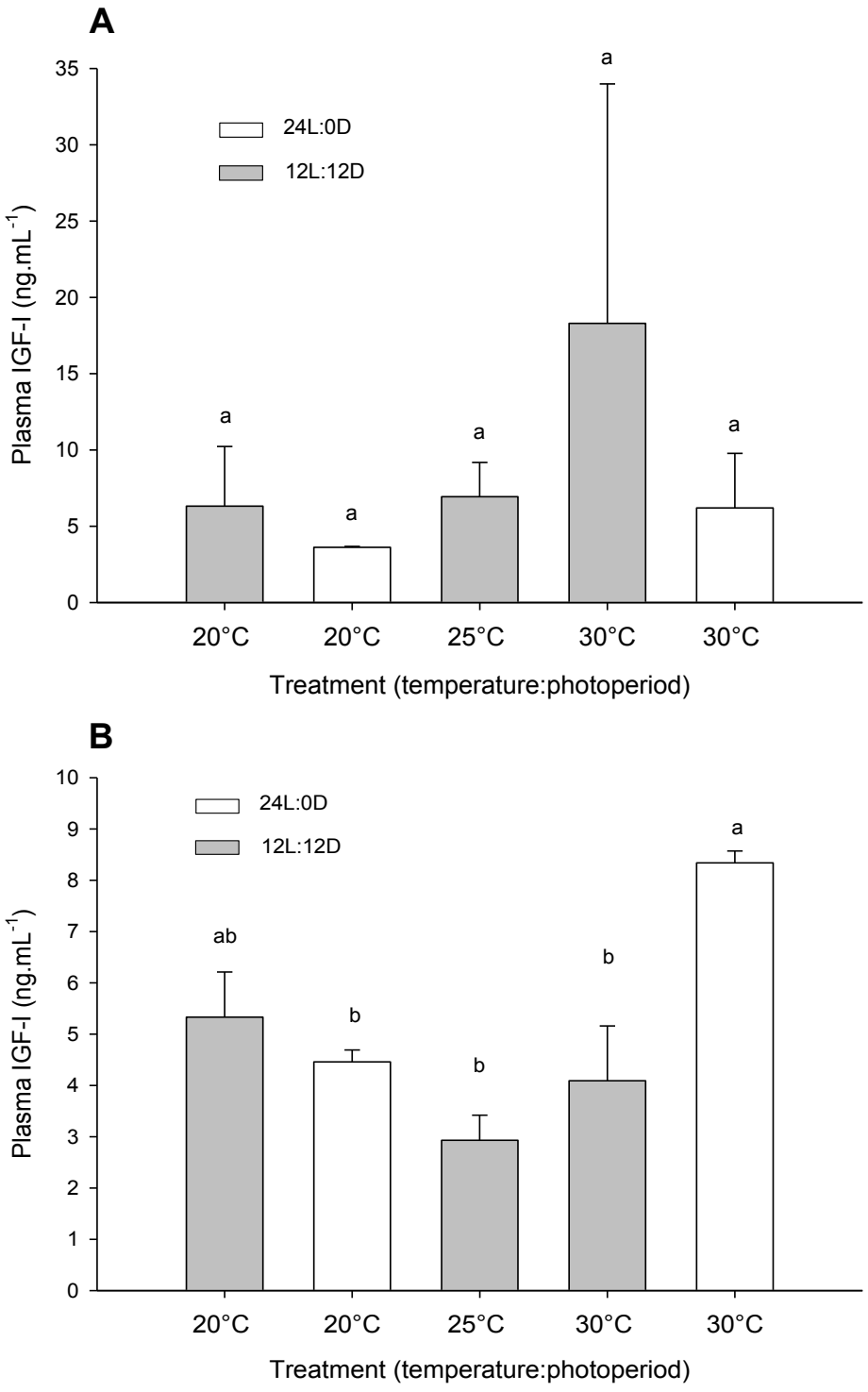


Figure 2.5. Experiment 1 - Mean plasma IGF-I concentration (ng.mL^{-1} ± SEM) of juvenile barramundi, taken at Day 20 (A) and Day 40 (B), held at 20^{o}C, 25^{o}C and 30^{o}C under 12L:12D and 24L:0D. Different letters denote significant differences (P<0.05).

2.4.2 Growth – Experiment 2

Feed intake ($g.day^{-1}$) was significantly higher at 29°C compared to 22°C and 24°C (one-way ANOVA; $P< 0.01$; F = 41.90; df = 2). At 29°C, feed intake significantly increased in fish exposed to 12L:12D compared to 24L:0D (one-way ANOVA; $P<0.01$; F = 130.50; df = 5) (Figure 2.6A). At 29°C, a reduced intake in feed was observed from day 0-5 in fish exposed to 24L:0D compared to 12L:12D. (Figure 2.7). Interestingly, observed trends of feed intake in juveniles oscillated, feeding heavily once every three days (Figure 2.7). Significant increases in FCE were observed in fish at 29°C compared to 22°C and 24°C (one-way ANOVA; $P<0.01$; F = 29.17; df = 5) (Figure 2.6B). At 22°C, FCE significantly increased in fish exposed to 12L:12D compared to 24L:0D (one-way ANOVA; $P<0.01$; F = 29.17; df = 5) (Figure 2.6B).

Initial wet weight and total length of fish were not significantly different between treatments (3.28 ± 0.01 g and 6.30 ± 0.06 cm) (one-way ANOVA weight; $P>0.05$; F =3.36; df = 5) (one-way ANOVA length; $P>0.05$; F = 2.32; df = 5). Throughout the trial, wet weight and total length significantly increased with increasing temperature (two-way ANOVA weight; $P<0.01$; F = 52.27; df = 8) (two-way ANOVA length; $P<0.01$; F = 98.19; df = 8) (Figure 2.8A and B). At 22°C and 24°C, no significant differences in growth were observed between photoperiods at day 20 or day 40 (Figure 2.8A and B). Photoperiod only impacted on growth at an elevated temperature of 29°C. At 29°C, wet weight and total length were significantly higher in fish exposed to 12L:12D compared to 24L:0D (two-way ANOVA weight; $P<0.01$; F = 818.01; df = 17) (two-way ANOVA length; $P<0.01$; F = 639.71; df = 17) (Figure 2.8A and B).

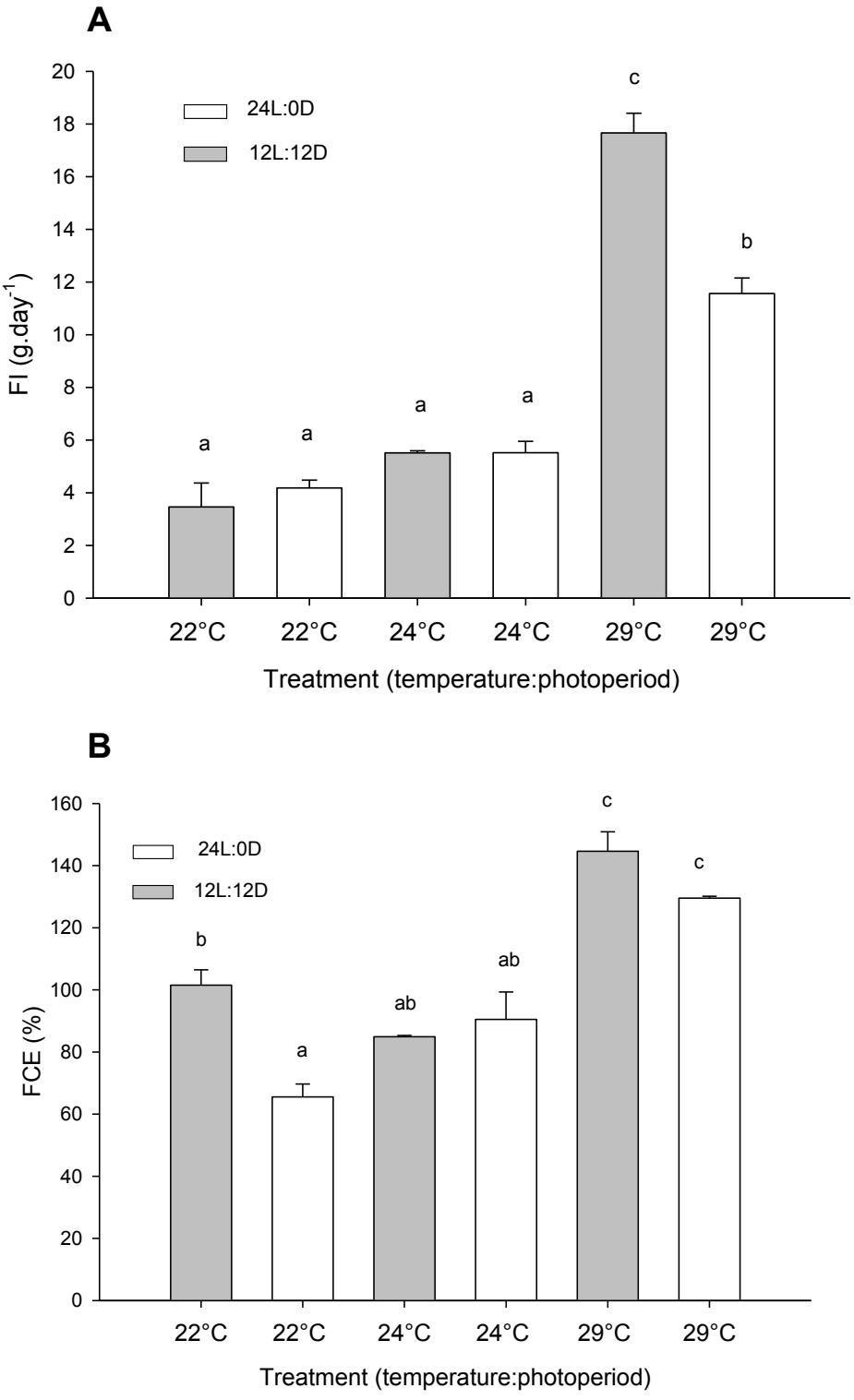


Figure 2.6. Experiment 2 - Feed intake (FI) ($g.d^{-1}$ ± SEM) (A) and feed conversion efficiency (FCE) (% ± SEM) (B) of juvenile barramundi held at 22°C, 24°C and 29°C under 12L:12D and 24L:0D. Different letters denote significant differences (P<0.05).

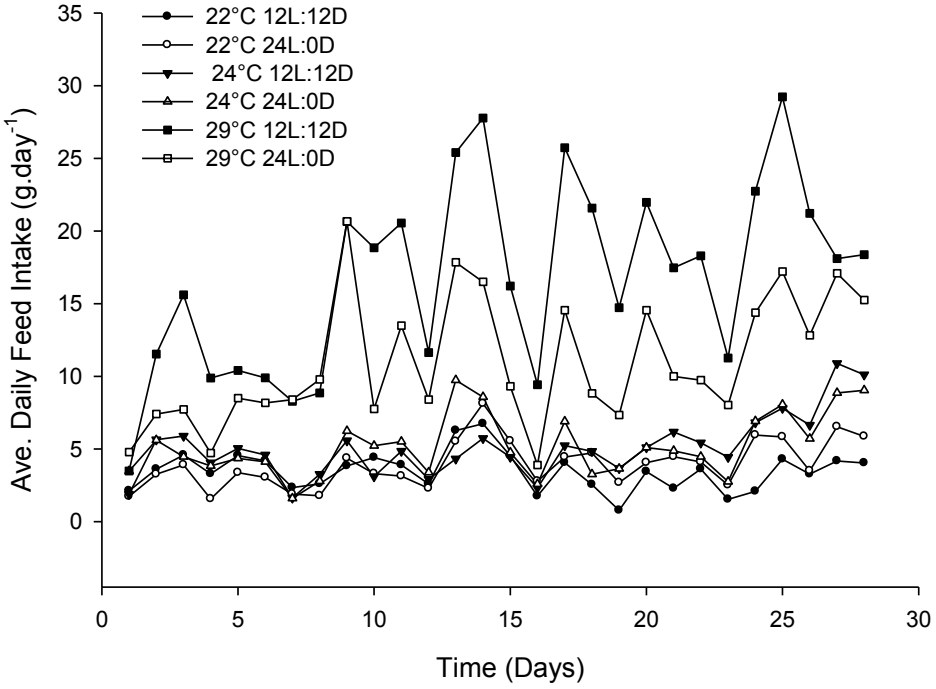


Figure 2.7. Experiment 2 - Average daily feed intake (g.day^{-1} ± SEM) of juvenile barramundi held at 22^{o}C, 24^{o}C and 29^{o}C under 12L:12D and 24L:0D.

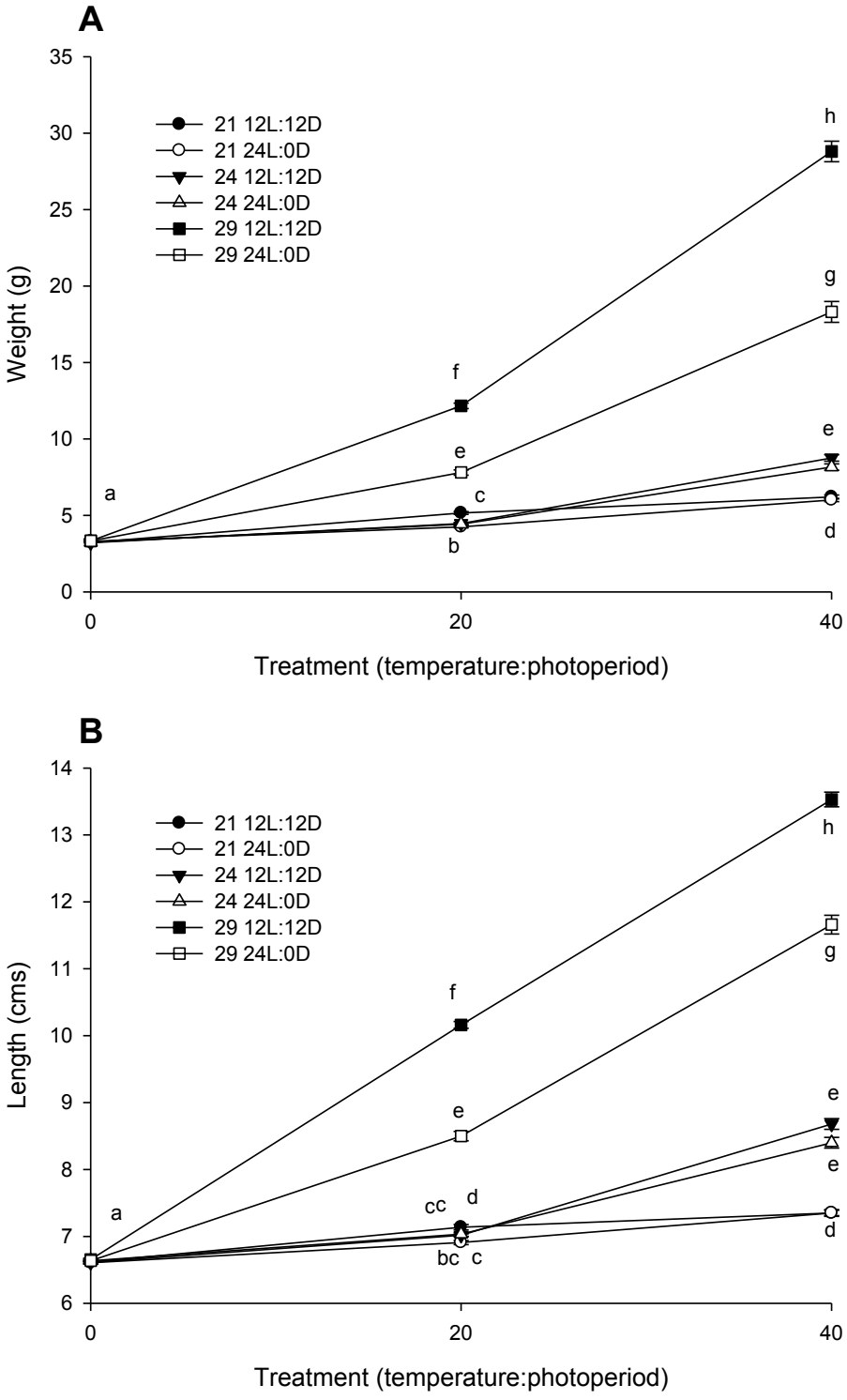


Figure 2.8. Experiment 2 - Mean wet weight (A) (g ± SEM) and mean total length (B) (cm ± SEM) of juvenile barramundi held at 22°C, 24°C and 29°C under 12L:12D and 24L:0D. Different letters denote significant differences (P<0.05).

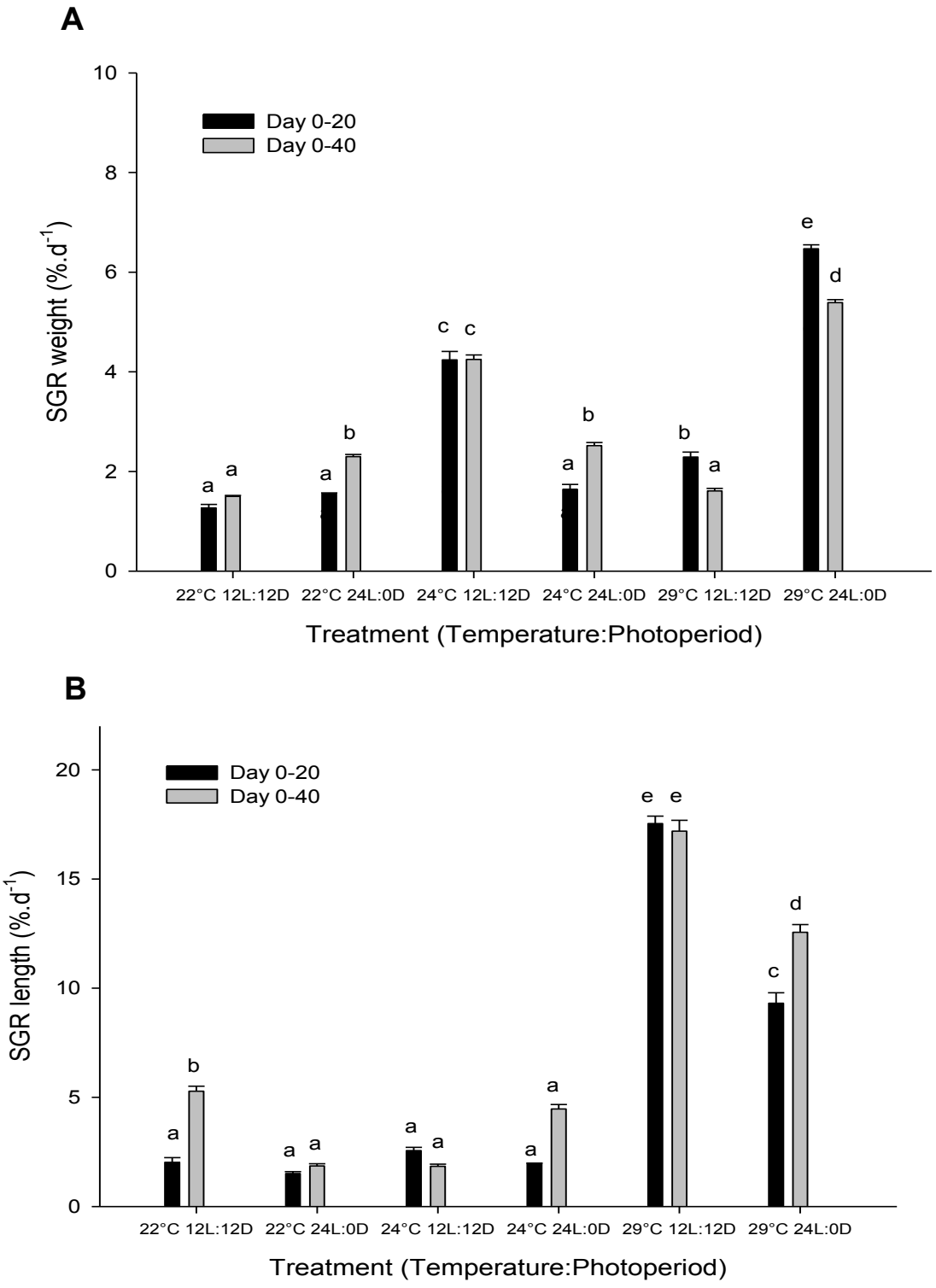


Figure 2.9. Experiment 2 - Mean specific growth rate; weight (SGR) (% bw.d^{-1}± SEM) (A) and length (SGR) (% lt.d^{-1} ± SEM) (B) of juvenile barramundi, at Day 20 and Day 40, held at 22^{o}C, 24^{o}C and 29^{o}C under 24L:0D and 12L:12D. Different letters denote significant differences (P<0.05).

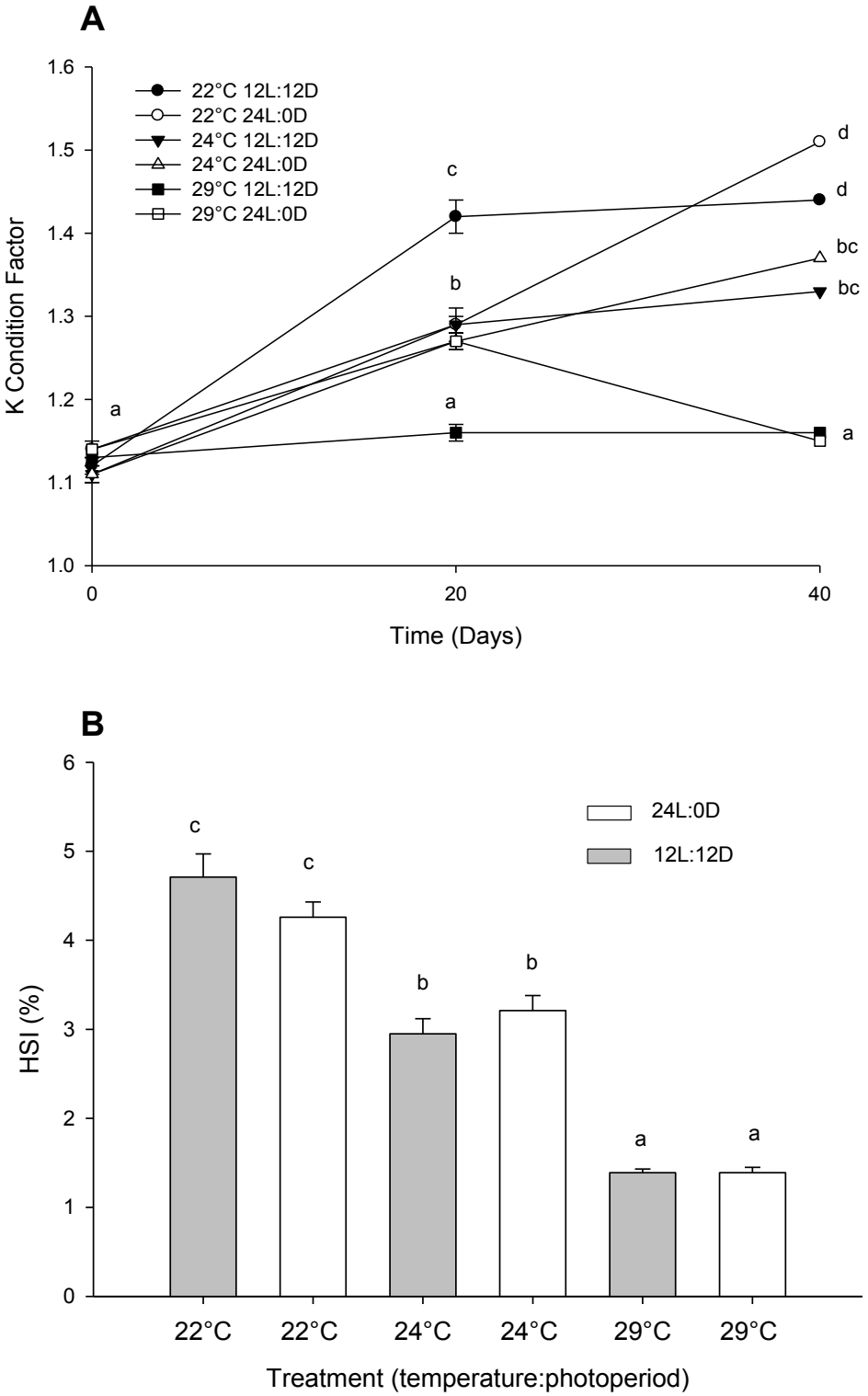


Figure 2.10. Experiment 2 - Mean condition factor (K ± SEM) (A) at Day 20 and Day 40; hepatosomatic index (HSI) (% ± SEM) (B) of juvenile barramundi taken at Day 40, held at 22°C, 24°C and 29°C under 12L:12D and 24L:0D. Different letters denote significant differences (P<0.05).

SGR (%.d^{-1}) significantly increased with increasing temperature (two-way ANOVA; P<0.01; F = 36.82; df = 5) (Figure 2.9A). At 22^{o}C and 24^{o}C, SGR did not significantly differ between photoperiod treatments. At 29^{o}C, SGR was significantly greater in fish exposed to 12L:12D compared to 24L:0D (two-way ANOVA; P<0.01; F = 626.15; df = 11) (Figure 2.9A). SGR length (%.d^{-1}) was significantly greater in fish held at 29°C compared to 22°C and 24°C (two-way ANOVA; P<0.01; F = 27.45; df = 5) (Figure 2.9B). At 22°C and 29°C, SGR length was significantly greater in fish held under 12L:12D compared to 24L:0D (two-way ANOVA; P<0.01; F = 434.22; df = 11) (Figure 2.9B).

Condition factor (K) was not significantly different between treatments at day 0, day 20. At day 40, condition factor significantly decreased with increased temperature, irrespective of photoperiod (two-way ANOVA; P<0.01; F = 50.36; df = 17) (Figure 2.10A). Interestingly, condition factor, at day 20, significantly decreased in fish held at 29^{o}C and exposed to 24L:0D compared to 12L:12D, but by day 40 this trend was no longer significant (two-way ANOVA; P<0.01; F = 50.36; df = 17) (Figure 2.10A). HSI (%) significantly decreased with increasing temperature, irrespective of photoperiod (two-way ANOVA; P<0.01; F = 88.96; df = 5) (Figure 2.10B).

Levels of plasma IGF-I at day 40 were significantly higher in fish exposed to 12L:12D at 29^{o}C compared to all other treatments (one-way ANOVA; P<0.01; F = 16.97; df = 5) (Figure 2.11).

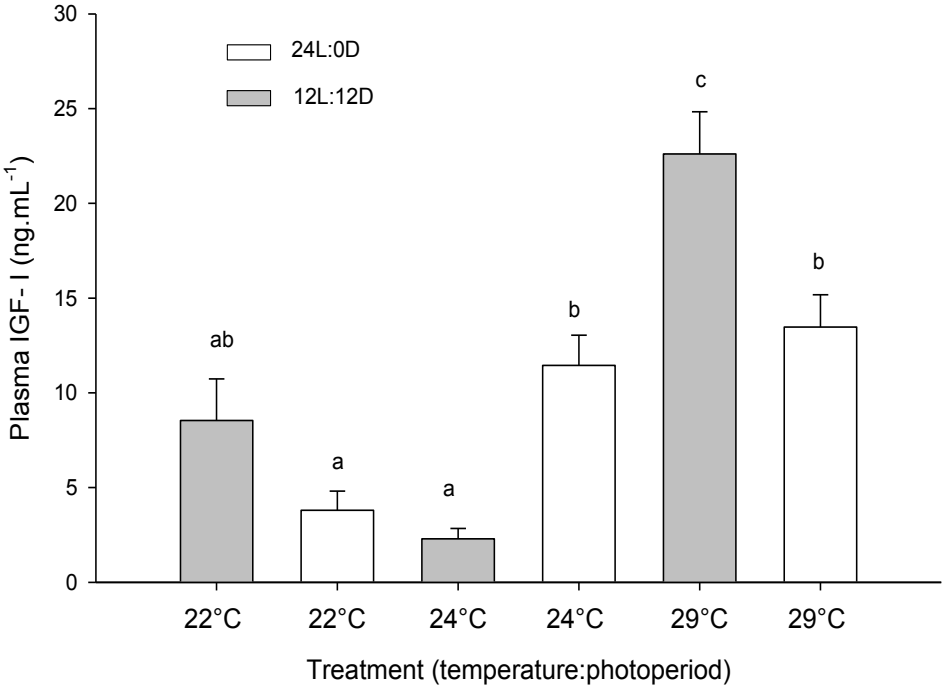


Figure 2.11. Experiment 2 - Mean plasma IGF-I concentration (ng.mL^{-1} ± SEM) from Day 40 of juvenile barramundi held at 22^{o}C, 24^{o}C and 29^{o}C under 12L:12D and 24L:0D. Different letters denote significant differences (P<0.05).

2.5 Discussion

The present study aimed to confirm enhanced growth effects of continuous light (24L:0D) on juvenile barramundi (Worrall et al., 2004) in addition to ascertaining effectiveness of photoperiod manipulation over varying water temperatures. Growth performance of juvenile barramundi (3-20g) exposed to 24L:0D and 12L:12D was investigated over a range of temperatures (20° - 30°C). Growth of barramundi significantly increased with increased water temperature. Only at higher temperatures of 29°C and 30°C did photoperiod manipulation affect growth performances of barramundi. This suggests photoperiod manipulation is ineffective between low water temperatures of 20°C and 25°C however contrasting results at higher water temperatures was observed here.

Temperature can affect the rate of biochemical reactions, retarding processes such as metabolism at low temperatures or increasing processes with increasing temperature until upper thermal limits are reached (Ibarz et al., 2005, 2007; Katersky and Carter, 2005). Metabolic processes of fish are sensitive to changes in temperature and a decrease in water temperature to below the optimum, results in reduced feed intake and growth. Fish eventually lose appetite and growth ceases in the lower part of the temperature tolerance range (Brett, 1979; Jobling 1994). Generally, feed intake, growth and growth efficiency increase with increasing temperature, reaching a peak at the optimum temperature before declining as temperature approaches the species thermal limit (Jobling 1993; Alvarez et al., 2010; Katersky and Carter, 2007a,b). Increased growth parameters (wet weight, total length, feed intake, SGR and FCE) were observed with increasing water temperatures (20°C - 30°C) in both experiment 1 and 2. These results were to be expected as optimal

temperatures for growth of barramundi of this size are between 27-33°C with thermal limits occurring at 39°C (Katersky and Carter, 2005, 2007b). Condition Factor (K) in both experiments were reduced with increasing water temperatures (20°C - 30°C) indicating more slender fish as temperatures increased. Although this being the case, markets for barramundi currently do not have preference in optimal body shape, considering body weight to be of more importance (pers comm. M Phillips, Pejo Enterprises). Additionally, currently commercial farmers place higher importance on the enhanced growth at higher temperatures than body shape (pers comm. M Phillips, Pejo Enterprises).

Barramundi exposed to 24L:0D at 30°C demonstrated 44% increase in wet weight and a 13% increase in total length compared 12L:12D in experiment 1. Increased growth observed under 24L:0D at 30°C was accompanied without significant increases in feed intake as well as significantly improved FCE compared to 12L:12D. This parallels findings in other species, such as largemouth bass (*Micropterus salmoides)*, haddock (*Melanogrammus aeglefinus)* and gilthead seabream (*Sparus aurata)* (Kissil et al., 2001; Petit et al., 2003 Trippel and Neil, 2003; Ginés et al., 2004). Increases in barramundi growth from extended photoperiod may have occurred through better utilization of feed rather than stimulation of feed intake (Gross et al., 1965; Boeuf and Le Bail, 1999).

If increased growth rates under 24L:0D, as seen in experiment 1, are gained from better feed conversion efficiencies there could be a number of reasons for this. Sustained activity of fish throughout continuous light has been observed to affect metabolism and body composition in fish (Jobling, 1993; Davison, 1997; Biswas et al., 2002).

Metabolism and fish's conservation of energy may be affecting feed conversion efficiencies when exposed to longer photoperiods (Biswas et al., 2002; Biswas and Takeuchi, 2003). Biswas et al., 2002 & 2002 (a) demonstrated fish held under shorter photoperiod cycles (3L:3D, 6L:6D) had higher oxygen consumption than longer photoperiod cycles of 12L:12D and 24L:24D. Oxygen consumption was higher during the light phase compared to the dark phase for all photoperiods but mean oxygen consumption/hour was lowest in longer photoperiods. Additionally, Biswas found the highest post-prandial increase in energy loss was recorded during the 3L:3D (145.88kJ/kg/day) and 6L:6D (141.19 kJ/kg/day) compared to the 12L:12D (128.70 kJ/kg/day) and 24L:24D (99.92 kJ/kg/day). He suggests these results indicate that higher energy conservation would be achieved if fish are exposed to longer rather than shorter photoperiod cycles. Conservation of energy when exposed to longer photoperiods may involve the liver as it is an important storage organ in barramundi. Significantly higher hepatosomatic index was observed in fish exposed to 24L:0D compared to 12L:12D at 30°C in experiment 1, although, this result was contradictory at 20°C in experiment 1.

Low water temperature may alter energy conservation in barramundi. HSI significantly increased as water temperature decreased in both experiments. Possibly fish are converting the majority of feed to energy storage in the liver at low water temperature ready for a period of rapid growth when thermal conditions are more favourable. Although low water temperatures decrease metabolism and may reduce digestive and absorptive performance (Prosser, 1991; Jobling, 1997) some "surplus" energy appears to be retained in the liver. This is further suggested as FCE was significantly lower at low temperatures of 20°C - 25°C compared to 29°C and 30°C, in both experiments. Lipid

and energy content was not measured in the current experiments however this would be useful in future studies to determine energy partitioning in barramundi.

Better conversion efficiencies may also be influenced by extended periods of day length allowing longer time frames for digestive processes to occur, improving digestion and retention efficiency. Fish's metabolic functions, digestive and absorptive capabilities involved with utilizing feed more efficiently could be attributed to changes in the endocrine/growth axis (Vera and Brown, 2009; Volkoff et. al., 2010). The hormone IGF-I is a central link in the endocrine/growth axis, influencing fish growth and development by acting on muscle and cartilage cell growth, stimulating nutrient uptake and protein synthesis while influencing a range of related hormones involved in the endocrine/growth axis (Le Bail et al., 1998; Pérez-Sánchez, 2000; Björnsson et al., 2002; Imsland et al., 2007). Circulating levels of IGF-I in fish are regulated in part by photoperiod, with plasma IGF-I levels increasing with increasing day length (M^{c}Cormick et al., 2000). Changes in fish growth are suggested to be reflected in alterations of IGF-I profiles, with levels of IGF-I positively correlating with growth (Mingarro et al., 2002; Taylor et al., 2005). In addition to IGF-I levels possibly influencing feed conversion efficiencies, observing IGF-I concentrations may provide a useful tool for monitoring barramundi growth rates as levels of IGF-I positively correlate with barramundi growth rates (Dyer et al., 2004; Worrall et al., (Chapter 5 and 6)).

In experiment 2, levels of plasma IGF-I were significantly increased in juveniles showing increased growth exposed to 12L:12D at 29°C. This pattern was consistent in experiment 1, with higher IGF-I levels measured in the treatment which resulted in the best growth

performance (24L:0D at 30°C). Although a clear effect of photoperiod was not determined in the current study, this parallels other studies where plasma IGF-I levels in barramundi were indicative of fish growth rates, with fish growing at faster rates having higher IGF-I levels Dyer et al., (2004). Additionally the current results concur with Taylor et al., (2005)'s findings of IGF values appearing to accurately reflect falling water temperatures.

Plasma IGF-I concentration is also suggested to be a better indicator of recent growth performance rather than growth measured over a longer period of time (Dyer et al., 2004). Plasma levels of IGF-I in both experiments were considerably lower than typical levels seen on subsequent experiments (fish approximately 20 g showing values around 30–50 $ng.mL^{-1}$ IGF-I). Although the current results concur with previous studies, IGF-I levels in the present study are very low and variable compared to other studies which could suggest that fish were compromised in some way. Indeed, in experiment 1 plasma levels of IGF-I seem to drop from day 20 to day 40. Unfortunately technical problems prevented the analysis of day 20 plasma samples for IGF-I in experiment 2. Further investigations into endocrine parameters would be beneficial to ascertain areas in which growth processes are being affected by photoperiod manipulation.

Barramundi held at 30°C and exposed to continuous light demonstrated improved growth performance in experiment 1. Contradicting these results in experiment 2, improved growth performance was observed in fish exposed to 12L:12D and held at 29°C, albeit and importantly to note, with significant increases in feed intake. Contradictory results in experiment 2 could be attributed to growth lag effects due to lowered feed intake over the first 5 days in fish exposed to 24L:0D (Ali et al., 2001). Barramundi observing a lag

effect in growth under 24L:0D, continued to show significantly reduced growth by day 40. A lag effect in growth and subsequent compensatory growth may not necessarily result in convergence of growth trajectories of normally growing fish (Jobling, 2010). Although, photoperiod manipulation did not cause significant stress response in the sub-tropical species, red sea bream (Biswas et al., 2008)., barramundi exposed to continuous light may demonstrate a stress response and subsequent reduction in feed intake when transferred from 12L:12D directly to continuous 24L:0D. Red sea bream (1.4 g) were exposed various photoperiods (6L:6D, 12L:12D, 16L:8D and 24L:0D) with 24L:0D treatments observing highest growth parameters without any differences in stress parameters such as cortisol and glucose levels (Biswas et al., 2008). As far as I am aware, gaps in knowledge regarding stress responses of photoperiod manipulation on barramundi are yet to be investigated.

2.6 Conclusion

The current results established growth performance of juvenile barramundi, reared from 2 to 20 g and exposed to 12L:12D and 24L:0D, significantly increase with increased water temperature. Photoperiod manipulation was observed to be ineffective at low water temperature between 20°C - 25°C. At 30°C photoperiod manipulation became effective, however this result was not replicated at a similar water temperature (29°C) in a subsequent experiment Contradicting results between 24L:0D and 12L:12D at 29°C/30°C were observed between both experiments. In Experiment 1, 24L:0D significantly increased growth without significant increases in feed intake compared to 12L:12D. In Experiment 2, growth was higher under 12L:12D compared to 24L:0D although importantly with significant increases in feed intake. Growth lag in fish

exposed to 24L:0D in experiment 2 is thought to be due to significant reduction in feed intake possibly due to stress from initial transfer to continuous light.

Higher growth performances in fish at 30°C and 24L:0D were indicative of significantly better feed conversion efficiencies. This suggests increased growth performances observed under 24L:0D alters parameters causing fish to metabolise feed more efficiently. Levels of plasma IGF-I significantly increased in barramundi demonstrating higher growth rates. Analysing components of the growth axis, such as IGF-I, may help to ascertain if photoperiod is altering growth at an endocrine level.

These results help towards establishing an optimal lighting regime for commercial barramundi farmers to increase growth rates of juvenile barramundi. Further studies are needed to confirm 24L:0D enhances growth of barramundi in addition to determining if growth enhancement is due to increased feed intake or extended photoperiod altering the endocrine/growth axis enabling better utilization of feed.

2.7 References

ABARE, 2008. Australian Bureau of Agricultural & Resource Economics Management. 2008, Australian Fisheries Statistics, Aquaculture, [Online], Available: http://www.abare.gov.au. [11 June 2010]

Ali, M., Cui, Y., Zhu, X., Wootton, R.J. 2001. Dynamics of appetite in three fish species (*Casterosteus aculeatus, Phoxinus phoxinus and Carassius auratus gibelio*) after feed deprivation. Aquaculture Research 32, 443-450.

Alvarez, A., Garcia, B.G., Valverde, J.C., Gimenez, F.A., Hernandez, M.D. 2010. Gastrointestinal evacuation time in gilthead seabream (*Sparus aurata*) according to the temperature. Aquaculture Research 41 (7), 101 – 1106.

Barlow, C.G., Pearce, M.G., Rodgers, L.J., Clayton, P., 1995. Effects of photoperiod on growth, survival and feeding periodicity of larval and juvenile barramundi *Lates calcarifer* (Bloch). Aquaculture 138, 159-168.

Biswas, A.K., Endo, M., Takeuchi, T., 2002. Effect of different photoperiod cycles on metabolic rate and energy loss of both fed and unfed young tilapia *Oreochromis niloticus*: Part I. Fisheries Science 68, 465-477.

Biswas, A.K. and Takeuchi, T. 2003. Effects of photoperiod and feeding interval on food intake and growth rate of Nile tilapia, *Oreochromis niloticus* L. Fisheries Science 69 (5), 1010-1016.

Biswas, A. K., Seoka, M., Inove, Y., Takii, K., Kumai, H. 2005. Photoperiod influences the growth, food intake, feed efficiency and digestibility of red sea bream (*Pagrus major*) Aquaculture 250, 666-673.

Biswas, A. K., Seoka, M., Ueno, K., Takii, K., Kumai, H. 2008. Stimulation of growth performance without causing stress response in young red sea bream, *Pagrus major*, (Temminck and Schlegel), by photoperiod. Aquaculture Research 39, 457- 463.

Bjornsson, B.T., Johansson, V., Benedet, S., Einarsdottir, I.E., Hildahl, J., Agustsson, T., Johsson, E. 2002. Growth hormone endocrinology of salmonids: regulatory mechanism and mode of action. Fish Physiology and Biochemistry 27, 227-242.

Boeuf, G., Le Bail, P.Y., 1999. Does light have an influence on fish growth? Aquaculture 177, 129-152.

Bolliet, V., Ali, M.A., Lapointe, F.J., Falcon, J., 1996. Rhythmic melatonin secretion in different teleost species: An *in vitro* study. Journal of Comparative Physiology 165B, 677-683.

Carter, C., Glencross, B., Katersky, R.S., Bermudes, M. 2010. The Snooks (Family: Centropomidae) In: Finfish Aquaculture: Species Selection for Diversification. (Ed Le Francois, N.R., Jobling, M., Carter, C., Bljer, P.U) CAB International, Walingford, UK. Chapter 14 pp 323 - 336

Davison, W. 1997. The effects of exercise training on teleost fish, a review of recent literature. Comparative Biochemistry and Physiology Part A: Physiology 117 (1), 67 - 75.

Dyer, A.R., Barlow, C.G, Bransden, M.P., Carter, C.G., Glencross, B.D., Richardson, N., Thomas, P.M., Williams, K.C., Carragher, J.F. 2004. Correlation of plasma IGF-I concentrations and growth rate in aquacultured finfish: a tool for assessing the potential of new diets. Aquaculture 236, 583-592.

Ekstrom, P., Meissl, H., 1997. The pineal organ of teleost fishes. Reviews in Fish Biology and Fisheries 7, 199-284.

El-Sayed, A. M., Kawanna, M. 2007. Effects of photoperiod on growth and spawning efficiency of Nile tilapia (*Oreochromis niloticus L.*) broodstock in a recycling system. Aquaculture Research 38, 1242-1247.

Gines, R., Afonso, J. M., Arguello, A. 2004. The effects of long-day photoperiod on growth, body composition and skin colour in immature gilthead sea bream (*Sparus aurata*). Aquaculture Research 35 (13), 1207-1212.

Gross, W.L., Roelofs, E.W., Fromm, P.O. 1965. Influence of photoperiod on groth of green sunfish, Lepomis cyanellus. Journal of the Fisheries Research Board of Canada 22 (6), 1379 - 1386.

Hovette, E., 2005. Influence of photoperiod and light intensity on growth and survival of *Lates calcarifer*. Honours Thesis, Good Fortune Bay, Queensland

Ibarz, A., Blasco, J., Beltrain, M., Gallardo, M.A., Sanchez, J., Sala, R., Fernandez-Borras, J. 2005. Cold induced alterations on proximate composition and fatty acid profiles of several tissues in gilthead sea bream (*Sparus aurata*). Aquaculture 249, 477 - 486.

Ibarz, A., Beltran, M., Fernandez-Borras, J., Gallardo, M.A., Sanchez, J., Blasco, J. 2007. Alterations in lipid metablism and use of energy depots of gilthead seabream (*Sparus aurata*) at low temperatures. Aquaculture 262, 470-480.

Imsland, A.K., Foss,A., Koedijk,R., Folkvord, A., Stefansson, S.O., Jonassen, T.M. 2007. Persistent growth effects of temperature and photoperiod in Atlantic cod *Gadus morhua.* Journal of Fish Biology 71 (5), 1371 – 1382.

Jobling, M., Jorgensen, E.H., Amesen, A.M., Ringo, E. 1993. Feeding, growth and environmental requirements of Arctic charr: a review of aquaculture potential. Aquaculture International 1, 20-46.

Jobling, M. 1995. The influence of environmental temperature on growth and conversion efficiency in fish. ICES CM 1995/P:4.

Jobling, M. 1997. Temperature and Growth: modulation of growth rate via temperature change. In: Global warming: Implications for Freshwater and Marine Fish pp. 225 – 253. Edited by D.M Wood and D.G McDonald. Cambridge University Press, Cambridge, UK.

Jobling, M. 2010. Are compensatory growth and catch-up growth two sides of the same coin? Aquaculture International 18, 501-510.

Kadri, S. 2003. Grilse reduction and beyond: growth benefits of photoperiod manipulation in cages. Bulletin of the Aquaculture Association of Canada 103 (1), 5-9

Katersky, R.S. & Carter, C.G. 2005. Growth efficiency of juvenile barramundi, *Lates calcarifer*, at high temperatures. Aquaculture 250, 775-780.

Katersky, R.S. & Carter, C.G. 2007(a). High growth efficiency occurs over a wide temperature range for juvenile barramundi, *Lates calcarifer*, fed a balanced diet. Aquaculture, 272**,** 444-450.

Katersky, R.S. & Carter, C.G. 2007(b) A preliminary study on growth and protein synthesis of juvenile barramundi, *Lates calcarifer*, at different temperatures. Aquaculture*,* **267,** 157-164.

Kissil, G.W., Lupatsch I., Elizur A., Zohar Y., 2001. Long photoperiod delayed spawning and increased somatic growth in gilthead seabream (*Sparus aurata*). Aquaculture 200, 363-379.

Le Bail, P.Y., Gentil, V., Noel, O., Gomez, J.M., Carre, F., Le Goff, P., Weil, C. 1998. Trends in comparative endocrinology and neurobiology - From molecular to integrative biology. Annuals of the New York Academy of Sciences 839, 157 - 161.

Mingarro, M., S. V. R. de Celis, 2002. Endocrine mediators of seasonal growth in Gilthead Sea bream (*Sparus aurata*): the growth hormone and somatolactin paradigm. General and Comparative Endocrinology 128(2): 102-111.

McCormick, S.D., Moriyama, S., Bjornsson, B.T. 2000. Low temperature limits photoperiod control of smelting in Atlantic salmon through endocrine mechanisms. American Journal of Physiology, Regulatory and Integrated Comparative Physiology 278, 1352 – 1361.

Perez-Sanchez, J. 2000. The involvement of growth hormone in growth regulation, energy homeostatis and immune function in the gilthead sea bream (*Sparus aurat*a): a short review. Fish Physiology and Biochemistry 22 (2), 135 – 144

Petit, G., Beauchaud, M., Attia, J., Buisson, B. 2003. Food intake and growth of largemouth bass (*Micropterus salmonides*) held under alternated light/dark cycle (12L:12D or exposed to continuous light. Aquaculture 228 (1-4), 397-401

Porter, M.J.R., Duncan, N.J., Mitchell, D., Bromage, N.R., 1999. The use of cage lighting to reduce plasma melatonin in Atlantic salmon (*Salmo salar*) and its effects on the inhibition of grilsing. Aquaculture 176, 237-244.

Porter, M.J.R, Stefansson, S.O., Nyhammer, G., Karlsen, O., Norberg, B. and Bromage, N.R. 2000. Environmental influences on melatonin secretion in Atlantic cod (*Gadus morhua L.*) and their relevance to commercial culture. Fish Physiology and Biochemistry 3, 191-200.

Porter, M.J.R., Duncan, N., Handeland, S.O., Stefansson, S.O., Bromage, N.R., 2001. Temperature, light intensity and plasma melatonin levels in juvenile Atlantic salmon. Journal of Fish Biology 58, 431-438.

Prosser, C.L. 1991. Environmental and Metabolic Animal Physiology: Comparative Animal Physiology and Ecology of Ectotherms. 4th Edn. Wiley-Leiss: New York.

Quigley, D.T.G, 2003. Early maturation in the Irish salmon farming industry. Bulletin of the Aquaculture Association of Canada 103 (1) 27-35

Taylor, J.F., Migaud, H., Porter, M.J.R., Bromage, N.R. 2005. Photoperiod influences growth rate and plasma insulin-like growth factor-I levels in juvenile rainbow trout, *Oncorhynchus mykiss*. General and Comparative Endocrinology 142, 169 – 185.

Trippel, E.A., Neil, S.R.E., 2003. Effects of photoperiod and light intensity on growth and activity of juvenile haddock (*Melanogrammus aeglefinus*). Aquaculture 217 (1-4), 633-645.

Tucker, J.W., Russell, D.J., Rimmer, M.A. 2002. Barramundi culture: A success story for aquaculture in Asia and Australia. World Aquaculture 33 (3), 53-59.

Vera, E.M. and Brown, C.L. 2009. Influence of the photoperiod on growth rate and insulin-like growth factor-I gene expression in Nile tilapia *Oreochromis niloticus*. Journal of Fish Biology 75, 130-141.

Volkoff, H., Canosa, L.F., Unniappan, S., Cerda-Reverter, J.M., Bernier, N.J., Kelly, S.P., Peter, R.E. 2005. Neuropeptides and the control of food intake in fish. General and Comparative Endocrinology 142, 3-19

Worrall, K., Carter, C., Porter, M.J.R. 2004. Effects of Photoperiod on the Growth of Juvenile Barramundi (*Lates calcarifer*). Hons Thesis, University of Tasmania.

Xiao, Y.S. 2000. Use of the original von Bertalanffy growth model to describe the growth of barramundi, *Lates calcarifer* (Bloch). Fishery Bulletin 98 (4), 835 – 841.

CHAPTER 3

Effects of Photoperiod and Feeding Regime on Growth of Juvenile Barramundi (*Lates Calcarifer*) (Bloch)

K. L. Worrall[a], C. G. Carter[b], M. J.R. Porter[c] , R. J. Wilkinson[a]

[a] National Centre for Marine Conservation and Resource Sustainability, University of Tasmania, Locked Bay 1370, Launceston, TAS, 7250, Australia

[b] Tasmanian Aquaculture and Fisheries Institute, University of Tasmania, Private Bag 49, Hobart, TAS 7001, Australia

[c] Ridley Aquafeed Pty Ltd, 12 Neon Street, Narangba Brisbane, QLD, 4504, Australia

Corresponding author. Tel.: +61 3 6324 3800; fax: +61 3 6324 3804

E-mail address: kworrall@utas.edu.au

Keywords: Asian sea bass; Barramundi; *Lates calcarifer*; Photoperiod; Growth; Feeding ration; Feeding frequency.

3.1 Abstract

This experiment investigated the effects of photoperiod and feeding regime on growth and endocrine parameters in juvenile barramundi (*Lates calcarifer*) (Bloch) over a period of 50 days. Barramundi (19.01 ± 0.42 g) were reared under 12L:12D or 24L:0D while being fed either twice or four times a day at a daily ration of 3% bw.d^{-1} (12 2R, 24 2R, 24 4R) or to satiation (12 2S, 24 2S, 24 4S). At a ration of 3% bw.d^{-1}, photoperiod and feeding regime did not have a significant effect on wet weight, total length, SGR weight, SGR length or FCE in juveniles. Photoperiod and feeding regime significantly affected growth and endocrine parameters when juveniles were fed to satiation. Significantly higher wet weight (141.54 ± 8.08 g), total length (22.53 ± 0.39 cm) were observed in fish reared under 24L 2S compared to 12L 2S (129.40 ± 8.98 g; 21.37 ± 0.48 cm;). Significantly higher wet weight and total length observed in fish reared under 24L 2S were observed without significant differences in feed intake, HSI or condition factor compared to 12 2S. This may suggest improved utilization of feed in barramundi reared under continuous light.

Further significant increases in growth were observed in fish reared under 24L:0D and fed additional feeds over what would normally be the scotophase (24 4S) compared to both 24 2S and 12 2S. Wet weight was significantly greater (15% higher) compared to fish reared under 24L:0D, fed to satiation twice during the photophase. Additionally, wet weight and total length was significantly greater (25.5 % in wet weight and 5% in total length) compared to 12L:12D, fed to satiation twice during the photophase. Albeit, increased wet weight in fish reared under 24 4S was concomitant with significantly

higher feed intake of 130.83 ± 8.82 g compared to 103.07 ± 3.87 and 97.92 ± 4.10 in 24 2S and 12L 2S respectively after 50 days.

Plasma insulin-like growth factor (IGF-I) concentrations reflected growth, with elevated concentrations occurring in fish with higher growth rates. No significant differences in IGF-I concentrations were observed in fish fed 3% $bw.d^{-1}$, irrespective of photoperiod (12 2R = 34.87 ± 3.27 $ng.mL^{-1}$; 24 2R = 33.74 ± 2.57 $ng.mL^{-1}$; 24 4R = 33.67 ± 2.85 $ng.mL^{-1}$). In contrast, when fed to satiation, significantly higher plasma IGF-I concentrations were observed in fish reared under 24L:0D (24 2S = 41.67 ± 2.73 $ng.mL^{-1}$; 24 4S = 46.35 ± 2.46 $ng.mL^{-1}$) compared to 12L:12D (12 2S = 30.50 ± 1.80 $ng.mL^{-1}$). This suggested up-regulation of the growth hormone-IGF-I axis in response to constant light, although being influenced by feed ration.

Current results help consolidate previous experiments indicating 24L:0D increases growth of juvenile barramundi when held at 30°C and fed to satiation twice daily. Additionally the current results expands this knowledge indicating barramundi will continue to feed over a 24 hour period when reared under 24L:0D. Further growth increases in barramundi are observed when reared under 24L:0D and fed twice during what would normally be the scotophase, albeit at a significantly lower FCE. Further research to optimise feeding regimes over a 24 hour continuous light period to achieve better feed conversion efficiencies will increase knowledge towards optimising artificial lighting techniques used to improved barramundi growth. The current results provide support for the application of photoperiod manipulation to enhance growth of juvenile

barramundi. In addition, this research provides feeding regime options for juvenile barramundi reared under 24L:0D.

3.2 Introduction

Photoperiod manipulation is used to improve commercial production of fish which increase economic benefits to industry (Porter et al., 1999, 2003; Taranger et al., 2006). Photoperiod manipulation is suggested to indirectly modify growth rates of fish by increasing food intake or feed utilization efficiency and/or muscle mass by exercise (Boeuf and Le Bail, 1999; Boeuf and Falcon, 2001, Volkoff et al., 2010). The majority of photoperiod manipulation research has being applied to temperate species, whereas recently these techniques have also been successful in sub-tropical and tropical species, such as red sea bream (*Pagrus major*), striped knifejaw (*Oplegnathus fasciatus)* and Nile tilapia (*Oreochromis niloticus)* (Biswas et al., 2005, 2008; El-sayed and Kawanna, 2007).

Recent research into photoperiod manipulation and juvenile barramundi (*Lates calcarifer*) (Bloch), demonstrated significant increases in growth without significant increases in feed intake when reared under 24L:0D (Worrall et al., 2004). In addition to photoperiod, feed ration and frequency also influences growth through feed intake and food utilization (Silva et al. 2007). The combined effects of photoperiod and feeding regime have been investigated in a number of species such as Nile tilapia and snapper (*Pagrus auratus*) with significantly improved growth rates being observed with optimisation of feeding frequencies over extended photoperiods (Biswas and Takeuchi, 2003; Zhou et al., 2003; Biswas et al., 2005; Tucker et al., 2006). Feeding frequency in barramundi has been studied in fry and juveniles in various rearing conditions, with

optimal feeds results ranging from two to three daily feeds (Harpaz, 2005; Biswas et al., 2010). Optimisation of feeding ration and frequency will depend on each fish species, its size and rearing conditions (Cho et al., 2003). Optimal feed rations and nutritional requirements for barramundi of varying weights at varying temperatures have been researched thoroughly (Williams and Barlow, 1999; Glencross et al., 2006; Katersky & Carter, 2007). Additionally, growth and feeding of larval (2-10 days old) and juvenile barramundi (11 – 12mm total length) reared under extended photoperiod has been investigated and it has been concluded that extended light had little benefit beyond the larval phases of growth (Barlow et al., 1995). Although, Barlow et al., (1995) investigated smaller juveniles being fed live feeds, in comparison to the current study in which juveniles (12 – 23 cm total length) are fed a commercial pelleted diet. This being said, they showed the effect of extending light hours on the growth rate of barramundi was size dependant.

The current experiment investigated the effects of feed ration (satiated vs rationed feed (3% bw.d^{-1})); and frequency (twice daily during the photophase or four times daily during continuous light), on growth of barramundi (12 – 23 cm total length) while reared under 12L:12D and 24L:0D. Key growth parameters (wet weight, total length, feed intake, feed conversion efficiency, specific growth rate and hepatosomatic index) were measured.

Enhanced growth under 24L:0D without increased feed intake may occur from improved feed conversion efficiencies (FCE) (Boeuf and Le Bail, 1999). Possible direct mechanisms for improved FCE include extended day length which provides a longer time frame for digestion and therefore improved assimilation of feed. Indirectly, extended day

length may alter enzymes allowing improved assimilation of feed both causing improved utilization of feed in fish (Harpaz et al., 2005). Improved feed conversion efficiencies may also be influenced by the endocrine/growth axis (Cruz and Brown, 2009; Volkoff et. al., 2010). The hormone IGF-I is a central link in the endocrine/growth axis, influencing fish growth and development by acting on muscle and cartilage cell growth, stimulating nutrient uptake and protein synthesis while influencing a range of related hormones involved in the endocrine/growth axis (Le Bail et al., 1998; Pérez-Sánchez, 2000; Björnsson et al., 2002; Imsland et al., 2007). Plasma IGF-I concentrations in fish are regulated in part by photoperiod, with concentrations increasing with increased day length (M^{c}Cormick et al., 2000). Changes in fish growth are suggested to be reflected in alterations of IGF-I profiles, with levels of IGF-I observed to positively correlate with growth (Mingarro et al., 2002; Taylor et al., 2005). In addition to IGF-I possibly influencing feed conversion efficiencies, observing IGF-I concentrations may provide a useful tool for monitoring barramundi growth rates as concentrations of IGF-I positively correlate with barramundi growth rates (Dyer et al., 2004; Chapter 3).

The aim of this experiment investigates synergistic effects of varied feeding regimes on the growth of barramundi when reared under 24L:0D and 12L:12D. Feeding barramundi a rationed vs satiated feed while reared under 12L:12D and 24L:0D will allow a better understanding of how feed intake is involved with growth increases observed under 24L:0D. Additionally, increasing feeding frequency from twice a day to four times a day in barramundi reared under 24L:0D, will indicate if barramundi will continue to feed over a 24 hour period as well as ascertain any further effects on growth. Ascertaining

circulating levels of the growth related hormone, IGF-I, will help to determine a possible endocrine mechanism for barramundi response to photoperiod.

3.3 Material and Methods

3.3.1. Experimental Design

Juvenile barramundi (fingerlings approximately 60 days old) from WBA Hatcheries, Adelaide (South Australia) were acclimated for a period of 5 days in 180 L aquaria at 30^{o}C in 10 ‰ seawater and held under 12L:12D. Fish were not fed during the acclimation period. A total of 480 fish (19.01 ± 0.42 g) were randomly stocked into 24 x 80 L tanks (20 fish per tank) maintained on 4 separate recirculation systems consisting of temperature control, mechanical filtration, biological filtration and foam fractionation (6 tanks per system). Initial mean stocking density was 5.64 kg/m^{3}for each replicate tank. Water was maintained at 10‰ salinity and delivered at a rate of 2.8L.min^{-1} with oxygen levels being maintained above 90% saturation. Water parameters (Appendix 1) was monitored daily and water changes occurring as necessary to keep water quality within the limits for barramundi (Tucker et al., 2002). Water quality parameters were consistent across all treatment tanks for the duration of the experiments. Particulate dacron filters were cleaned daily and water exchange was less than 10% per day (to replace water discarded during cleaning and siphoning uneaten feed).

Each system was maintained at 30^{o}C with control over the water temperature achieved using submersible heaters in each reservoir, each controlled with an individual

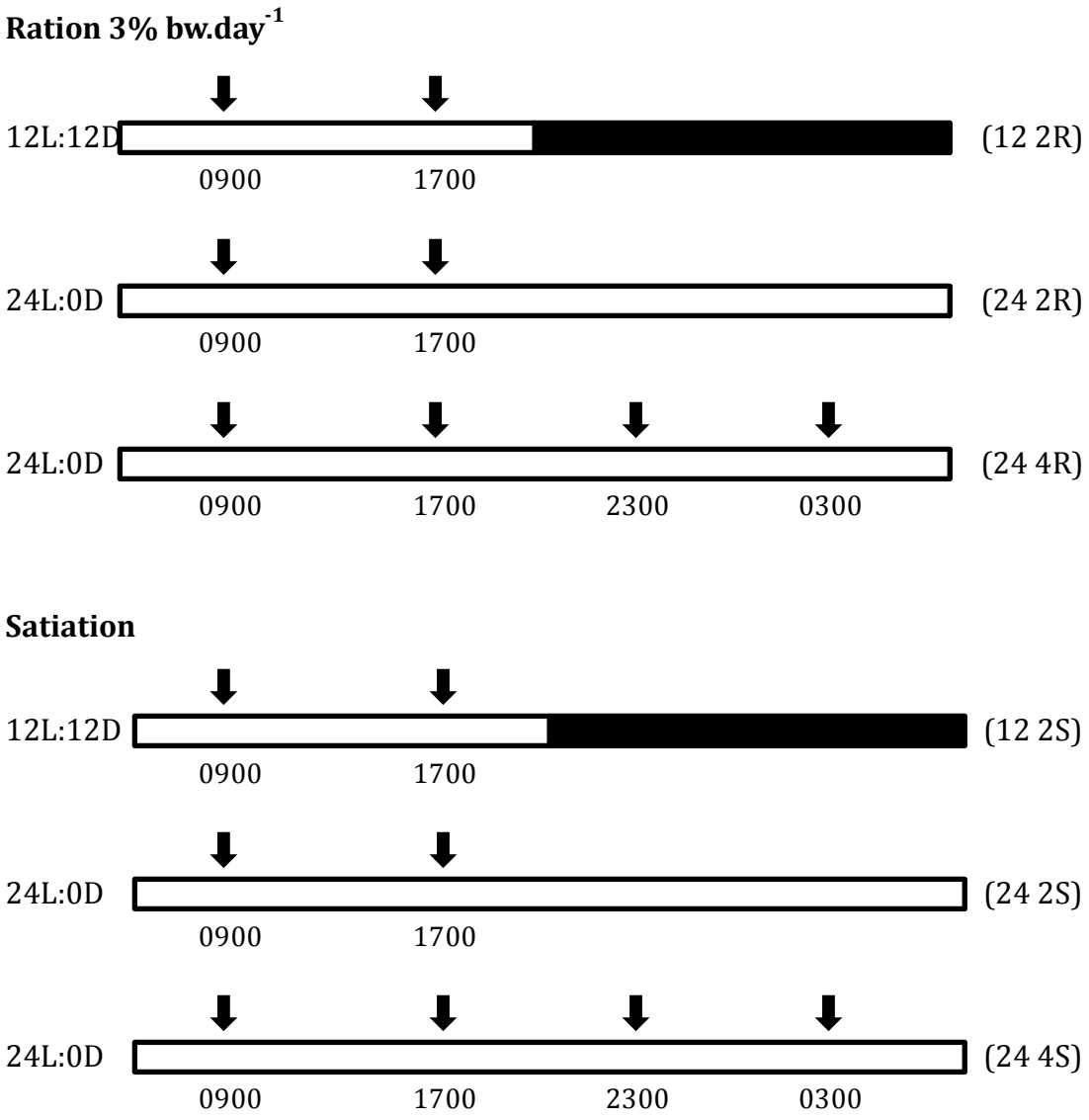
thermostat. Temperature was recorded every half hour with StowAway Tidbit Temperature Loggers (Onset Computer Company, Bourne, MA, USA) as well as each system being manually checked daily. Diurnal variation in water temperature in each re-circulation system was ± 0.5°C of the intended temperature.

Experimental treatments were partitioned into photoperiods using individual tank covers which incorporated individual halogen waterproof lights set on individual timers allowing manipulation of light. Artificial lighting was supplied by 20 W Halogen white light, with timers set to turn on lights, without a dimming effect, at 0700 and turn off at 1900 for the 12L:12D treatments. Average light intensity sampled throughout a number of locations within the water column of each experimental tank was 540 lux (~9 μmol s^{-1} m^{-2}) which was measured using a Li-COR Underwater Quantum sensor (LI-192SA).

Fish were maintained on experimental treatments for a period of 50 days. During this time fish were hand fed a commercial high protein pelleted diet ranging from 1 mm at the beginning of the trial and progressing to 4 mm. (Marine Start 1-3mm, Marine Float 54/10 4mm, crude protein 52% and crude Fat 16%, Ridley Aquafeed, Australia). Feed intake was recorded for each tank after each feed with any uneaten pellets being siphoned out and counted at the end of each feed.

The experiment comprised of six treatments with selected combinations of photoperiod, feed ration and feeding frequency (Figure 3.1): photoperiods 24L:0D or 12L:12D; ration 3% bw.day^{-1} or satiation; and feeding frequency (2 feeds per day at 0900 and 1700 or 4 feeds per day at 0900, 1700, 2300 and 0300).

Figure 3.1. Experimental treatments detailing photoperiod and feeding frequency at both feeding regimes – Ration 3% bw.d^{-1} and Satiation.



Photoperiod is depicted by light and dark bars. Feeding frequency is depicted by arrows at specific times. Abbreviated titles for treatments are depicted at the end of each line and show hours of light (12L or 24L), feeds per day (2 or 4) and feed ration (R, ration or S, satiation).

3.3.2 Sampling Procedures

Sampling involved netting all fish from experimental tanks and transferring them to a 20 L tank containing iso-eugenol at 40 $mg.L^{-1}$ (AQUI-S, New Zealand Ltd). An initial sample of 20 fish from acclimation tanks were anaesthetized, measured for wet weight (to nearest 0.1 g) and total length (cm), blood sampled for circulating plasma IGF-I then euthanized for lipid analysis. Whole livers were used for nutritional tissue analysis using standard methods from Bligh and Dyer (1959) where tissue was freeze dried to a constant weight and analysed for crude lipid. Every two weeks all 20 fish from each tank were anesthetized and measured for total length (cm) and wet weight (nearest 0.1 g) then returned back to each tank, while on day 30 and at the completion of the trial (day 50) 10 fish were sampled for blood and euthanized for further analysis as above. The total biomass (g) of fish in each tank was measured every 14 days and feed weights for 3% $bw.d^{-1}$ ration treatments were adjusted accordingly.

Blood (approximately 1 mL) was drawn from the caudal artery of fish (10 fish at Day 30 and Day 50) for analysis of insulin-like growth factor I (IGF-I). Blood was collected using heparinised (ammonium heparin, Sigma; 4mg/ml) syringes (1 ml Terumo syringes, 25G Terumo hypodermic needles) then centrifuged at 3500 rpm at $4^{o}C$, for 15mins and stored at -20°C until assayed. Plasma IGF-I levels were analysed using a commercially available RIA kit as previously described by Wilkinson et al., (2006) (GroPep, Adelaide, Australia).

3.3.3. Calculations

The following equations were used to calculate feed intake (FI), feed conversion efficiency (FCE), specific growth rate of weight and length (SGR weight and SGR length), condition factor (K) and hepatosomatic index (HSI) for each replicate tank (n=4) on day 0, 30 and 50;

FI (g) = total dry feed intake/fish

FCE (%) = 100 x (wet weight gain/total dry feed intake).

SGR weight (% $bw.d^{-1}$) = 100x (lnW_2-lnW_1)/time (days)

Where, W_1 and W_2 were the initial and final wet weight (g), respectively.

SGR length (% $lt.d^{-1}$) = 100 x (TL_2-TL_1)/time (days)

Where, TL_1 and TL_2 indicate the initial and final total length (cm) respectively.

$K = 100 \times (W / TL^3)$

Where, W = wet body weight (g) and TL = total body length (cm)

HSI (%) = 100 x (wet weight of liver (g)/wet body weight (g))

3.3.4. Statistical Analysis

Statistical analysis was carried out using SPSS 16.0 for windows (SPSS Inc.). For each treatment, mean ± standard error was calculated from 20 fish in each of the four replicate tanks (n=4). The overall mean for growth data, feed intake, total lipid and hormone levels of each replicate tank was analysed using one-way ANOVA, where there were

significant differences a Tukeys post hoc test was performed to identify differences between pairs of treatment groups. A two-way nested ANOVA was also applied in order to calculate the overall effects of time, feeding regime and photoperiod on growth data with tanks nested within photoperiod and feeding regimes. Differences were considered to be significant if $P<0.05$. Values are presented as means ± standard error (SEM).

3.4 Results

3.4.1 Growth – Treatments fed to satiation (12 2S; 24 2S; 24 4S)

Feed intake (g) was significantly higher in fish reared under 24 4S compared to 24 2S and 12 2S (one-way ANOVA; $P<0.01$; F = 36.08; df = 3), which were not significantly different from each another (Figure 3.2A). FCE was significantly reduced in fish reared under 24 4S compared to 24 2S and 12 2S, which were not significantly different from one another (one-way ANOVA; $P< 0.05$; F = 5.93; df = 2) (Figure 3.2B).

Initial weights and lengths of fish were not significantly different between treatments (18.63 ± 0.09 g and 11.98 ± 0.02 cm) (one-way ANOVA weight; $P>0.05$; F = 1.26; df = 23) (one-way ANOVA length; $P>0.05$; F = 2.34; df = 23). At day 30, 24 4S demonstrated greater wet weight and total lengths compared to 12L 2S and 24 2S, which did not significantly differ from each other. At day 50, fish reared under 24 4S continued to demonstrated significantly greater wet weight and total length in comparison to 12 2S and 24 2S. Whereas, at day 50, significantly greater wet weight and total length was observed in fish reared under 24 2S compared to 12 2S. (weight = two-way ANOVA; $P<0.01$; F = 604.01; df = 8) (length = two-way ANOVA; $P<0.01$; F = 913.42; df = 8) (Figure 3.3 A and B).

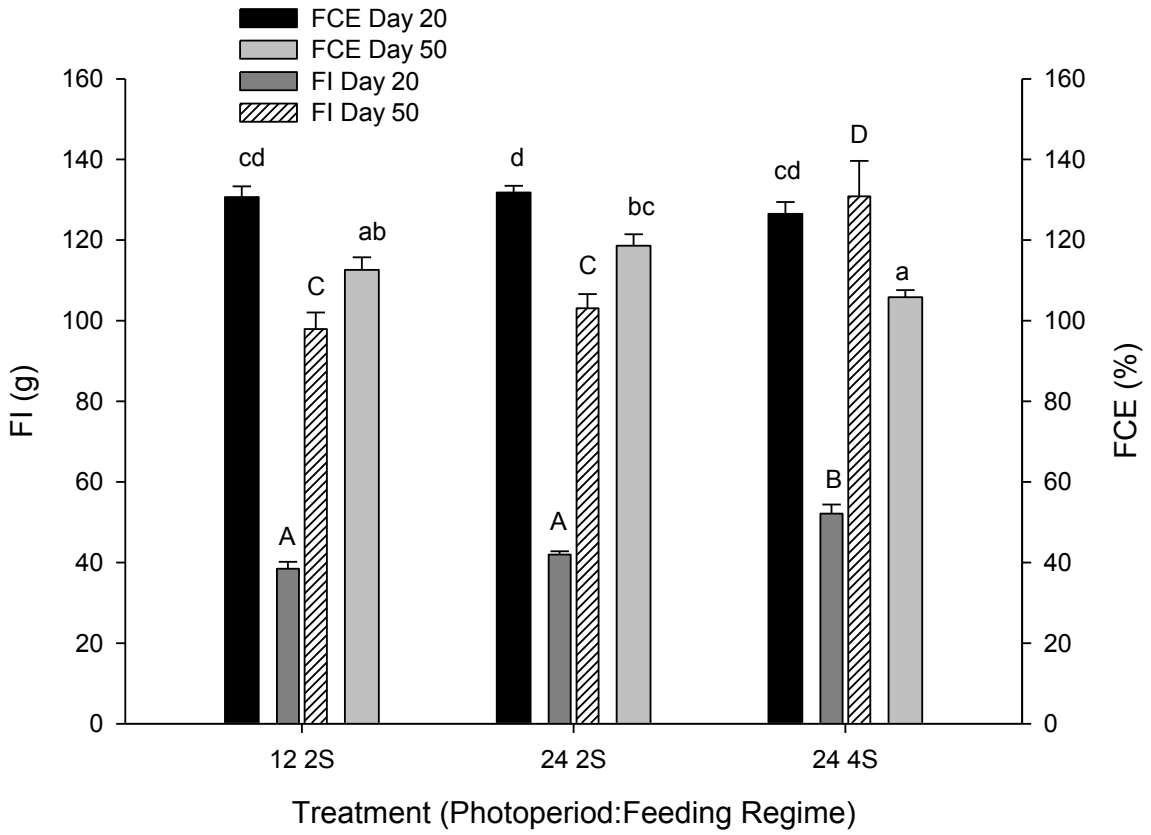


Figure 3.2. Feed Intake (FI) (g ± SEM) per fish (A) and Feed conversion efficiency (FCE) (% ± SEM) (B) of juvenile barramundi held under 12L:12D and 24L:0D fed to satiation over 12 hours (12 2S and 24 2S) and 24 hours (24 4S) at Day 30 and Day 50. Different letters denote significant differences (P<0.05).

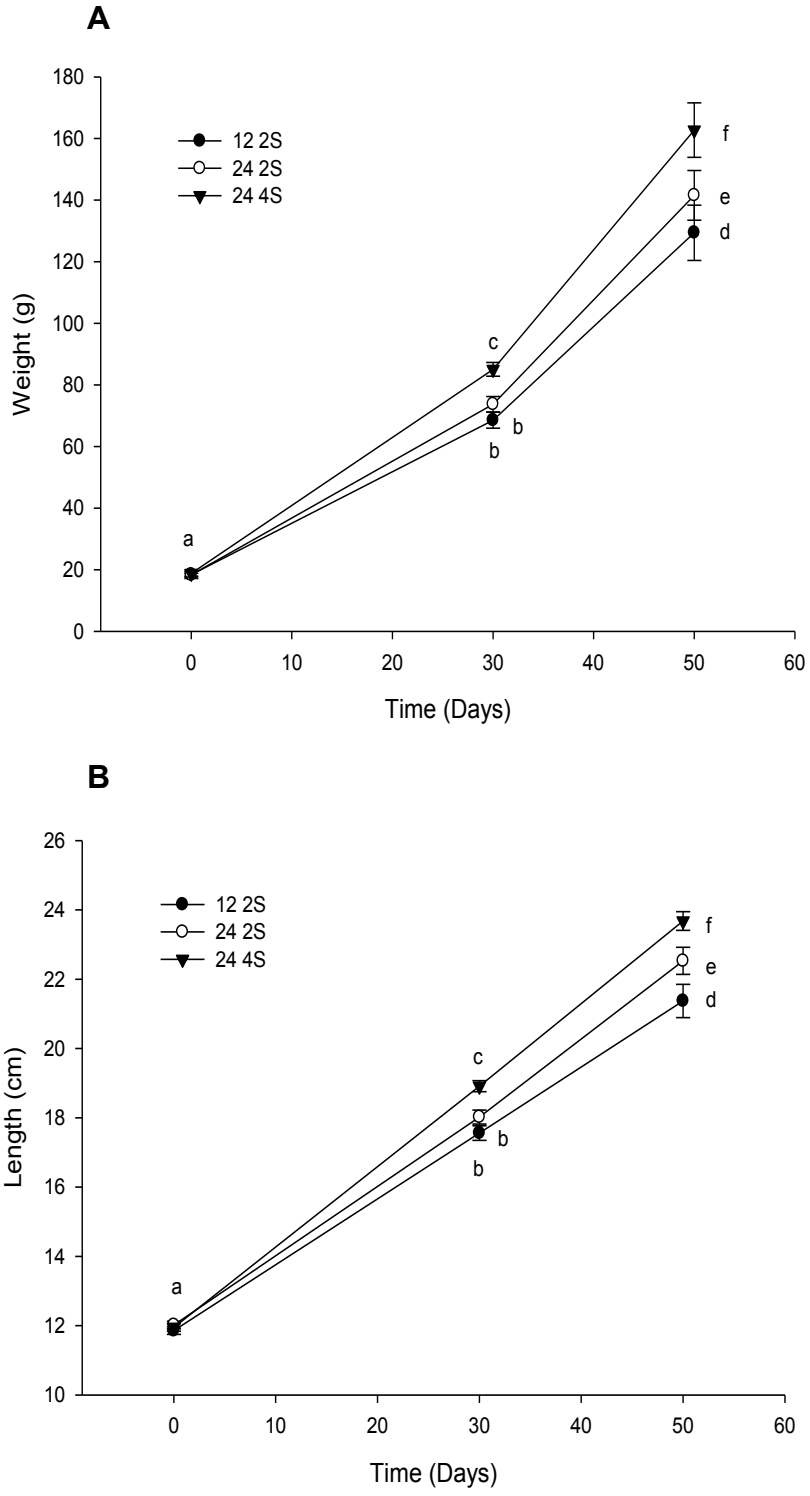


Figure 3.3. Mean wet weight (A) (g ± SEM) and mean total length (B) (cm ± SEM) of juvenile barramundi held under 12L:12D and 24L:0D fed to satiation over 12 hours (12 2S and 24 2S) and 24 hours (24 4S). Different letters denote significant differences (P<0.05).

SGR weight (% $bw.d^{-1}$) was significantly greater in fish reared under 24L 4S compared to 12 2S although not significant compared to 24 2S at both day 30 and day 50 (two-way ANOVA; $P<0.05$; F = 94.56; df = 5) (Figure 3.4A). No significant differences of SGR length were observed between photoperiod or feeding regime (two-way ANOVA; $P<0.01$; F = 11.58; df = 5) (Figure 3.4B).

Condition factor (K) was not significantly different between treatments at day 1, 30 and 50, although was significantly lower at day 1 in comparison to day 30 or 50. (two-way ANOVA; $P< 0.01$; F = 12.17; df = 8) (Figure 3.5A).

At day 1 and 30, HSI did not significantly differ between treatments. At day 50, a significantly greater HSI was observed in fish reared under 12 2S compared to both treatments exposed to 24L:0D (two-way ANOVA; $P< 0.05$; F = 3.26; df = 5) (Figure 3.5A). Crude lipid levels (%) at day 50 were significantly higher in fish reared under 24 4S compared to both 12 2S and 24 2S which were not significantly different from each other (one-way ANOVA; $P< 0.01$; F = 7.668; df = 4) (Figure 3.5B).

Concentrations of plasma IGF-I on day 30 did not significantly differ between treatments. At day 30 and day 50, plasma IGF-I concentrations did not significantly differ in fish reared under 12 2S. At Day 50, plasma IGF-I concentrations were significantly higher in fish reared under 24L:0D compared to 12L:12D. No significant differences in plasma IGF-I concentrations were observed between 24 2S and 24 4S at either day 30 or day 50. (two-way ANOVA; $P<0.01$; F = 8.86; df = 2) (Figure 3.6).

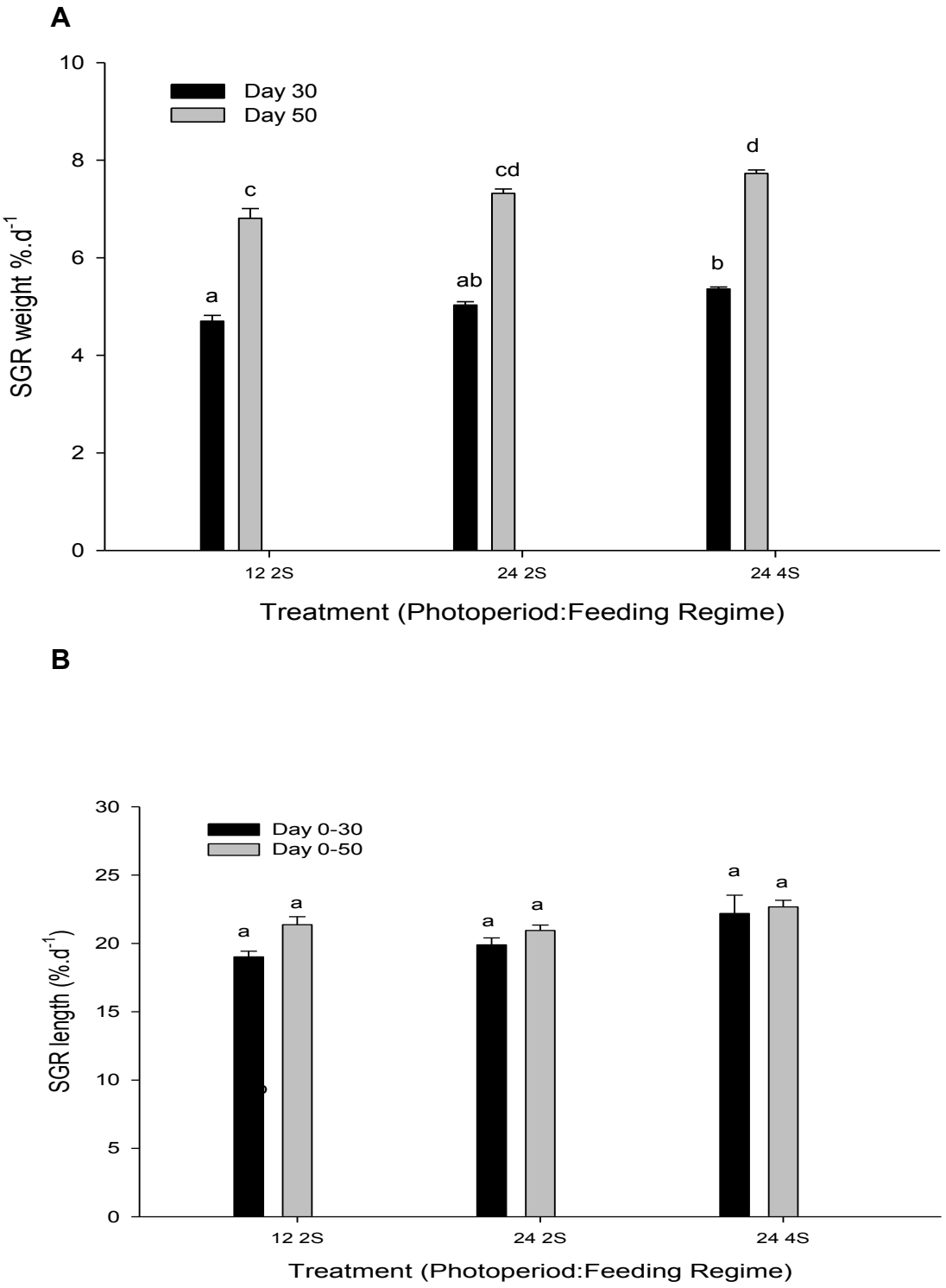
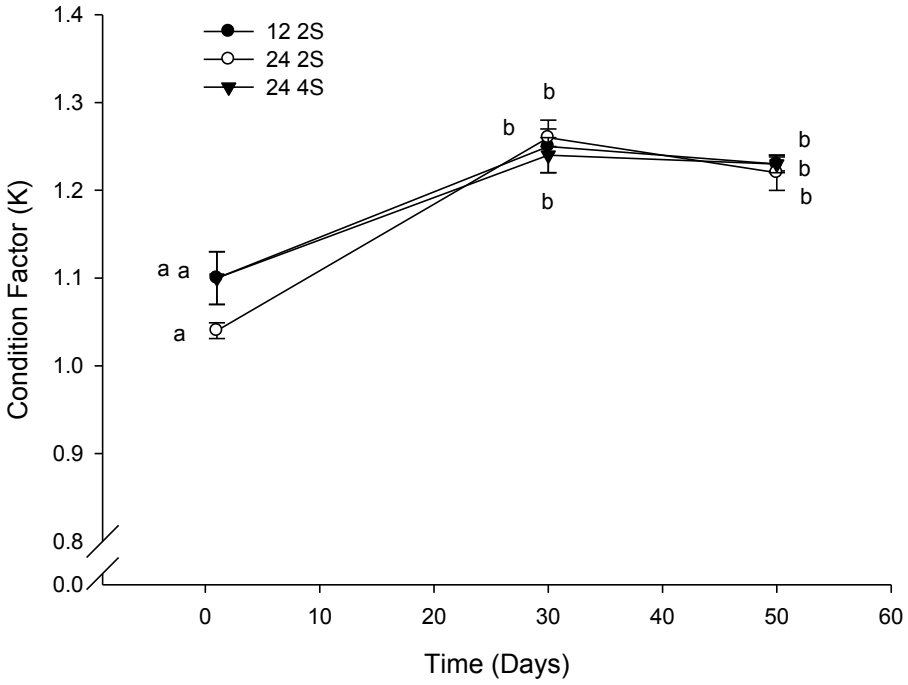
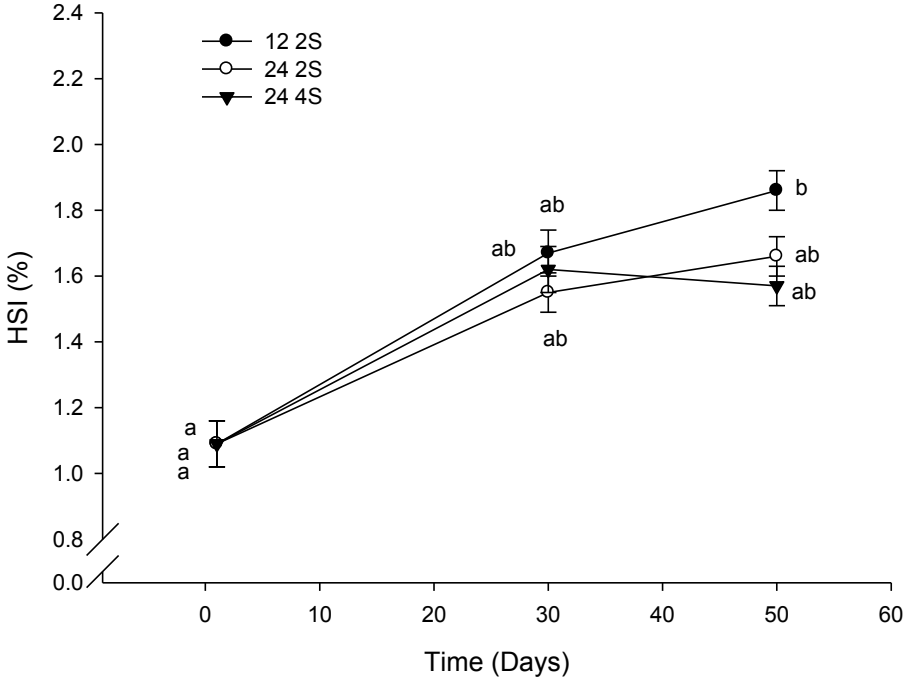


Figure 3.4. Mean specific growth rate; weight (SGR) (% bw.d^{-1} ± SEM) (A) and length (SGR) (% lt.d^{-1} ± SEM) (B) of juvenile barramundi, at Day 30 and Day 50, held under 24L:0D and 12L:12D being fed to satiation over 12 hours (12 2S and 24 2S) and 24 hours (24 4S). Different letters denote significant differences (P<0.05).


12 2S
24 2S
24 4S
Condition Factor (K)
Time (Days)
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.0
0
10
20
30
40
50
60
a a
a
b
b
b
b
b
b

B


12 2S
24 2S
24 4S
HSI (%)
Time (Days)
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.0
0
10
20
30
40
50
60
a
a
a
ab
ab
ab
b
ab
ab

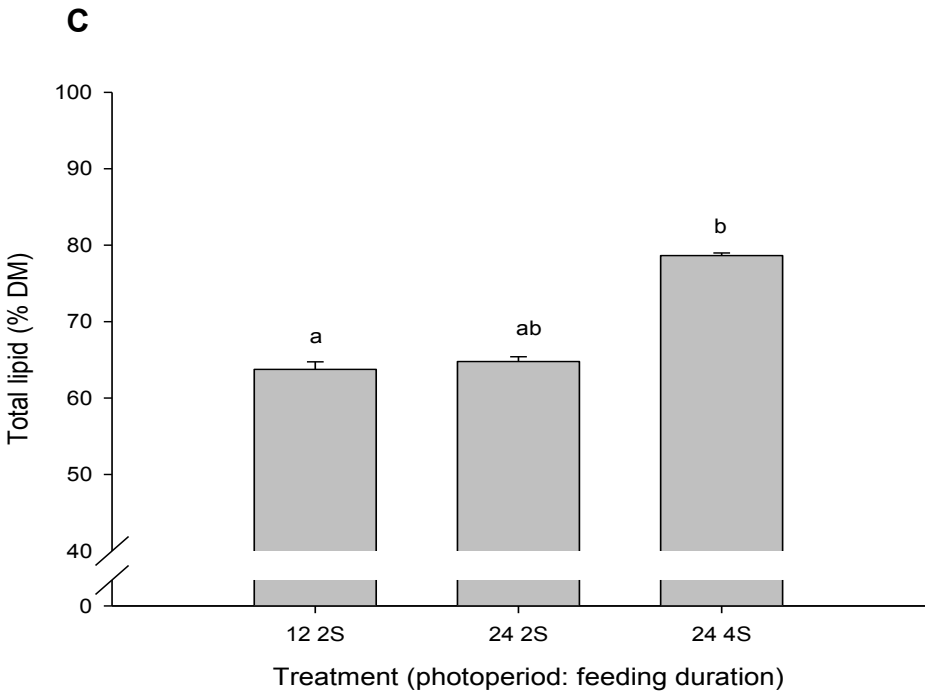


Figure 3.5. Condition factor (K ± SEM) (A), hepatosmatic index (HSI) (% ± SEM) (B) and of juvenile barramundi, at Day 1, 30 and Day 50 and mean total lipid (% DM ± SEM) of liver on Day 50 (C), held under 24L:0D and 12L:12D being fed to satiation over 12 hours (12 2S and 24 2S) and 24 hours (24 4S). Different letters denote significant differences (P<0.05).

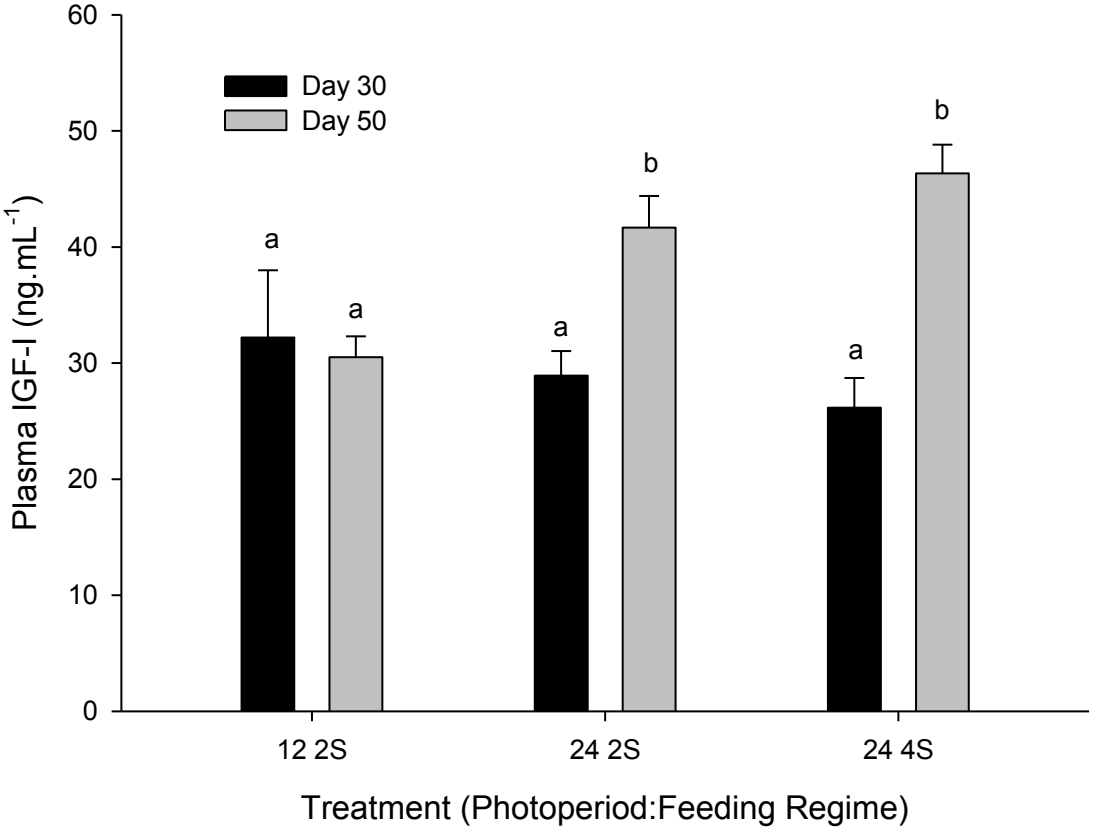


Figure 3.6. Mean plasma IGF-I concentration (ng.mL^{-1} ± SEM) of juvenile barramundi, taken at Day 30 and Day 50 held under 12L:12D and 24L:0D being fed to satiation over 12 hours (12 2S and 24 2S) and 24 hours (24 4S). Different letters denote significant differences (P<0.05).

3.4.2 Growth – Treatments fed Rationed 3% bw.day^{-1} (12 2R, 24 2R, 24 4R)

Initial weight and length of fish were not significantly different between treatments (18.63 ± 0.09 g and 11.98 ± 0.02 cm) (one-way ANOVA; $P>0.05$; F = 1.26; df = 23) (one-way ANOVA; $P>0.05$; F = 2.34; df = 23). At day 30 and day 50, no significant differences in weight or length between treatments were observed, irrespective of photoperiod or feeding regimes (two-way ANOVA; $P>0.05$; F = 477.34; df = 8) (two-way ANOVA; $P>0.05$; F = 459.06; df = 8) (Figure 3.7A and B). In conjunction with weight and length, no significant differences between treatments were observed in SGR weight and SGR length (two-way ANOVA; $P>0.05$; F = 2.59; df = 5); condition factor (two-way ANOVA; $P>0.05$; F = 1.18; df = 5); feed intake (one-way ANOVA; $P>0.05$; F = 1.07; df = 2); feed conversion efficiency (one-way ANOVA; $P>0.05$; F = 2.34; df = 2) and plasma IGF-I levels (one-way ANOVA; $P>0.05$; F = 2.47; df = 5)(Table 3.1). Significantly lowered HSI were observed in juveniles reared under 24 2R (1.37 ± 0.06) compared to 12 2R (1.71 ± 0.10) and 24 4R (1.68 ± 0.02) (one-way ANOVA; $P<0.05$; F = 4.88; df = 2) (Table 3.1).

Significantly reduced wet weight (g) and feed intake (g) was observed in fish fed a rationed feed of 3% bw.day^{-1} compared to fish fed to satiation at both day 30 and day 50 (two-way ANOVA; $P>0.01$; F = 198.53; df = 11) (Figure 3.8A and B).

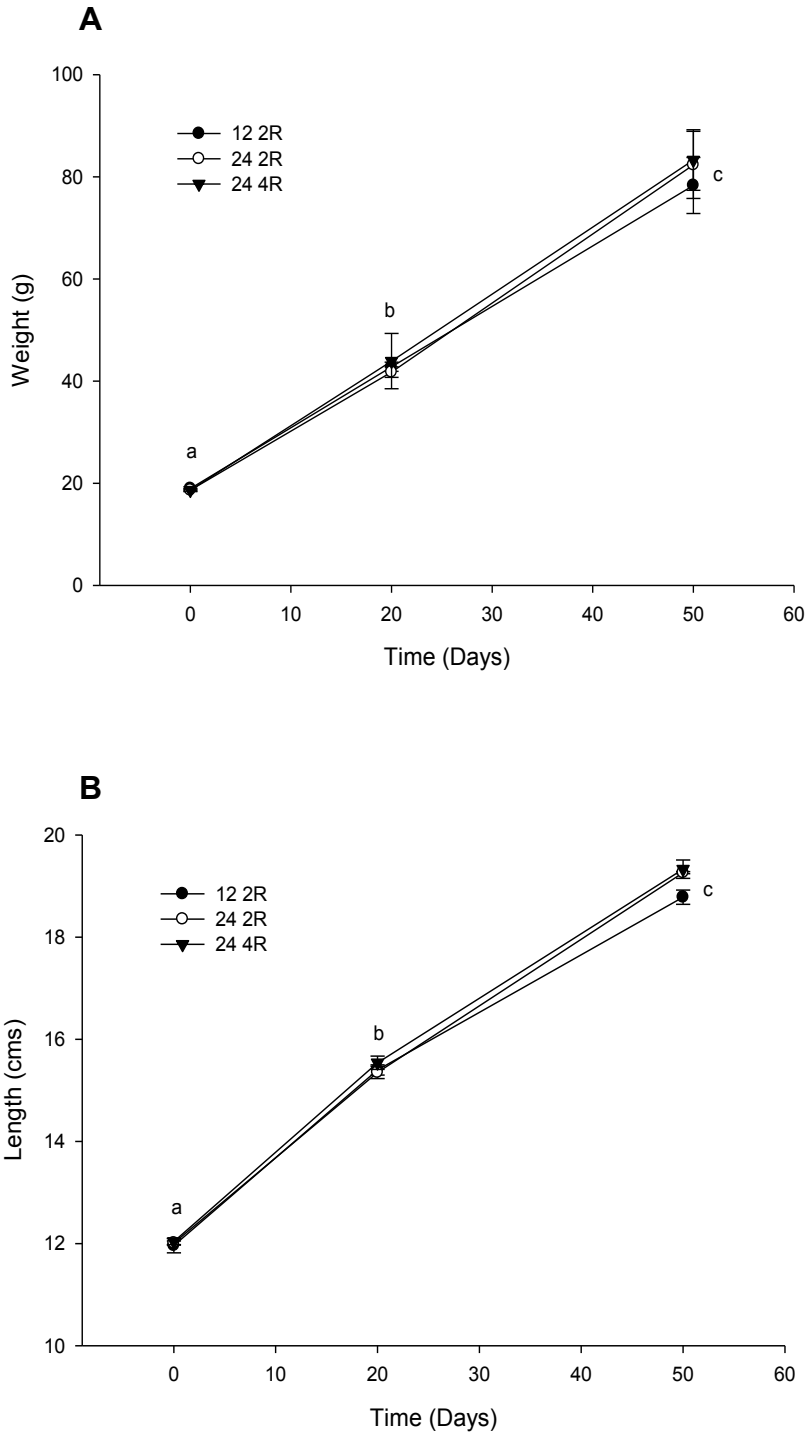
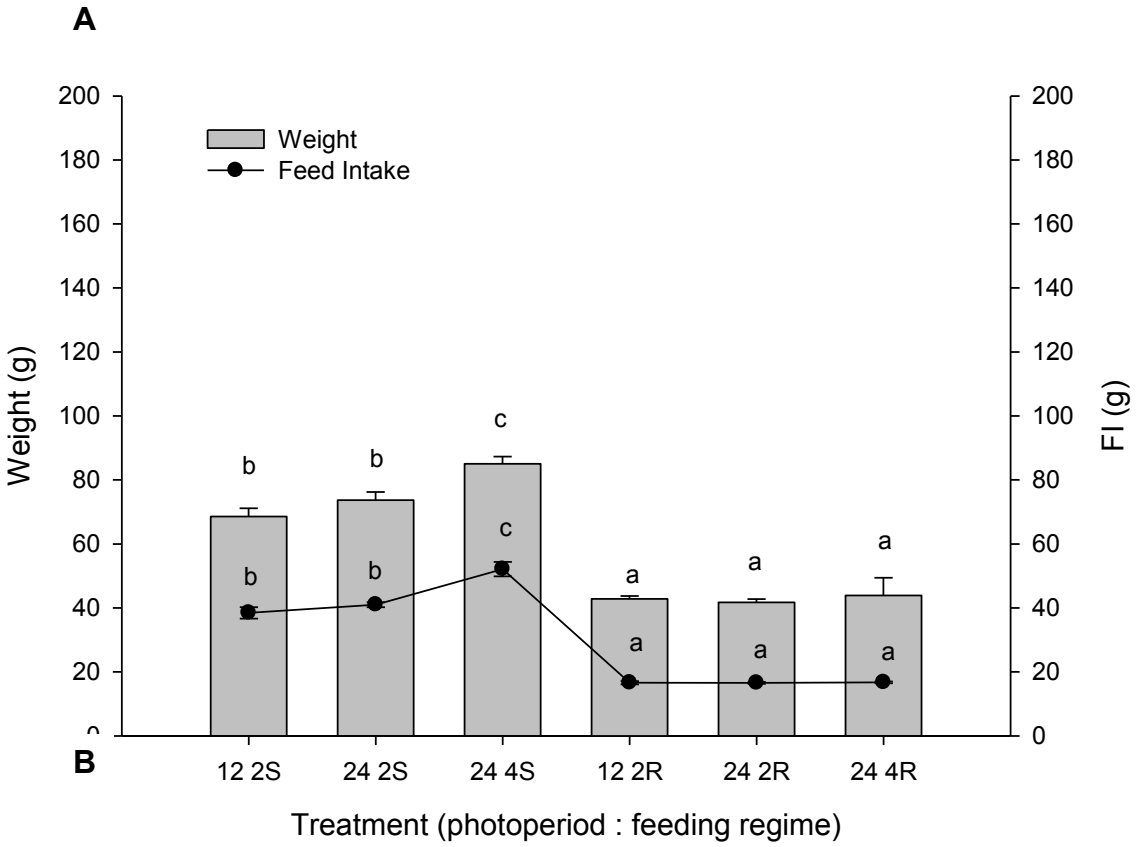


Figure 3.7. Mean wet weight (g ± SEM) (A) and mean total length (cm ± SEM) (B) of juvenile barramundi held under 12L:12D and 24L:0D fed 3% bw.d^{-1} over 12 hours (12 2R and 24 2R) and 24 hours (24 4R). Different letters denote significant differences (P<0.05).


A
Weight
Feed Intake
Weight (g)
FI (g)
0
20
40
60
80
100
120
140
160
180
200
b
b
c
a
a
a
12 2S
24 2S
24 4S
12 2R
24 2R
24 4R
Treatment (photoperiod : feeding regime)

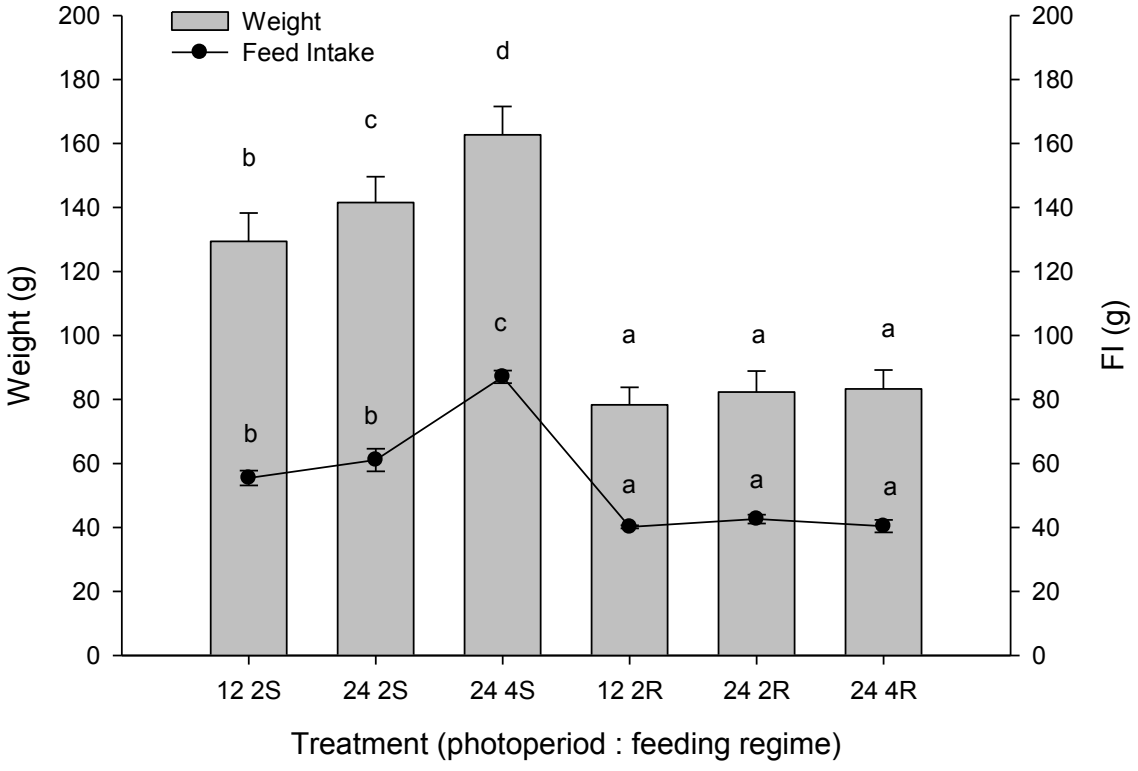

B
Weight
Feed Intake
Weight (g)
FI (g)
0
20
40
60
80
100
120
140
160
180
200
b
c
d
a
a
a
12 2S
24 2S
24 4S
12 2R
24 2R
24 4R
Treatment (photoperiod : feeding regime)

Figure 3.8. Mean wet weight (g ± SEM) and feed intake (g ± SEM) of juvenile barramundi held under 12L:12D and 24L:0D fed to satiation (12 2S, 24 2S and 24 4S) and 3% bw.d^{-1} (12 2R, 24 2R and 24 4R) from Day 30 (A) and Day 50 (B). Different letters denote significant differences (P<0.05).

Table 3.1. Performance of juvenile barramundi fed a ration of 3% bw.day^{-1}, exposed to either 12L:12D or 24L:0D and fed either twice per day or four times over a 24 hour period.

	12L 2R	**24L 2R**	**24L 4R**
Mean body weight $_{initial}$ (g)	18.96 ± 0.35^{a}	18.64 ± 0.28^{a}	18.64 ± 0.21^{a}
Mean body weight $_{Day\ 30}$ (g)	42.80 ± 0.91^{a}	41.77 ± 1.03^{a}	43.91 ± 5.42^{a}
Mean body weight $_{Day\ 50}$ (g)	78.30 ± 5.49^{a}	82.32 ± 6.57^{a}	83.28 ± 5.93^{a}
Mean length $_{initial}$ (cm)	11.96 ± 0.14^{a}	12.01 ± 0.04^{a}	12.04 ± 0.06^{a}
Mean length $_{Day\ 30}$ (cm)	15.40 ± 0.10^{a}	15.35 ± 0.12^{a}	15.54 ± 0.13^{a}
Mean length $_{Day\ 50}$ (cm)	18.78 ± 0.14^{a}	19.26 ± 0.02^{a}	19.33 ± 0.18^{a}
SGR $_{weight\ Day\ 30}$ (% bw.day^{-1})	2.71 ± 0.03^{a}	2.68 ± 0.04^{a}	2.89 ± 0.03^{a}
SGR $_{weight\ Day\ 50}$ (% bw.day^{-1})	2.84 ± 0.08^{a}	2.95 ± 0.00^{a}	2.95 ± 0.02^{a}
SGR $_{length\ Day\ 30}$ (% lt.day^{-1})	0.84 ± 0.05^{a}	0.82 ± 0.04^{a}	0.86 ± 0.03^{a}
SGR $_{length\ Day\ 50}$ (% lt.day^{-1})	0.90 ± 0.03^{a}	0.95 ± 0.00^{a}	0.95 ± 0.01^{a}
K $_{initial}$	1.11 ± 0.04^{a}	1.07 ± 0.00^{a}	1.06 ± 0.01^{a}
K $_{Day\ 30}$	1.15 ± 0.01^{a}	1.13 ± 0.01^{a}	1.15 ± 0.05^{a}
K $_{Day\ 50}$	1.19 ± 0.01^{a}	1.14 ± 0.01^{a}	1.15 ± 0.05^{a}
HSI $_{Day\ 30}$	0.71 ± 0.03^{a}	0.55 ± 0.03^{a}	0.69 ± 0.04^{a}
HSI $_{Day\ 50*}$	1.71 ± 0.10^{b}	1.37 ± 0.06^{a}	1.68 ± 0.02^{b}
Feed intake $_{Day\ 50}$ (g)	56.79 ± 0.26^{a}	59.21 ± 1.37^{a}	57.16 ± 2.16^{a}
FCE(%)	107.64 ± 4.32^{a}	117.06 ± 1.18^{a}	112.51 ± 1.22^{a}
Plasma IGF-I (ng.ml^{-1})$_{Day\ 30}$	22.88 ± 2.12^{a}	26.44 ± 1.63^{a}	24.68 ± 1.66^{a}
Plasma IGF-I (ng.ml^{-1})$_{Day\ 50}$	33.59 ± 4.21^{a}	33.74 ± 2.15^{a}	33.69 ± 2.08^{a}

See Figure 3.1 for abbreviations describing photoperiod, feeding ration and frequency. Data represent means ± SEM of n=4 replicate tanks. Different letters denote significant differences (P<0.05).

3.5 Discussion

Photoperiod manipulation to enhance wet weight and length in juvenile barramundi (18 – 160 g) was validated in the current study. This confirms previous experiments demonstrating continuous light (24L:0D) significantly enhances growth of juvenile barramundi compared to 12L:12D when fed to satiation (Worrall et al., 2004; Chapter 2). No significant increases in feed intake were observed in juveniles reared under 24L:0D compared to 12L:12D when fed to satiation twice daily. Although feed intake does appear to influence the effectiveness of photoperiod manipulation as 24L:0D did not enhance growth of juveniles when fed a reduced ration of 3% bw.day^{-1}. This will be discussed in the Chapter 3.5.2.

In addition, when reared under 24L:0D, juvenile barramundi continue to feed during what would normally be the scotophase. Additional feeding in fish exposed to 24L:0D resulted in further significant increases in growth of barramundi, albeit concomitant with significantly increased feed intake and reduced feed conversion efficiency.

3.5.1 Treatments fed to Satiation (12 2S; 24 2S; 24 4S)

Juveniles reared under 24 2S demonstrated 7.5% increase (although not significant) in weight at day 30 and a significant 9.5% increase on day 50 compared to 12 2S. This increase in growth was observed without significant increases in feed intake compared to 12 2S. The current results contradict Barlow et al., (1995), observing no growth advantages in rearing juvenile barramundi (34 mm total length) under extended light regimes of 24L:0D in comparison to 12L:12D. In addition, Barlow et al., (1995) observed juveniles exposed to 24L:0D consumed approximately 40% more food

(zooplankton) in comparison to 12L:12D. Although juveniles exposed to experimental regimes in Barlow et al., (1995) were not weaned onto commercial pelleted feeds, these contradictory results may be due to different sizes of juveniles as well as quality of feeds, differences between live and commercial pelleted feeds, suggesting diet may influence the effectiveness of photoperiod manipulation in barramundi. Additionally, the study by Barlow et al., (1995) continued for a period of 13 days which was not long enough for the effect to become evident.

A number of studies concur with the current results, finding extended photoperiod increases growth rates of tropical species (Almazan-Rueda et. al., 2004; Biswas et. al., 2006; Falcon et al., 2010) Additionally, growth increases under extended photoperiod without increases in feed intake have also been observed sub-tropical species, gilthead sea bream (Gines et al., 2004) and tropical species, Nile tilapia (El-Sayed and Kawanna, 2007). Gilthead sea bream (25.6 g) significantly improved growth when held under 16L: 8D without significant increases in feed intake compared to the control treatment (ranging from 10L:14D to 13L:11D). Petit et al., (2003) observed improved feed conversion efficiencies in red sea bream (*Pagrus major*) under continuous light in comparison to 12L:12D photoperiod. Biswas et al., (2005) also demonstrated improved feed conversion efficiencies in the tropical species, largemouth bass (*Micropterus salmoides*) when held under longer photoperiods.

At day 30, improvements in growth were observed in 24 2S compared to 12 2S but did not become significant until day 50. This may suggest growth enhancing effects of 24L:0D takes longer than 30 days to become significant in juveniles at this particular size

(~12 cm TL). This could explain why Barlow et al., (1995) did not observe any benefits to barramundi growth when reared under 24L:0D for a period of 13 days. Sanchez-Vazquez et al., (1996) suggests fish exposed to constant lighting retain previous feeding rhythms, indicating rhythms are not due only to environmental cues, but also to endogenous clocks. A determinate amount of time may be needed to alter the entrainment of endogenous rhythms such as activity, feeding and endocrine parameters in a tropical species like barramundi. Impacts on the entrainment of fish's endogenous clocks can also be affected by specific ages of fish and initial application of photoperiod manipulation (Boeuf and Le Bail, 1999). In previous studies by Worrall et al., (2004), barramundi (~6.5 cm TL) exposed to 24L:0D for 40 days demonstrated a 15% increase in wet weight per day compared to 9.75% increase in growth per day when exposed to 12L:12D. The current study exposed barramundi (~12 cm TL) to 24L:0D for 50 days and observed a 13.4% increase in wet weight per day compared to 12.1% increase in weight in fish reared under 12L:12D. Size of barramundi and initial commencement of photoperiod manipulation may affect the entrainment of fish's endogenous clocks. Altering endogenous rhythms in barramundi may improve utilization of nutrients; digestibility of protein, lipid and energy allowing fish to utilize feed more efficiently (Biswas et al., 2005). Subjecting barramundi to 24L:0D at an earlier stage may allow an earlier dampening within entrainment of endogenous clocks. This may explain larger growth increments observed in barramundi when exposed to 24L:0D at an earlier stage of development.

Additional feeds throughout, what would normally be the scotophase in fish reared under 24L:0D (24 4S), attributed to significant growth increases compared to all other

treatments, 15% increase in weight compared to 24 2S and 25.5% increase in weight compared to 12 2S. Similarly, Biswas et al., (2010) found growth of barramundi fry in brackish water improved when fed three or four times compared to one and two times daily. This contradicts previous studies of feeding regimes in barramundi suggesting no growth benefits in feeding small barramundi more than twice a day (Williams and Barlow, 1999), although feeding frequency effects on growth performances change with size of fish with smaller fish being fed smaller meals more frequently and feed amounts required for optimal growth diminishing as fish grows. The current growth increases in fish reared under 24 4S were concomitant with significant increases in feed intake and lowered FCE. The increase in feed intake seen in fish reared under 24 4S may attribute to the significantly higher crude lipid levels found in the liver compared to 12 2S and 24 2S. An increase in feed intake would be assumed in fish demonstrating higher growth rates; however, a lowered FCE observed in 24 4S suggests fish are not utilizing feed as efficiently as fish under 12 2S and 24 2S.

Feeding fish at greater frequencies than they can accommodate could lead to inefficient use of feed and poor feed conversion (Schnaittacher et al., 2005). Booth et al., (2008) found poorer feed conversion ratio (FCR) in fish fed four times daily was due to increased gastrointestinal overload where intake of the next meal occurred before the previous bolus had been subjected to adequate gastric attack. This may be case in the current study where juveniles reared under 24L:0D and fed to satiation four times over a 24 hour period are being overfed. Barramundi being fed four times to satiation over a 24 hour period may be too shorter intervals between meals with feed is passing through the digestive tract too quickly resulting in less effect digestion (Liu and Liao, 1999) and

assimilation. Alternatively, increased swimming and foraging activity under continuous light may lead to added energy expenditure and lowered FCE (Johansen and Jobling, 1998).

A number of studies have found time of feeding to affect food intake and growth performance (Boujard et al., 1995; Bolliet et al., 2004; Volkoff et al., 2010). Timing of feeding may attribute to a lower FCE observed in barramundi under 24L 4S with feeds during what would normally be the scotophase, not being utilized as efficiently as "usual" feeding regimes during the light phase. Although, Harpaz et al., (2005) found that feeding time factor had almost no affect on barramundi growth rate, only feeding ration significantly affect growth rate. In saying this, they observed brush border proteolytic enzyme activity was higher in barramundi fed during the day time and the activity of digestive enzymes and the absorption of the digested feed have a decisive influence on growth rate.

Optimisation of feeding regimes when rearing fish under 24L:0D and feeding over what would normally be the scotophase will enable improved FCE to be achieved along with increases in growth observed in the current study. Optimisation of feeding regimes may include; feeding times, intervals of feeding times, feeding behaviour, stomach capacity of fish and how quickly appetite returns after feeding, gastric evacuation rates, metabolic rate and protein turnover, all which influence the feed conversion efficiency and growth (Carter et al., 2001; Biswas and Takeuchi, 2003; Reddy and Leatherland, 2003). Additionally, determining energy expenditure spent in extra activity under 24L:0D would enable optimising lighting regimes as it is suggested without a period of total darkness,

fish condition, growth and feed conversion rates are compromised (Boeuf and Le Bail, 1999; Gines et al., 2004).

Significant growth differences observed in 24 4S compared to all other treatments were apparent from day 30 onwards compared to 24 2S which only demonstrated significant growth differences at day 50. This suggests combined effects of extended photoperiod and increased feeding frequency enhances growth of juvenile barramundi earlier than photoperiod alone (comparing 24 2S and 12 2S). Feeding time may influence the phase or amplitude of some endocrine cycles involved in the rhythmic secretion, activation or synthesis of digestive or metabolic enzymes (Sanchez-Muros et al., 2003). Feeding juveniles throughout what would normally be the scotophase (when reared under continuous light) may have further influenced either amplitude or endocrine cycles involved with digestive processes thereby influencing earlier growth increases seen in 24 4S (30 days) over 24 2S (50 days).

The ability to continually feed barramundi throughout a 24 hour period may reduce size variation and thereby reduce agonistic interactions and even cannibalism, which is a significant and time consuming problem in commercial barramundi culture. Size variations and behaviour was not measured or observed in this experiment, although this warrants further investigations due to the potential applied benefits to commercial industry. Wang et al. (1998) hypothesized higher feeding frequencies would increase opportunities for subordinate fish to feed because first-feeding dominants might become satiated and less aggressive, resulting in greater food intake of subordinate fish and a reduction in individual size variation. Alternatively, feeding throughout a 24 hour period

may increase fish dominance as fewer feeds are observed to provide less opportunity for the dominant fish to dominate the food supplied with one meal being shared out more evenly (Thorpe et al., 1990; Carter et al., 1994).

Apart from feeding regimes, application of extended photoperiod (24L:0D) only, may be altering endogenous rhythms and endocrine parameters in barramundi, allowing fish to utilize feed more efficiently. Observing levels of IGF-I further suggests endocrine parameters are being altered, as plasma IGF-I levels were significantly higher in fish demonstrating greater growth under 24L:0D compared to 12L:12D. As IGF-I is a central link in the endocrine/growth axis, higher levels will influencing fish growth and development by acting on muscle and cartilage cell growth, stimulating nutrient uptake and protein synthesis while influencing a range of related hormones involved in the endocrine/growth axis (Le Bail et al., 1998; Pérez-Sánchez, 2000; Björnsson et al., 2002; Imsland et al., 2007). The specific endocrine pathways behind growth increases observed under extended day length are unclear, however, numerous research points to hormone concentrations being affected by light regimes (Reddy and Leatherland, 2003; Falcon et al., 2007). Any single or combined interaction of growth/appetite related hormones might contribute to variations in food intake, conversion efficiency and growth rates (Facciolo et al., 2009; Volkoff et al., 2010).

3.5.2 Treatments fed a ration 3% bw.day^{-1} (12 2R, 24 2R and 24 4R)

The relationship between ration and wet weight growth is best described by a curvi-linear relationship and reduced will occur at sub-optimum rations (Johnston et al. 2003). This was shown to be the case with juvenile barramundi fed a rationed feed of 3% bw.d^{-1},

demonstrating significantly reduced growth in comparison to treatments fed to satiation. 3% bw.d^{-1} is a low ration for juvenile barramundi (~18 to 80 g) as optimal rations range from 9 % bw.day^{-1} for 10 g juveniles to 3.8% bw.day^{-1} for 50 g juveniles when held at 29°C and fed a 15MJ digestible energy diet kg^{-1} diet (Williams and Barlow, 1999; Glencross et al., 2006). Additionally Harpaz et al., (2005) found feeding barramundi (~20g) 2% biomass per day resulted in much poorer growth in comparison to 4% biomass per day which was close to optimal with the addition of more feed only resulting in a drastic reduction in the feed utilization efficiency.

Low growth rates observed at 3% bw.d^{-1} suggests barramundi met their nutrient and energy requirements for maintenance but there was only a limited amount remaining for somatic growth. Juveniles were allocated this ration to ensure all feed was consumed, allowing determination of growth influences seen under 24L:0D to be due to factors other than increased feed intake. This suggests the enhancing growth effect of continuous light is dependent on receiving adequate feed, far in excess of maintenance requirements, to utilize this feed more efficiently. Further investigations replicating this study with increased rations would provide a better understanding towards the affects of feed on photoperiod manipulation.

Significantly lower HSI levels were observed in juveniles under 24 2R compared to 12 2R and 24 4R. This was also observed in red sea bream and gilthead sea bream, with significantly higher lipid digestibility and lowered lipid content when exposed to 16L:8D and 24L:0D photoperiods compared to 12L:12D (Gines et al., 2004; Biswas et. al., 2005) This may be indicative of juveniles using lipids to mobilize energy to compensate for a

greater energy demand for growth and an elevated metabolic rate when held under 24L:0D. Although, juveniles reared under 24 4R did not demonstrate lowered HSI, possibly due to increased feeding frequencies and significantly greater feed intake, thereby receiving adequate feed to balance energy consumption.

3.6 Conclusion

Continuous photoperiod (24L:0D) significantly increases wet weight and length of juvenile barramundi when fed to satiation compared to 12L:12D. No significant differences in feed intake was observed between fish reared under 24L:0D and 12L:12D when fed to satiation twice daily.

Increased growth in fish reared under 24L:0D significantly increased plasma levels of IGF-I. This could indicate an up-regulation of the growth hormone/IGF-I axis in response to extended daylight which may also have implication on influencing improved utilization of feed in barramundi. Further significant growth increases in barramundi are observed if fed to satiation four times over what would normally be the scotophase when exposed to continuous light, albeit with lowered FCE suggesting fish are being overfed and cannot assimilate feed as efficiently during the "scotophase". Photoperiod did not significantly affect growth when fed a low ration, suggesting adequate feed intake may influence the effectiveness in extended photoperiod enhancing somatic growth. Results from this study provide further information towards achieving optimal lighting and feeding regimes for commercial aquaculture to improve somatic growth of juvenile barramundi. Further research investigating how photoperiod alters the photoreceptive

hormone melatonin as well as the growth hormone/IGF-I axis will allow a better understanding and ability to use photoperiod manipulation on tropical fish.

3.7 References

Almazan-Rueda, P., Schrama, J.W., Verreth, J.A.J., 2004. Behavioural responses under different feeding methods and light regimes of the African catfish (*Clarias gariepinus*) juveniles. Aquaculture 231, 347-359.

Barlow, C.G., Pearce, M.G., Rodgers, L.J., Clayton, P., 1995. Effects of photoperiod on growth, survival and feeding periodicity of larval and juvenile barramundi *Lates calcarifer* (Bloch). Aquaculture 138, 159-168.

Biswas, A.K. and Takeuchi, T. 2003. Effects of photoperiod and feeding interval on food intake and growth rate of Nile tilapia, *Oreochromis niloticus* L. Fisheries Science 69 (5), 1010-1016.

Biswas, A. K., Seoka, M., Inove, Y., Takii, K., Kumai, H. 2005. Photoperiod influences the growth, food intake, feed efficiency and digestibility of red sea bream (*Pagrus major*) Aquaculture 250, 666-673.

Biswas, A. K., Seoka, M., Tanaka, Y., Takki, K., Kumai, H. 2006. Effect of photoperiod manipulation on the growth performance and stress response of juvenile red sea bream (*Pagrus major*). Aquaculture 258 (1-4), 350-356.

Biswas, A. K., Seoka, M., Ueno, K., Takii, K., Kumai, H. 2008. Stimulation of growth performance without causing stress response in young red sea bream, *Pagrus major*, (Temminck and Schlegel), by photoperiod. Aquaculture Research 39, 457- 463.

Biswas, A., Seoka, M., Inagaki, H., Takii, K. 2010. Reproduction, growth and stress response in adult sea bream, *Pagrus major*, exposed to different photoperiods at spawning season. Aquaculture Research 41 (4), 519-527

Bjornsson, B.T., Johansson, V., Benedet, S., Einarsdottir, I.E., Hildahl, J., Agustsson, T., Johsson, E. 2002. Growth hormone endocrinology of salmonids: regulatory mechanism and mode of action. Fish Physiology and Biochemistry 27, 227-242.

Bligh, E. G., Dyer, W. J. 1959. A rapid method of total lipid extractions and purification. Canadian Journal of Biochemistry and Physiology 37 (8), 911-917.

Boeuf, G., Falcon, J., 2001. Photoperiod and Growth in Fish. Vie Milieu 51, 247-266.

Boeuf, G., Le Bail, P.Y., 1999. Does light have an influence on fish growth? Aquaculture 177, 129-152.

Bolliet, V., Jarry, M., Boujard, T. 2004. Rhythmic pattern of growth and nutrient retention in response to feeding time in the rainbow trout. Journal of Fish Biology 64 (6) 1616-1624.

Booth, M.A., Tucker, B.J., Allan, G.L., Fielder, S. 2008. Effect of feeding regime and fish size on weight gain, feed intake and gastric evacuation in juvenile Australian snapper, *Pagrus auratus*. Aquaculture 282 (1-4), 104 – 110.

Boujard, T. 1995. Diel rhythms of feeding activity in the European catfish, *Silurus glanis*. Physiology and Behaviour 58 (4) 641-645.

Carter, C.G., Mccarthy, I.D., Houlihan, D.F., Johnstone, R., Walsingham, M.V. & Mitchell, A.I. (1994) Food consumption, feeding behaviour and growth of triploid and diploid Atlantic salmon, *Salmo salar* L., parr. *Canadian Journal of Zoology,* **72,** 609-617.

Carter, C.G., Bransden, M.P. 2001. Relationships between protein-nitrogen flux and feeding regime in greenback flounder, *Rhombosolea tapirina* (Gunther). Comparative Biochemistry and Physiology A – Molecular and Integrative Physiology 130 (4), 799-807.

Cho, S.H., Lim, Y.S., Lee, J.H., Lee, J.K., Park, S., Lee, S.M. 2003. Effects of feeding rate and feeding frequency on survival, growth, and body composition of *Plecoglossus altivelis*. Journal of the World Aquaculture Society 34 (1), 85 - 91.

Cruz, E.M. and Brown, C.L. 2009. Influence of the photoperiod on growth rate and insulin-like growth factor-I gene expression in Nile tilapia *Oreochromis niloticus*. Journal of Fish Biology 75, 130-141.

El-Sayed, A. M., Kawanna, M. 2007. Effects of photoperiod on growth and spawning efficiency of Nile tilapia (*Oreochromis niloticus L.*) broodstock in a recycling system. Aquaculture Research 38, 1242-1247.

Dyer, A.R., Barlow, C.G, Bransden, M.P., Carter, C.G., Glencross, B.D., Richardson, N., Thomas, P.M., Williams, K.C., Carragher, J.F. 2004. Correlation of plasma IGF-I concentrations and growth rate in aquacultured finfish: a tool for assessing the potential of new diets. Aquaculture 236, 583-592.

Facciolo, R. M., Crudo, M., Giusi, G., Alo, R., Canonaco, M. 2009. Light and Dark dependent Orexinergic Neuronal Signals Promote Neurodegenerative Phenomena Accounting for Distinct Behavioural Responses in the Teleost *Thalassoma pavo*. Journal of Neuroscience Research 87 (3), 748-757.

Falcon, J., Besseau, L., Sauzet, S., Boeuf, G. 2007. Melatonin effects on the hypothalamo-pituitary axis in fish. Trends in Endocrinology and Metabolism 18 (2), 81-88.

Falcon, J., Migaud, H., Munoz-Cueto, J.A., Carrillo, M. 2010. Current knowledge on the melatonin system in teleosts fish. General and Comparative Endocrinology 165, 469-482.

Gines, R., Afonso, J. M., Arguello, A. 2004. The effects of long-day photoperiod on growth, body composition and skin colour in immature gilthead sea bream (*Sparus aurata*). Aquaculture Research 35 (13), 1207-1212.

Glencross, B. 2006. The nutritional management of barramundi, *Lates calcarifer* – a review. Aquaculture Nutrition 12 (4), 291 – 309.

Harpaz, S., Hakim, Y., Barki, A., Karplus, I., Slosman, T., Eroldogan, O.T. 2005. Effects of difference feeding levels during day and/or night on growth and brush border enzyme activity in juvenile *Lates calcarifer* reared in freshwater re-circulating tanks. Aquaculture 248 (1 – 4), 325 – 335.

Imsland, A.K., Bjornsson, B.T., Gunnarsson, S., Foss,A., Stefansson, S.O. 2007. Temperature and salinity effects on plasma insulin-like growth factor-I concentrations and growth in juvenile turbot (*Scophthalmus maximus*). Aquaculture 271 (1-4), 546 – 552.

Johansen, S.J.S., Jobling, M. 1998. The influence of feeding regime on growth and slaughter traits of cage reared Atlantic salmon. Aquaculture International 6 (1), 1 – 17.

Johnston, G., Kaiser, H., Hecht, T., Oellermann, L. 2003. Effect of ration size and feeding frequency on growth, size distribution and survival of juvenile clownfish, *Amphiprion percula*. Journal of Applied Ichthyology 19 (1), 40 – 43.

Katersky, R.S. & Carter, C.G. 2007(a). High growth efficiency occurs over a wide temperature range for juvenile barramundi, *Lates calcarifer*, fed a balanced diet. Aquaculture, 272**,** 444-450.

Le Bail, P.Y., Gentil, V., Noel, O., Gomez, J.M., Carre, F., Le Goff, P., Weil, C. 1998. Trends in comparative endocrinology and neurobiology - From molecular to integrative biology. Annuals of the New York Academy of Sciences 839, 157 - 161.

Liu, F., Liao, I.C. 1999. Effect of feeding regime on the food consumption, growth and body composition in hybrid striped bass *Morone saxatilis x M. Chrysops*. Fisheries Science 65, 513 – 519.

McCormick, S.D., Moriyama, S., Bjornsson, B.T. 2000. Low temperature limits photoperiod control of smolting in Atlantic salmon through endocrine mechanisms. American Journal of Physiology – Regulatory Integrative and Comparative Physiology 278 (5), R1352-R1361.

Mingarro, M., S. V. R. de Celis, 2002. Endocrine mediators of seasonal growth in Gilthead Sea bream (*Sparus aurata*): the growth hormone and somatolactin paradigm. General and Comparative Endocrinology 128(2): 102-111.

Perez-Sanchez, J. 2000. The involvement of growth hormone in growth regulation, energy homeostasis and immune function in the gilthead sea bream (*Sparus aurata*): a short review. Fish Physiology and Biochemistry 22 (2), 135-144.

Petit, G., Beauchaud, M., Attia, J., Buisson, B. 2003. Food intake and growth of largemouth bass (*Micropterus salmonides*) held under alternated light/dark cycle (12L:12D or exposed to continuous light. Aquaculture 228 (1-4), 397-401

Porter, M.J.R., Duncan, N.J., Mitchell, D., Bromage, N.R., 1999. The use of cage lighting to reduce plasma melatonin in Atlantic salmon (*Salmo salar*) and its effects on the inhibition of grilsing. Aquaculture 176, 237-244.

Porter, M.J.R, Woolcott, H.M, Pankhurst, N.W. 2003. The use of additional lighting and artificial photoperiods to recondition early maturing Atlantic salmon (*Salmo salar*) in Tasmania. Fish Physiology and Biochemistry 28, 391-393

Reddy, P.K., Leatherland, J.F. 1995. Influence combination of time of feeding and ration level on the diurnal hormone rhythms in rainbow trout. Fish Physiology and Biochemistry 14, 25-36.

Sanchez-Muros, M.J., Corchete, V., Suarez, M.D., Cardenete, G., Gomez-Milan, E., de la Higuera, M. 2003. Effect of feeding method and protein source on *Sparus aurata* feeding patterns. Aquaculture 224 (1-4) 89-103.

Sanchez-Vazquez, F. J., Madrid, J.A., Zamora, S., Iigo, M., Tabata, M. 1996. Demand feeding and locomotor circadian rhythms in the goldfish, *Carassius auratus*: Dual and independent phasing. Physiology and Behaviour 60 (2), 665-674.

Schnaittacher, G., King, W., Berlinsky, D.L. 2005. The effects of feeding frequency on growth of juvenile Atlantic halibut, *Hippoglossus hippoglossus*. Aquaculture Research 36 (4), 370 – 377.

Silva, C.R., Gomes, L.C., Brandao, F.R. 2007. Effect of feeding rate and frequency on tambaqui (*Colossoma macropomum*) growth, production and feeding costs during the first growth phase in cages. Aquaculture 264 (1-4), 135-139.

Taranger, G.L., Aardal, L., Hansen, T., Kjesbu, O.S. 2006. Continuous light delays sexual maturation and increases growth of Atlantic cod (*Gadus morhua L.*) in sea cages. ICES Journal of Marine Science - Gadoid mariculture: development and future challenges 63 (2), 365-375.

Taylor, J.F., Migaud, H., Porter, M.J.R., Bromage, N.R. 2005. Photoperiod influences growth rate and plasma insulin-like growth factor – I levels in juvenile rainbow trout, *Oncorhynchus mykiss*. General and Comparative Endocrinology 142 (1-2), 169-185.

Thorpe, J.E., Talbot, C., Miles, M.S., Rawlings, C. & Keay, D.S. (1990) Food consumption in 24 hours by Atlantic salmon (*Salmo salar L.*) in a sea cage. Aquaculture, 90**,** 41-47.

Tucker, J.W., Russell, D.J., Rimmer, M.A. 2002. Barramundi culture: A success story for aquaculture in Asia and Australia. World Aquaculture 33 (3), 53-59.

Tucker, B.J., Booth, M.A., Allan, G.L., Booth, D., Fielder, D.S. 2006. Effects of photoperiod and feeding frequency on performance of newly weaned Australian snapper *Pagrus auratus*. Aquaculture 258 (1-4), 514-520.

Volkoff, H., Hoskins, L.J., Tuziak, S.M. 2010. Influence of intrinsic signals and environmental cues on the endocrine control of feeding in fish: Potential application in aquaculture. General and Comparative Endocrinology 167, 352 – 359.

Wang, N., Xuliang Xu, Kestemont, P. 2009. Effect of temperature and feeding frequency on growth performances, feed efficiency and body composition of pikeperch juveniles (*Sander lucioperca*). Aquaculture 289, 70-73.

Williams, K., Barlow, C.G. 1999. Dietary requirements and optimal feeding practices for barramundi (*Lates calcarifer*). Queensland Department of Primary Industries, Queensland, Australia.

Wilkinson, R.J., Porter, M., Woolcott, H., Longland, R., Carragher, J.F. 2006. Effects of aquaculture related stressors and nutritional restriction on circulating growth factors (GH, IGF-I and IGF-II) in Atlantic salmon and rainbow trout. Comparative Biochemistry and Physiology, Part A 145, 214 – 224.

Worrall, K., Carter, C., Porter, M.J.R. 2004. Effects of Photoperiod on the Growth of Juvenile Barramundi (*Lates calcarifer*). Hons Thesis, University of Tasmania.

Zhou, Z., Cui, Y., Xie, S., Zhu, X., Lei, W., Xue, M., Yang, Y. 2003. Effect of feeding frequency on growth, feed utilization and size variation of juvenile gibel carp (*Carassius auratus gibelio*). Journal of Applied Ichthyology 19, 244-249.

Chapter 4

The Effects of Continuous Photoperiod (24L:0D) on Growth in Commercially Farmed Juvenile Barramundi (*Lates calcarifer)* (Bloch)

K. L. Worrall[a], C. G. Carter[b], M. J.R. Porter[c] , M. Phillips[d], R. J. Wilkinson[a]

[a] National Centre for Marine Conservation and Resource Sustainability, University of Tasmania, Locked Bay 1370, Launceston, TAS, 7250, Australia

[b]Tasmanian Aquaculture and Fisheries Institute, University of Tasmania, Private Bag 49, Hobart, TAS 7001, Australia

[c] Ridley Aquafeed Pty Ltd, 12 Neon Street, Narangba Brisbane, QLD, 4504, Australia

[d] Pejo Enterprises Pty Ltd, Innisfail, QLD, Australia

Corresponding author. Tel.: +61 3 6324 3800; fax: +61 3 6324 3804

E-mail address: kworrall@utas.edu.au

Keywords: Barramundi; IGF-I; Growth; *Lates calcarifer*; Photoperiod

4.1 Abstract

The effects of photoperiod manipulation on growth of juvenile barramundi (*Lates calcarifer*) (Bloch) were investigated in a pilot study (Experiment 1) and subsequent commercial scale experiment (Experiment 2). Barramundi from Experiment 1 (24.99 ± 1.29 g) and Experiment 2 (35.96 ± 0.20 g) were reared in cages within freshwater earthen ponds under commercial conditions and exposed to one of two photoperiod regimes, ambient photoperiod (~12L:12D) or continuous light (24L:0D). Juveniles reared under 24L:0D demonstrated significantly higher final weight gains of 31.42 g in Experiment 1 (n = 2) and 14.48 g in Experiment 2 (n = 4) when compared to 12L:12D. Increased weight in fish exposed to 24L:0D were concomitant with significant increases in total length, specific growth rate and feed conversion efficiency. No significant differences were observed in feed intake between treatments in either experiment. Juveniles exposed to 24L:0D also demonstrated significant increases in plasma IGF-I levels compared to 12L:12D. Relative expression of IGF-I and IGF-II mRNA did not significantly differ between 12L:12D and 24L:0D, whereas IGF-II mRNA was significantly higher than IGF-I mRNA irrespective of photoperiod. It is proposed endocrine parameters in fish (such as IGF-I), are possibly are being altered when exposed to 24L:0D, which leads to fish utilizing feed more efficiently resulting in improved growth. Current results point towards growth performances of juvenile barramundi under commercial conditions, reared in cages within freshwater ponds, can potentially be stimulated by photoperiod manipulation in the form of artificial lighting. This knowledge will provide the potential for commercial barramundi farmers to install artificial light to increases growth rates of barramundi and thereby improve production efficiencies.

4.2 Introduction

Barramundi aquaculture is an important and rapidly expanding industry within Australia, being typically cultured from fingerlings to market size in freshwater ponds, recirculation systems or sea cages (Katersky and Carter, 2007; Carter et al., 2010). Currently, Australian commercial production of barramundi does not use photoperiod manipulation techniques to improve growth rates. The ability to use photoperiod manipulation techniques on commercial barramundi farms could help improve production and economic gain.

In Chapter 2, 3 and Worrall et al., (2004), continuous light (24L:0D) significantly enhanced growth performances of juvenile barramundi when reared in re-circulation systems, held at a temperature of 30°C and fed to satiation. This was achieved without significant increase in feed intake and demonstrated improved feed conversion efficiencies. Photoperiod manipulation has been successfully used to improve growth in a number of juvenile finfish species, both temperate and tropical (Petit et al., 2003; Biswas and Takeuchi, 2003, El-Sayed and Kawanna, 2007; Martinez-Charez et al., 2008) with the efficacy of photoperiod manipulation varying due to environmental influences such as water temperature, developmental stages and food availability (Hovette, 2005, Purchase et al., 2000; Simensen et. al., 2000).

Somatic growth in fish is under complex endocrine control that directly and indirectly involves several hormones, including insulin-like growth factor-I (IGF-I) (Duan et al., 1997; Duan, 1998). Determining concentrations of IGF-I is a useful tool in detecting or predicting changes in growth of fish, as levels of IGF-I have been observed to positively

correlate with growth rate (Duan et al., 1997; Duan, 1998; Dyer et al., 2004). Production and secretion of plasma IGF-I can be directly or indirectly stimulated by cues such as photoperiod, temperature and food availability (Pierce et al., 2005; McCormick et al., 2007; Cruz et al., 2009) thereby permitting us to potentially explain growth differences observed in fish maintained under 12L: 12D and 24L:0D.

Currently the Australian barramundi industry uses photoperiod manipulation to manipulate spawning events, whereas the installation of artificial lighting to improve growth of juvenile barramundi may also be beneficial to commercial farmers. Increasing growth rates of barramundi would benefit industry by reducing time to harvest; and/or juveniles attaining larger sizes before winter.

This study aimed to investigate whether artificial lighting techniques, previously used in re-circulation systems to enhance the growth of juvenile barramundi, could be transferred to commercial on-farm cages in freshwater earthen ponds. To achieve this objective, two separate trials were conducted - a pilot study (Experiment 1) and a repeated experiment with greater replication of cages (Experiment 2). The successful pilot study warranted an expanded replicated experiment to validate results. In both experiments, juvenile barramundi were subjected to two photoperiod regimes, ambient day length (approximately twelve hours light and twelve hours dark (12L:12D) and twenty four hours light (24L:0D) while being held at ambient water temperatures in cages within a commercial freshwater pond in Queensland, Australia.

4.3 Material and Methods

4.3.1. Experiment 1/Pilot Study

A pilot study was conducted using floating cages held within an 80 m x 40 m x 1.6 m freshwater earthen pond at PEJO Enterprises (Innisfail, Queensland). The use of cages within the pond enabled replication in each experiment and ensured fish were subjected to the same fluctuations in ambient temperature and water quality. A total of 4 x 2 m^3 experimental square cages were suspended from a floating pontoon attached to a fixed walkway in the pond (Figure 4.1). Cages consisted of 10 mm nylon nets attached to PVC piping with a square wire sinker dropped to the bottom of each cage to keep the net open. Each cage had a lid of wire mesh attached to PVC piping to prevent predation of fish by birds. A total of 450 fingerlings were stocked into each cage (24.99 ± 1.29 g; 12.57 ± 0.23 cm; stocking density 5.62 kg/m^3).

The experiment was conducted for 120 days from 20th February 2007 to 15th July 2007, when average water temperatures ranged from 27.70 ± 0.15°C to 24.01 ± 0.13°C with diurnal variation being within ± 0.5°C. Temperature was recorded every hour with StowAway Tidbit Temperature Loggers (Onset Computer Company, Bourne, MA, USA). The pond was aerated using two 2 hp paddlewheel aerators, positioned to provide an even flow through the pond and cages. Fish were fed a commercial barramundi diet (Marine Float 54/10, Crude protein 52% and Crude Fat 16%, Ridley Aquafeed, Australia) twice daily to visual satiation as judged by cessation of feeding activity at the water surface. Feed intake, as feed supplied, was recorded daily for each cage. Water parameters (Appendix 1) and disease monitoring was routinely carried out throughout the experiment in accordance with on-farm practices.

Artificial lighting was supplied by one 500 Watt Tungsten Halogen flood light positioned 2 m above the water's surface for each 24L:0D cage (Figure 4.1 and 4.2). Average light intensity at the water's surface for lit cages during the night was 750 lux (10.57 μmol s^{-1} m^{-2}) which was measured using a Li-COR Underwater Quantum sensor (LI-192SA).

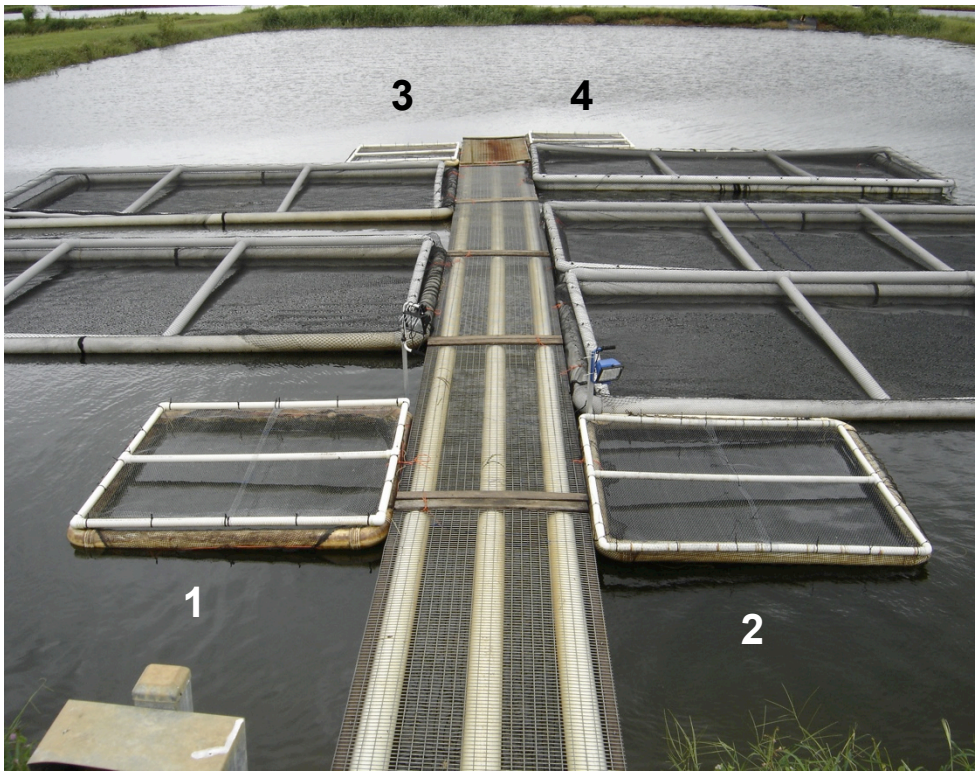


Figure 4.1. Experiment 1 (Pilot study) – Two $2m^3$ cages exposed to 24 hours light (24L:0D) (Cage 1 and 2) and two $2m^3$ cages exposed to ambient day length (12L:12D) (Cage 3 and 4).

Figure 4.2. Experiment 2 (walkway one) - Two $2m^3$ cages exposed to 24 hours light (24L:0D) (Cage 1 and 2) and two $2m^3$ cages exposed to ambient day length (12L:12D) (Cage 3 and 4). Reverse positioning of lights occurring on the second replicate walkway (Cages 5 to 8).

4.*3.2. Experiment 2*

The experimental design and facilities were similar to the Pilot study except that cage replication was increased to n = 4 cages for each treatment. A total of eight 2 m^3 square cages were suspended from 2 identical floating pontoons that were attached to fixed walkways in the pond (4 cages/walkway) (Figure 4.2). A total of 200 fingerlings were stocked into each cage, (average wet weight 35.96 ± 0.20 g and average total length 13.96 ± 0.07 cm - stocking density 3.59kg/m^3). The trial was conducted for 100 days from 22nd October 2007 to 2nd February 2008 where average water temperatures ranged from 27.70 ± 0.15°C to 30.71 ± 0.04°C with diurnal variation being within ± 0.5°C.

4.3.3 Sampling Procedures

4.3.3.1 General

At the beginning of the experiment (day 1), random sub-samples of 60 fish obtained from an onsite pond were measured for initial weight and total length. This population was used to stock all experimental cages. Fish were sampled on days 1, 100 and 120 in Experiment 1 and days 1, 60 and 100 in Experiment 2 and were removed from cages, anaesthetised using iso-eugenol at 40 $mg.L^{-1}$ (AQUI-S, New Zealand Ltd) and were measured for wet weight (to nearest 0.1 g) and total length (mm). On day 1 and 100, 30 fish were blood sampled for circulating plasma IGF-I then euthanized for lipid and molecular analysis. Blood (approximately 300 μL) was drawn from the caudal vein of fish using heparinised (ammonium heparin, Sigma; 4mg/ml) syringes (1ml Terumo syringes, 25G Terumo hypodermic needles) then centrifuged at 3500 rpm at 4^oC, for 15 min and stored at -20°C until assayed for IGF-I. Plasma IGF-I levels were analysed as described by Wilkinson et al., (2006) using a commercially available RIA kit (GroPep,

Adelaide, Australia). Standard AOAC methods were used for nutritional tissue analysis, fillets of white muscle from one side of the fish and whole livers were dissected, freeze dried to a constant weight and used for analysis of total lipid (Bligh and Dyer (1959). Samples of 1 cm^3 sections were taken from the head area of the liver and from white muscle taken anterior to the dorsal fin and stored in a RNA preservation reagent, stored at -20^oC until molecular analysis could be conducted.

4.3.3.2 RNA isolation and preparation

Total RNA was extracted from liver and white muscle stored in an RNA preservation reagent (25 mM sodium citrate, 10 mM EDTA, 10M ammonium sulphate, pH 5.2) and purified using TRI Reagent® (Molecular Research Center, Cincinnati, OH, USA) including DNAse treatment (DNA-*free*™, Ambion, Austin, TX, USA). RNA yield (A_{260}) and purity ($A_{260/230}$ and $A_{260/280}$) were determined spectrophotometrically and the integrity of the RNA was estimated from gel electrophoresis on a 1% agarose gel.

4.3.3.3 Reverse transcription

First strand cDNA was synthesised from total RNA (5 µg) using a SensiMix kit (Qantace, NSW, Australia) with Oligo (dT)18 priming according to the manufacturer's instructions. The reactions were incubated at 65°C for 10mins then 42°C for 50 min before the reverse transcriptase enzyme was inactivated at 70°C for 15 min. First strand cDNA reactions (20 uL) were diluted to 80 uL using nuclease-free water (Sigma-Aldrich, NSW, Australia) and stored at -20°C until quantitative PCR was performed.

4.3.3.4 Quantitative PCR

Real-time PCR primers were designed from partial sequences obtained using degenerate PCR primers and from the full length cDNA sequences obtained using RACE. Quantitative PCR was performed using SYBR® Green chemistry on a MyiQ™ Real-Time PCR Detection System (Bio-Rad, NSW, Australia). Each reaction (25 µL) contained primers (200 nM each), 1× SensiMix*Plus* SYBR and Fluorescein PCR master mix (Quantace) and 2 µL cDNA. All samples were assayed for each gene in duplicate with no-template controls and a 5-step, 2-fold cDNA dilution series for PCR efficiency calculation on the same plate. The reaction was incubated at 95°C for 10 min to activate the heat-activated Taq DNA polymerase followed by 40 cycles of 95°C for 15 s, 55°C for 30 s and 72°C for 25 s. At the end of the 40 cycles a melt curve analysis was performed to test the specificity of reaction.

4.3.3.5 Relative expression

mRNA expression levels were normalized using the geometric mean of three stably expressed reference genes (eukaryotic elongation factor 1 alpha (EF1A), beta actin (ß-actin), and RNA polymerase II (RPL2) as determined by the geNorm software (Vandesompele, 2002)**.** Automated analysis of real-time quantitative PCR data was performed using qBase software (Hellemans, 2007) which employs a modified delta delta-Ct relative quantification model with PCR efficiency correction and multiple reference gene normalisation.

4.3.4. Calculations

The following equations were used to calculate specific growth rate (SGR), condition factor (K), feed conversion efficiency (FCE) and hepatosomatic index (HSI) for each replicate cage.

SGR (% bw.d^{-1}) = 100 x ($\ln W_2$-$\ln W_1$)/time (days)

Where, W_1 and W_2 = the initial and final wet weight (g) respectively.

K = 100 x (W / L^3)

Where, W= wet weight (g) and L = standard total body length (cm)

FI ($g.d^{-1}$) = total dry feed intake/time (days)

FCE (%) = 100 x (wet weight gain (W_2-W_1)/dry feed intake (FI)).

HSI (%) = 100 x (wet weight of liver (g)/wet weight (g))

4.3.5. Statistical Analysis

Statistical analyses were carried out using SPSS 15.0 for windows (SPSS Inc.). In experiment 1, mean ± standard error for each replicate cage (n = 2) for each treatment was calculated from 60 fish at day 1 and 100 fish on completion (day 120). In experiment 2, mean ± standard error for each replicate cage (n = 4) for each treatment was calculated from 200 fish at day 1, day 60 and day 100. The overall mean ± standard error for growth data, feed intake and IGF-I concentrations of each replicate tank was compared using t-tests with differences considered to be significant if $P<0.05$. Two-way ANOVA was used in order to calculate overall effects of time and photoperiod of growth parameters, with significant differences followed by a Tukey's multiple comparison tests to identify differences between pairs of treatment groups. Values are presented as means ± standard error (SEM).

4.4 Results

4.4.1 Growth – Experiment 1/Pilot Study

Feed intake did not significantly differ between treatments with each cage being fed 31.2 kg over 120 days. FCE improved significantly in juveniles exposed to 24L:0D compared to 12L:12D (t-test; $P<0.01$; t = 53.33; df = 2) (Figure 4.5A). Initial wet weights of juveniles were 24.99 ± 1.29 g and total length 12.57 ± 0.23 cm. On completion of the experiment, a significant difference in growth was observed between juveniles exposed to 24L:0D and 12L:0D. At day 120, wet weight significantly increased in juveniles exposed to 24L:0D (356.90 ± 5.93 g), demonstrating a 9.5% increase compared to 12L:12D (325.48 ± 5.68 g) (two-way ANOVA; $P<0.01$; F = 1341.34; df = 5) (Figure 4.3A). Similarly, at day 120, total length significantly increased in juveniles exposed to 24L:0D (28.83 ± 0.19 cm) demonstrating a 5.5% increase compared to 12L:12D (27.32 ± 0.01 cm) (two-way ANOVA; $P<0.01$; F = 1751.99; df = 5) (Figure 4.3B). At day 100 and day 120, significantly greater SGR was observed in juveniles exposed to 24L:0D compared to 12L:12D (two-way ANOVA; $P<0.05$; F = 28.83; df = 3) (Figure 4.4A). Initial condition factor (K) did not significantly different between treatments. At days 100 and 120, a significantly lower condition factor was observed in juveniles exposed to 24L:0D compared to 12L:12D (two-way ANOVA; $P<0.01$; F = 27.49; df = 3) (Figure 4.4B).

HSI did not significantly differ between 24L:0D (1.65 ± 0.06%) or 12L:12D (1.63 ± 0.06%). Total lipid levels did not significantly differ in livers or white muscle of juveniles exposed to either treatment (Figure 4.5B).

At day 120, significantly higher plasma IGF-I concentrations were observed in juveniles exposed to 24L:0D compared to 12L:12D (t-test; $P<0.05$; t = -2.520; df = 56), whereas relative expression of IGF-I mRNA did not significantly differ between 24L:0D and 12L:12D (Figure 4.6 A and B). Relative expressions of IGF-II mRNA were significantly elevated compared to IGF-I mRNA, irrespective of photoperiod at day 120 (one way ANOVA; $P<0.05$; F = -1.42; df = 7) (Figure 4.6B). At day 120, relative expressions of IGF-I and IGF-II mRNA did not significantly differ between 24L:0D and 12L:12D, with large variations being observed in 12L:12D treatments (Figure 4.6B).

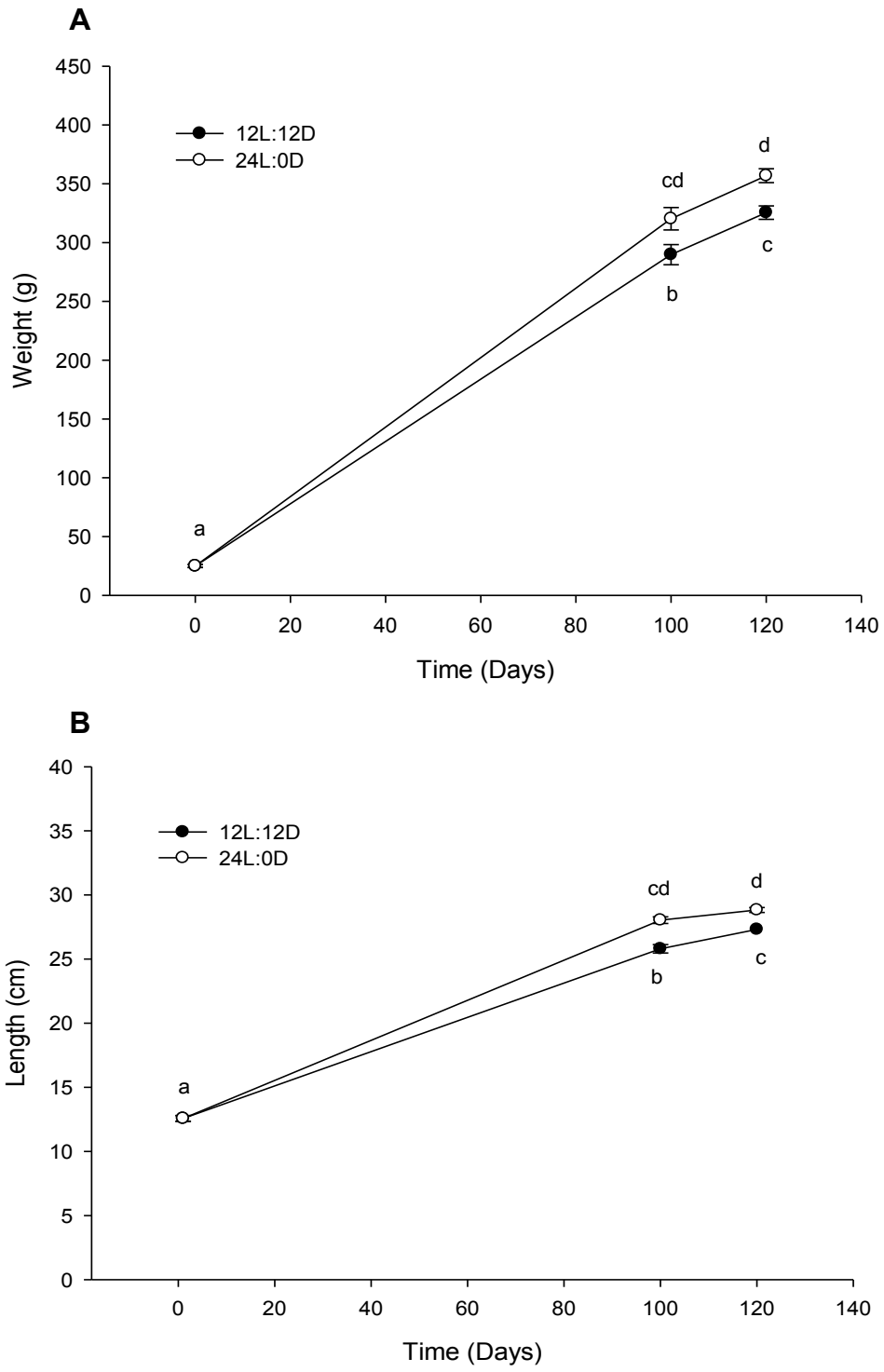


Figure 4.3 Mean wet weight ± SEM (A) (g) and mean total length ± SEM (B) (cm) of juvenile barramundi held under ambient day length (~12L:12D) and constant light (24L:0D) at day 1, 100 and 120. Different letters denote significant differences (P<0.05).

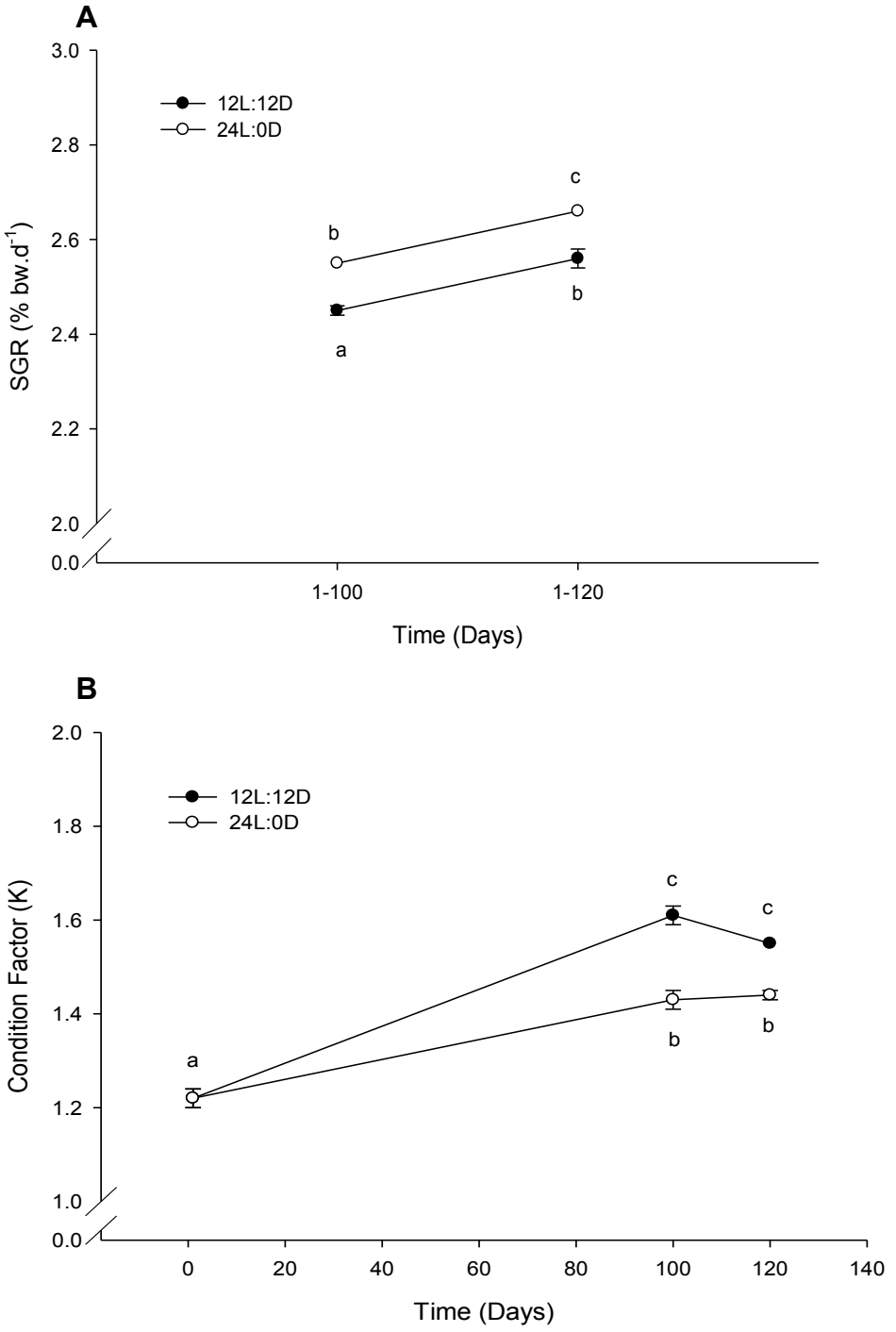


Figure 4.4 Mean specific growth rate ± SEM (% bw.d^{-1}) at day 1-100 and 1-120(A) and mean condition factor ± SEM (K) on day 1, 100 and 120 and (B) of juvenile barramundi held at two photoperiods; ambient day length (~12L:12D) and constant light (24L:0D). Different letters denote significant differences (P<0.05).

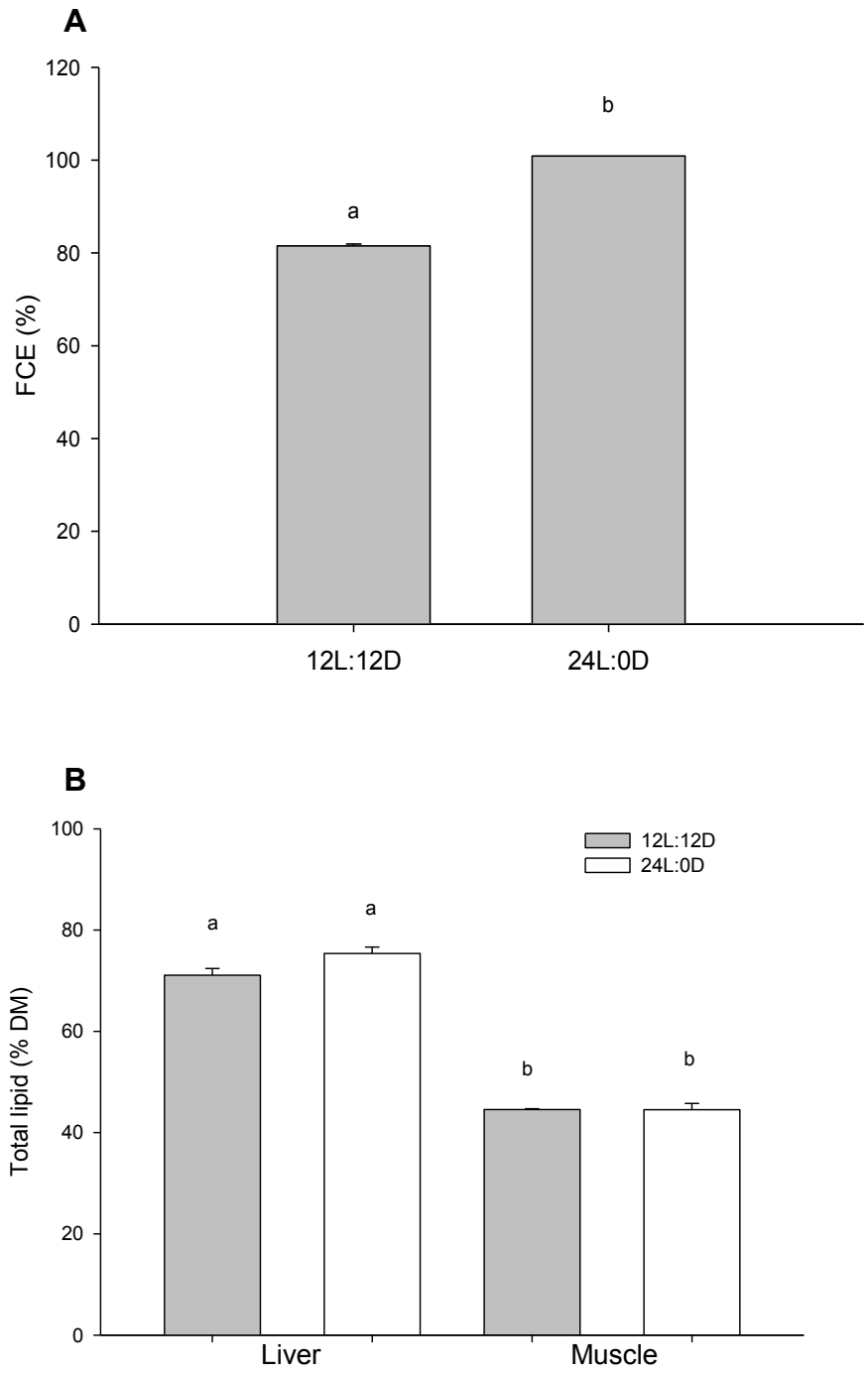


Figure 4.5 Mean feed conversion efficiency ± SEM (FCE %) at day 120 (A) and mean total lipid analysis ± SEM (B) on liver and white muscle tissue of juvenile barramundi held at two photoperiods; ambient day length (~12L:12D) and constant light (24L:0D) at day 120. Different letters denote significant differences (P<0.05).

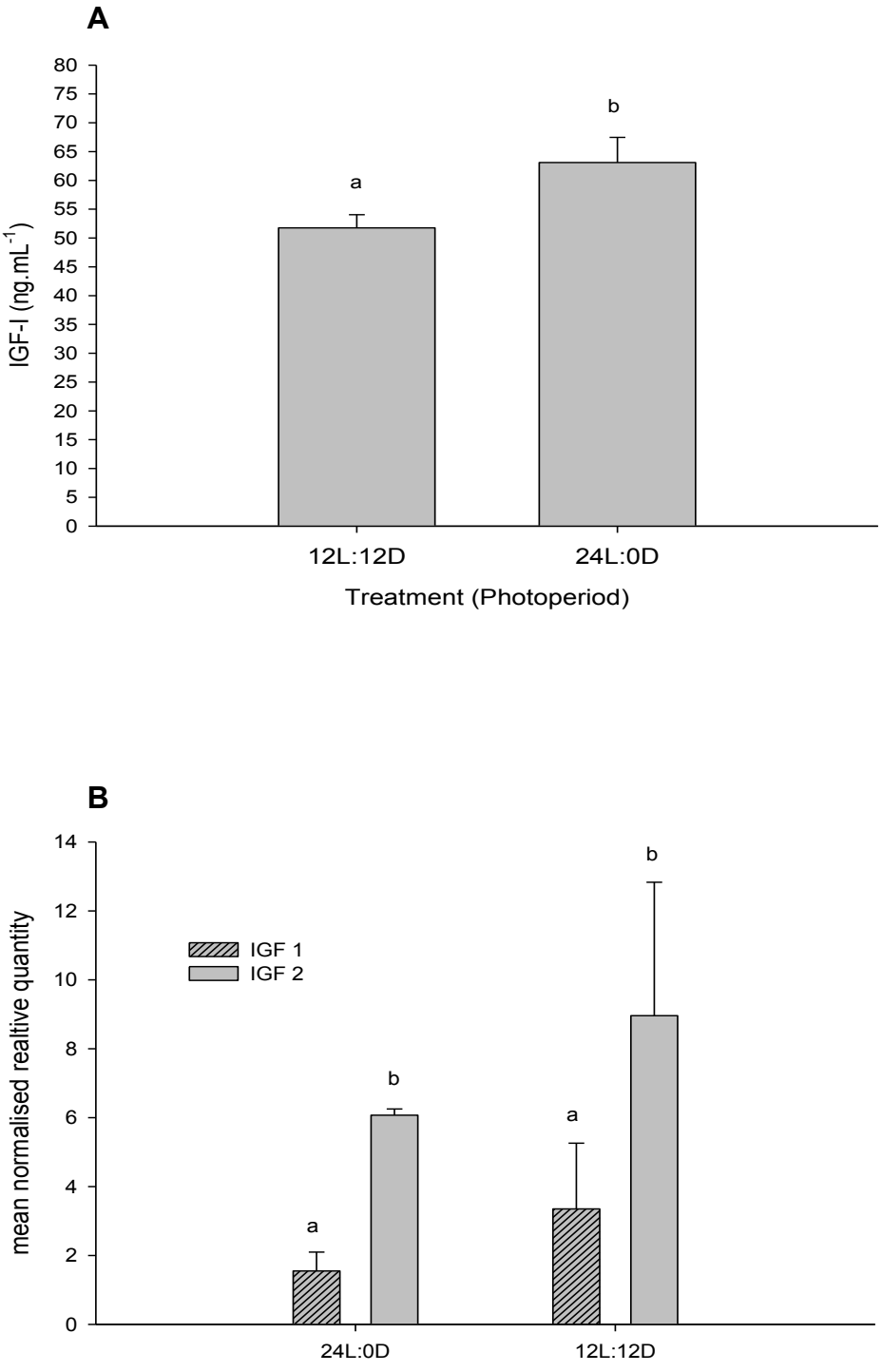


Figure 4.6. Mean plasma IGF-I concentration ± SEM (ng.mL^{-1}) at day 120 (A) and mean normalised relative quantity of IGF-I mRNA and IGF-II mRNA ± SEM (B) in liver of juvenile barramundi, taken at Day 120 held under 12L:12D and 24L:0D. Different letters denote significant differences (P<0.05).

4.4.2 Growth – Experiment 2

Feed intake did not significantly differ between treatments with all cages being fed 37.9 kg over 100 days. FCE was not significantly different between 24L:0D (98.70 ± 3.45%) and 12L:12D (89.19 ± 3.24%) at day 100.

Initial wet weight (35.95 ± 0.20 g) and total length (13.96 ± 0.07 cm) did not significantly differ between treatments (one-way ANOVA; $P>0.05$; F = 1.333; df = 7) (one-way ANOVA; $P>0.05$; F = 0.948; df = 7). At day 100, significant increases in wet weight were observed in juveniles exposed to 24L:0D (238.30 ± 2.85 g) compared to 12L:12D (223.37 ± 2.46 g) (two-way ANOVA; $P<0.01$; F = 1145.74; df = 5) (Figure 4.7A). Similarly, at day 100, significant increases in total length were observed in juveniles exposed to 24L:0D (26.67 ± 0.10 cm) compared to 12L:12D (26.12 ± 0.08 cm) (two-way ANOVA; P <0.01; F = 4606.48; df = 5) (Figure 4.7B). At day 60 and 100, SGR did not significantly differ between experimental treatments (Figure 4.8B). Initial condition factor (K) did not differ between treatments. At day 60, condition factor in fish exposed to 24L:0D was significantly higher compared to 12L:12D although by day 100 this trend altered with no significant differences in condition factor observed between treatments (two-way ANOVA; $P<0.01$; F = 28.68; df = 5) (Figure 4.8A).

HSI significantly decreased in both treatments over the course of the experiment, with no significant differences being observed between 24L:0D and 12L:12D (two-way ANOVA; $P<0.01$; F = 49.05; df = 3) (Figure 4.9A). Total lipid (% DM) in barramundi liver was significantly higher in juveniles exposed to 24L:0D compared to 12L:12D at day 100 (t-test; $P<0.05$; t = -2.714; df = 49) (Figure 4.9B). At day 60, no significant differences in

plasma IGF-I concentrations were observed. At day 100, plasma IGF-I concentrations were significantly higher in juveniles exposed to 24L:0D compared to 12L:12D (two-way ANOVA; $P < 0.05$; $F = 3.50$; $df = 5$) (Figure 4.10).

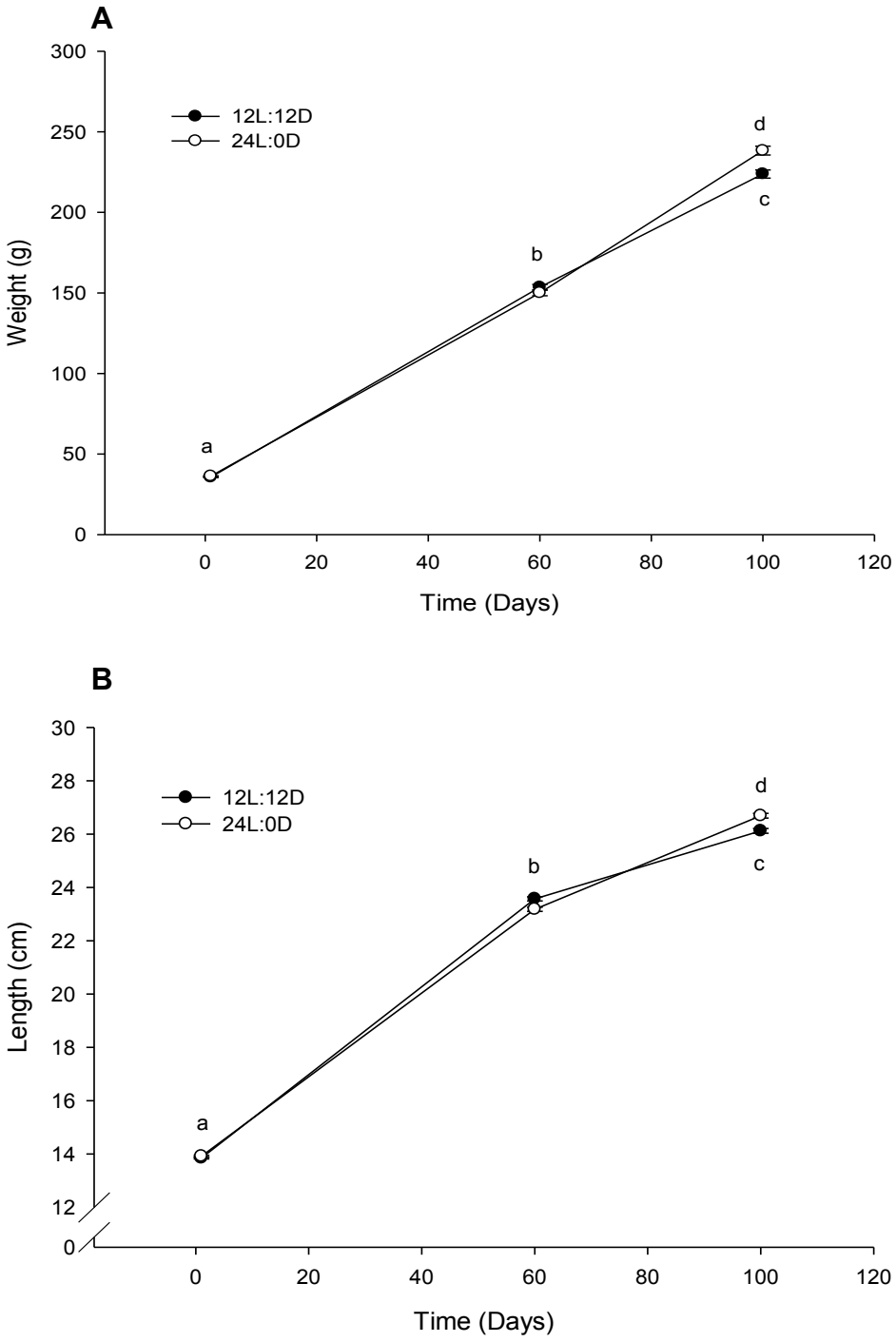


Figure 4.7 Mean wet weights ± SEM (g) (A) and mean total lengths ± SEM (cm) (B) of juvenile barramundi held at two photoperiods; ambient day length (~12L:12D) and constant light (24L:0D) at day 1, 60 and 100. Different letters denote significant differences (P<0.05).

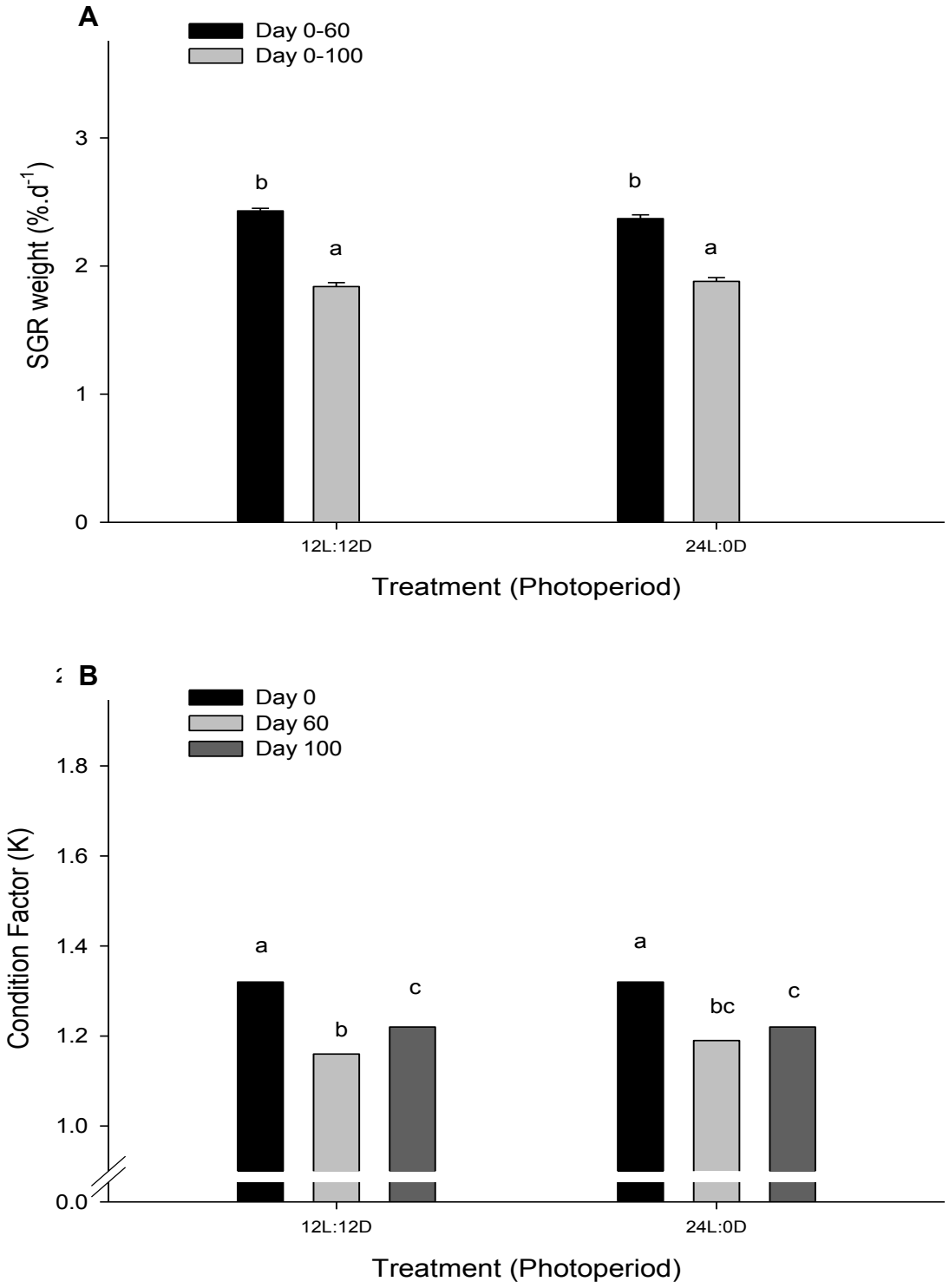


Figure 4.8 Mean specific growth rate ± SEM (SGR % bw.d^{-1}) at day 1-60 and 1-100 (A) and mean condition factor ± SEM (K) at day 1, 60 and 100 (B) of juvenile barramundi held at two photoperiods; ambient day length (~12L:12D) and constant light (24L:0D). Different letters denote significant differences (P<0.05).

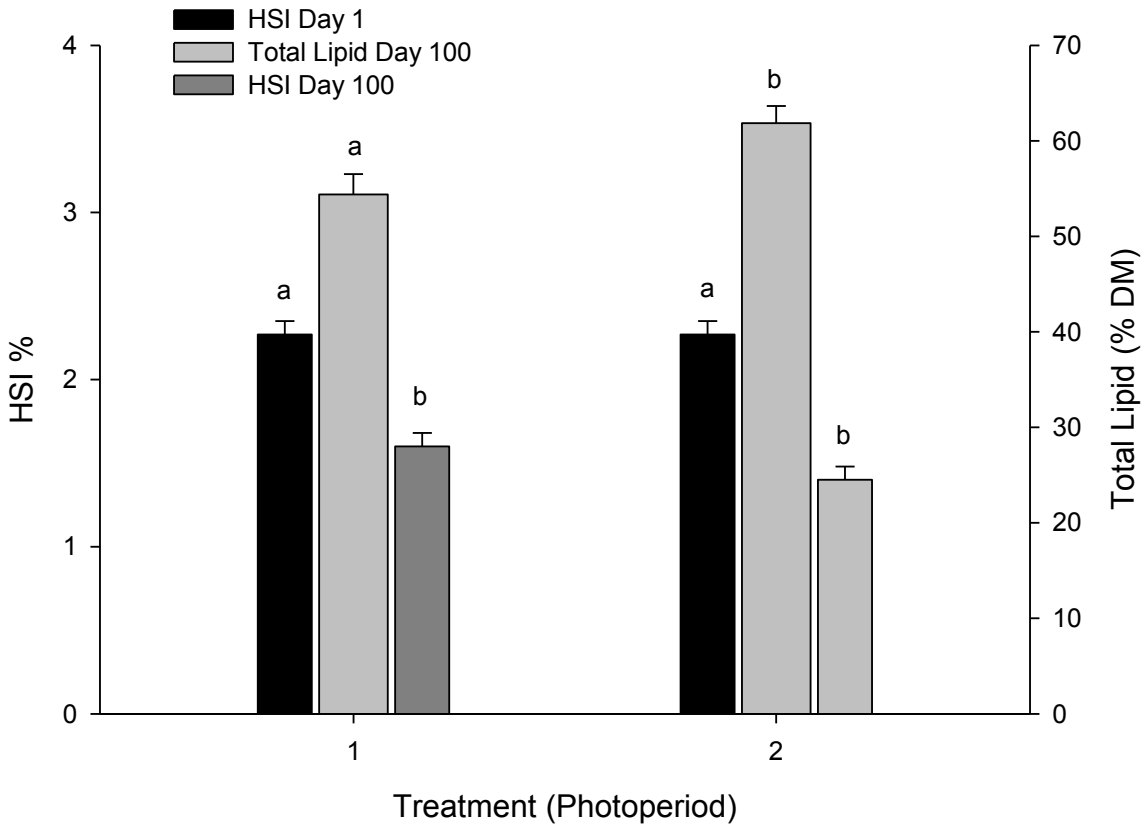


Figure 4.9 Mean HSI (%) ± SEM (A) at Day 1 and Day 100 and mean total lipid ± SEM (% DM) of livers at Day 100 in juvenile barramundi held under ambient day length (~12L:12D) and constant light (24L:0D). Different letters denote significant differences (P<0.05).

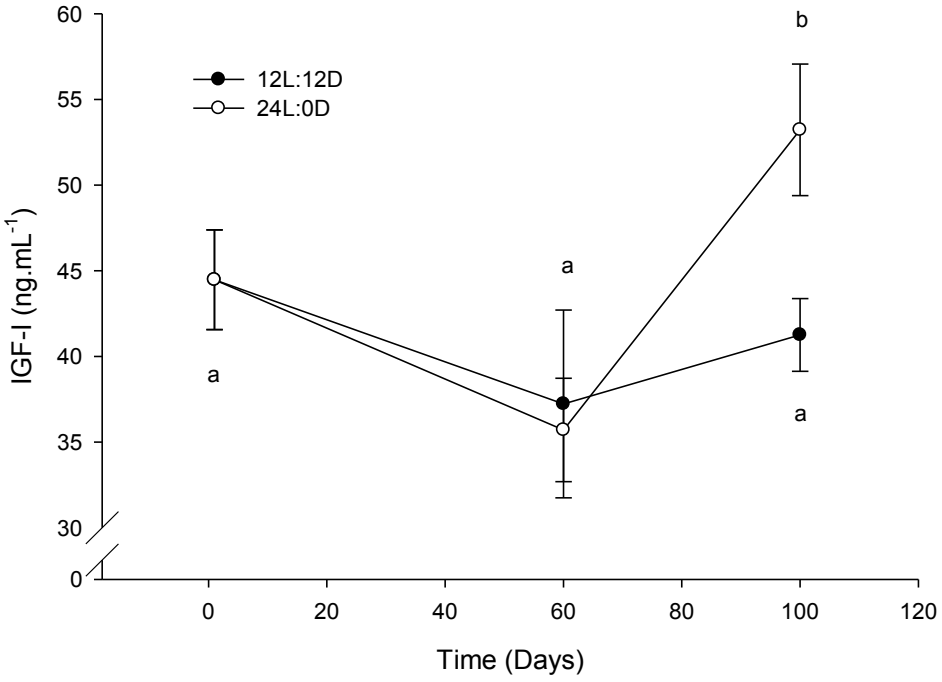


Figure 4.10 Mean plasma IGF-I ± SEM (ng.mL^{-1}) at Day 1, 60 and Day 100 in juvenile barramundi held under ambient day length (~12L:12D) and constant light (24L:0D). Different letters denote significant differences (P<0.05).

4.5 Discussion

The current results demonstrated continuous light (24L:0D) enhanced growth performance of juvenile barramundi without increases in feed intake when reared in cages held in earthen freshwater ponds. This being said, the ability to obtain an accurate measurement of feed intake in a commercial situation is difficult as uneaten pellets were unable to be collected and counted. Continuous light also influenced feed conversion efficiencies, HSI, total lipid levels and endocrine concentrations of plasma IGF-I. These results parallel previous experiments in small scale recirculation systems (Chapters 2 and 3), indicating photoperiod manipulation techniques are also applicable to commercial freshwater earthen ponds. The positive effect of continuous (24L:0D) photoperiod on growth performances is comparable to that observed previously in a number of other tropical fish species (El-Sayed and Kawanna, 2007; Biswas et al., 2008; Martinez-Charez et al., 2008).

Increased growth performance of fish reared under 24L:0D was unrelated to increases in feed intake, indicating photoperiod alone alters fishes ability to utilize feed more efficiently. This parallels findings in other species, such as largemouth bass, *Micropterus salmoides* (Petit et al., 2003); haddock, *Melanogrammus aeglefinus* (Trippel and Neil, 2003) and gilthead seabream, *Sparus aurata L.* (Kissil et al., 2001; Ginés et al., 2004). Additional evidence to fish improving feed utilization under 24L:0D relates to no significant differences being observed between HSI at 24L:0D and 12L:12D, with total lipid levels being significantly higher in 24L:0D fish in Experiment 2. This indicates fish reared under 24L:0D are not using stored lipids in the liver to mobilise energy to compensate for a greater energy demand for growth and an elevated metabolic

rate. As plasma concentrations of IGF-I were significantly altered in fish reared under 24L:0D, this points to continuous light up-regulating the GH-IGF-I growth axis therefore potentially influencing the fishes ability to utilize feed more efficiently.

Central to the hormonal control of growth is the GH–IGF-I axis. Hormonal stimulation under continuous light may directly affect fish growth by signalling fish to increase secretion of plasma GH (McCormick et al., 1995; Bjornsson, 1997; Cruz and Brown, 2009). Plasma GH in turn stimulates the production of plasma IGF-I (Duan et al., 1998; Pierce et al., 2005). GH and IGF-I have both metabolic and growth-promoting functions (Bjornsson, 1997; Duan, 1998). In both experiments increased plasma IGF-I concentrations were concomitant with growth increases observed in barramundi reared under 24L:0D. This concurs with results from Dyer et al., (2004) who also found plasma IGF-I concentrations were positively correlated to growth rates in barramundi. This being said, Dyer et al., (2004) observed significant increases in growth of juvenile barramundi with increased feed rations of 2%, 4% and 10%, observing circulating IGF-I concentrations increased with increasing ration size. In the current experiment, ration was not an influencing factor as no significant increases in feed intake occurred, indicating significant increases in IGF-I concentrations were due to an extended photoperiod of 24L:0D.

Interestingly, tissue levels of IGF-I mRNA did not reflect increased levels of plasma IGF-I concentrations in fish exposed to 24L:0D. In this experiment hepatic IGF-I mRNA may not have provided an accurate reflection of circulating IGF-I protein. Plasma IGF-I concentrations and mRNA may not correlate as the liver is not the only source of IGF-I

involved in the regulation of growth, but it is also produced locally by some tissues such as the muscle, stomach, pancreas and brain (Pierce et al., 2005). Additionally, the IGF system is complex, consisting of several components including IGF-I, IGF-II, six binding proteins (IGFBP) and their specific proteases, the type 1 (IGF-1R) receptor which mediate the effects of IGF-I and IGF-II and would influence the level of free circulating IGF-I in the plasma (Reinke et al., 2010). Due to these range of factors which may influence the biological activity of circulating IGF-I, tissue levels of IGF-I mRNA may yet prove to be a good indicator of barramundi growth, although mRNA levels can also be influenced by a range of factors including feeding regimes and temperatures (Cruz and Brown, 2009). Additionally, in the current study, wide variations of IGF-I and IGF-II mRNA within photoperiods were demonstrated. Similar results have been observed in Nile tilapia, with different levels of social hierarchy (dominant, intermediate and subordinate individuals) being the suggested cause (Cruz and Brown, 2009). Alternatively, additional analysis of IGFBP's and IGF-1R would provide a better indication of IGF biological activity when analysing plasma IGF-I concentrations.

Improved feed utilization may be influenced directly through longer photoperiods allowing a slower and more efficient digestive process, which may improve digestion and retention efficiency (Biswas et al., 2008). Continuous light has been shown to increase digestion, absorption and transport of free amino acids which stimulate greater protein synthesis in Atlantic salmon (Rungruangsak-Torrissen et al., 2009). Alternatively, improved utilization of feed may be influenced indirectly through prolonged activity or exercise whilst held under extended photoperiod as sustained activity can affect metabolism and body composition (Jobling, 1993; Petit et al., 2003; Biswas et al., 2004;

Davidson, 1997). Feed conversion efficiencies may also be influenced by changes in metabolic functions produced by 24L:0D altering/stimulating the endocrine/growth axis and associated hormones (Biswas et al., 2002; Biswas and Takeuchi, 2003).

The effectiveness of continuous light on enhancing growth parameters in juvenile barramundi may depend on the fish's stage of development and/or initial commencement of photoperiod manipulation. In experiment 1, growth increases of up to 10% were observed in juveniles (24.99 g) reared under 24L:0D. In experiment 2, growth increases of up to 6% were observed in juveniles (35.95 g) reared under 24L:0D. The effectiveness of photoperiod manipulation may alter with developmental stages of barramundi, as endocrine and mRNA parameters are shown to be age/size-dependant in fish (Porter et al., 2000; Xu and Volkoff, 2009).

Alternatively, initial commencement of continuous light may involve a specific time frame before influencing growth parameters. Rungruangsak-Torrissen et al., (2009), found Atlantic salmon took 70 days to adjust or "adapt" to its new environment of continuous light being indicated by the analysis of enzymes, trypsin and chymotrypsin. Experiment 2 demonstrating 6% increases in growth under 24L:0D continued for a period of 100 days whereas Experiment 1 demonstrating up to 10% increase in growth continued for a period of 120 days. This warrants further investigation; as barramundi may show an "adjustment" period before continuous light begins to become effective which may have involvement with entrainment of circadian rhythms involved with photoperiod.

Growth variations between Experiment 1 (Chapter 4.4.1) and 2 (Chapter 4.4.2) may also be explained by natural environmental factors involved with commercial culture. For example, Experiment 2 (Chapter 4.4.2) demonstrated higher water temperatures of 27°C to 30°C (optimal for barramundi growth) whereas Experiment 1 temperatures decreased from 27°C to 24°C and yet observed approximately 4% increase in growth in comparison. At optimal water temperatures for barramundi and results from previous experiments, an expected greater growth increment in experiment 2 would be assumed, especially as previous results demonstrated juveniles held at 24°C did not significantly increase in growth when exposed to 24L:0D (Chapter 2). Due to the nature of commercial trials, growth variations seen between Experiments 1 and 2 may also have occurred from natural events such as increased occurrences of algal blooms seen in higher temperatures. Algal blooms in conjunction with higher water temperature of the water will influence the oxygen availability which can have impacts on fish growth.

4.6 Conclusion

These results demonstrated growth performance of juvenile barramundi reared under commercial farm conditions of freshwater ponds can be significantly improved by photoperiod manipulation in the form of artificial lighting. Importantly, the increased growth performance of fish reared under 24L:0D was independent of feed intake. This suggested enhanced growth from 24L:0D was due to hormonal stimulation of IGF-I caused by extended photoperiod and not by increased feed intake. Improved feed conversion efficiencies may be influenced at all levels of nutrient and energy balance (digestion, respiration and excretion / metabolism) by increased day lengths.

Due to the nature of commercial trials the ability to achieve an accurate measure of feed intake was limited, therefore a controlled experiment is needed to obtain an accurate measurement of feed intake of juveniles, ascertaining if fish are feeding more efficiently or consuming additional feed. Further research investigating the potential for extended feeding intervals throughout a 24 hour period in continuous light would be advantageous. Additionally, further studies are needed to determine the effectiveness of continuous light on various life stages of barramundi. The current results help towards establishing optimal light regimes for growth of commercial barramundi. In saying this, the current study has been undertaken in freshwater earthen ponds and the effectiveness of artificial lighting and photoperiod manipulation would need to be tested on individual commercial farms to determine economic viability.

From a production point of view, up to 10% growth advantage in barramundi would equate to increases in the biomass harvested. As an example, a banquet sized barramundi (1kg) takes from between 12 – 14 months to reach harvest weight depending on farm and environmental conditions. A barramundi growing to 1kg in 14 months equates to 71 g/month. With a 10% increase in weight, barramundi will grow to 1.1kg in 14 months, equating to 78.5 g/month. A 10% increase in weight of barramundi will allow farmers to either harvest 1 month earlier, now being able to produce a 1kg fish in less than 13 months or alternatively harvest fish at 1.1kg. To give an example of economic gains, a farm producing 30 tonnes of barramundi per year and receiving an average wholesale price of $15.00 per kilo would equate to $450,000AUD. Whereas with a 10% increase in biomass per year would equate to 33 tonnes per year at $15.00 per kilo would equate to $495,000. Potentially these figures could increase as current results have demonstrated

from 6 – 10% increases in growth of barramundi whereas with further optimising of artificial lighting regimes and continuation of these lighting regimes until fish are harvested, could equate to further economic gains.

4.7 Reference

Biswas, A.K., Endo, M., Takeuchi, T., 2002. Effect of different photoperiod cycles on metabolic rate and energy loss of both fed and unfed young tilapia *Oreochromis niloticus*: Part I. Fisheries Science 68, 465-477.

Biswas, AK., Takeuchi, T. 2003. Effects of photoperiod and feeding interval of food intake and growth rate of Nile tilapia, *Oreochromis niloticus*. Fisheries Science 69 (5), 1010 - 1016.

Biswas, A.K., Maita,M., Yoshizaki, G., Takeuchi, T. 2004. Physiological responses in Nile tilapia exposed to different photoperiod regimes. Journal of Fish Biology 65 (3), 811 – 821.

Biswas, A. K., Seoka, M., Ueno, K., Takii, K., Kumai, H. 2008. Stimulation of growth performance without causing stress response in young red sea bream, *Pagrus major*, (Temminck and Schlegel), by photoperiod. Aquaculture Research 39, 457- 463.

Bjornsson, B.T. 1997. The biology of salmon growth hormone: from daylight to dominance. Fish Physiology and Biochemistry 17 (1-6) 9-24.

Bligh, E. G., Dyer, W. J. 1959. A rapid method of total lipid extractions and purification. Canadian Journal of Biochemistry and Physiology 37 (8), 911-917.

Carter, C., Glencross, B., Katersky, R.S., Bermudes, M. 2010. The Snooks (Family: Centropomidae) In: Finfish Aquaculture: Species Selection for Diversification. (Ed Le Francois, N.R., Jobling, M., Carter, C., Bljer, P.U) CAB International, Walingford, UK. Chapter 14 pp 323 - 336

Cruz, E.M.V., Brown, C.L. 2009. Influence of the photoperiod on growth rate and insulin-like growth factor-I gene expression in Nile tilapia (*Oreochromis niloticus)*. Journal of Fish Biology 75 (1), 130-141.

Duan, C. 1997 The Insulin-like Growth Factor System and Its Biological Actions in Fish. American Zoology 37, 491-503.

Duan, C. 1998. Nutritional and Developmental regulation of insulin-like growth factors in fish. American Society for Nutritional Sciences 128, 306S - 314S.

Dyer, A.R., Barlow, C.G, Bransden, M.P., Carter, C.G., Glencross, B.D., Richardson, N., Thomas, P.M., Williams, K.C., Carragher, J.F. 2004. Correlation of plasma IGF-I concentrations and growth rate in aquacultured finfish: a tool for assessing the potential of new diets. Aquaculture 236, 583-592.

El-Sayed, A. M., Kawanna, M. 2007. Effects of photoperiod on growth and spawning efficiency of Nile tilapia (*Oreochromis niloticus L.*) broodstock in a recycling system. Aquaculture Research 38, 1242-1247.

Gines, R., Afonso, J. M., Arguello, A. 2004. The effects of long-day photoperiod on growth, body composition and skin colour in immature gilthead sea bream (*Sparus aurata*). Aquaculture Research 35 (13), 1207-1212.

Hellemans, J., Mortier, G., De Paepe, A., Speleman, F. , Vandesompele, J. , 2007. qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. Genome Biology 8.

Hovette, E., 2005. Influence of photoperiod and light intensity on growth and survival of *Lates calcarifer*. Honours Thesis, Good Fortune Bay, Queensland.

Jobling, M., Jorgensen, E.H., Amesen, A.M., Ringo, E. 1993. Feeding, growth and environmental requirements of Arctic charr: a review of aquaculture potential. Aquaculture International 1, 20-46.

Katersky, R.S., Carter, C.G. 2007. A preliminary study on growth and protein synthesis of juvenile barramundi, *Lates calcarifer* at different temperatures. Aquaculture 267, 157-164.

Kissil, G.W., Lupatsch I., Elizur A., Zohar Y., 2001. Long photoperiod delayed spawning and increased somatic growth in gilthead seabream (*Sparus aurata*). Aquaculture 200, 363-379.

Martinez-Chavez, C.C., Al-Khamees, S., Campos-Mendoza, A., Penman, D.J., Migaud, H. 2008., Clock-Controlled Endogenous Melatonin Rhythms in Nile Tilapia (*Oceochromis niloticus niloticus*) and African Catfish (*Clarias gariepinus*). Chronobiology International 25, 31-49.

McCormick, S.D., Shrimpton, J.M., Moriyama, S., Bjornsson, B.T. 2007. Differential hormonal responses of Atlantic salmon parr and smolt to increased daylength: A possible developmental basis for smolting. Aquaculture 273 (2-3), 337-344.

Petit, G., Beauchaud, M., Attia, J., Buisson, B. 2003. Food intake and growth of largemouth bass (*Micropterus salmonides*) held under alternated light/dark cycle (12L:12D or exposed to continuous light. Aquaculture 228 (1-4), 397-401

Pierce, A.L., Shimizu, M., Beckman, B.R., Baker, D.M., Dickhoff,W.W. 2005.
Time course of the GH/IGF axis response to fasting and increased ration in Chinook salmon (*Oncorhynchus tshawytscha*). General and Comparative Endocrinology 140, 192–202

Porter, M.J.R, Stefansson, S.O., Nyhammer, G., Karlsen, O., Norberg, B. and Bromage, N.R. 2000. Environmental influences on melatonin secretion in Atlantic cod (*Gadus morhua* L.) and their relevance to commercial culture.

Purchase, C.F., Boyce, D.L., Brown, J.A. 2000. Growth and survival of juvenile yellowtail flounder *Pleuronectes ferrugineus* under different photoperiods. Aquaculture Research 31 (6), 547-552.

Reinecke, M. 2010. Influences of the environment on the endocrine and paracrine fish growth hormone – insulin-like growth factor-I system. Journal of Fish Biology 76, 1233 – 1254.

Rungruangsak-Torrissen, K., Sunde, J., Berg, A.E., Nordarden, U., Fjelldal., G., Oppedal, F. 2009. Digestive efficiency, free amino acid pools and quality of growth performance in Atlantic salmon (*Salmo salar L.*) affect by light regimes and vaccine types. Fish Physiology and Biochemistry 35, 255 – 272.

Simensen, L. M., Jonassen, T. M., Imsland, A. K., Stefansson, S. O. 2000. Photoperiod regulation of growth of juveniles Atlantic halibut *(Hippoglossus hippoglossus* L.) Aquaculture 190 (1-2), 119-128.

Trippel, E.A., Neil, S.R.E., 2003. Effects of photoperiod and light intensity on growth and activity of juvenile haddock (*Melanogrammus aeglefinus*). Aquaculture 217: 633-645

Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., Speleman, F., 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. . Genome Biology.

Wilkinson, R.J., Porter, M., Woolcott, H., Longland, R., Carragher, J.F. 2006. Effects of aquaculture related stressors and nutritional restriction on circulating growth factors (GH, IGF-I and IGF-II) in Atlantic salmon and rainbow trout. Comparative Biochemistry and Physiology, Part A 145, 214 – 224.

Worrall, K., Carter, C., Porter, M.J.R. 2004. Effects of Photoperiod on the Growth of Juvenile Barramundi (*Lates calcarifer*). Hons Thesis, University of Tasmania.

Xu, M., Volkoff, H. 2009. Molecular characterization of ghrelin and gastrin-releasing peptide in Atlantic cod (*Gadus morhua*): cloning, localization, developmental profile and role in food intake regulation. General and Comparative Endocrinology 160, 250 – 258.

CHAPTER 5

Diurnal Endocrine Profiles of Melatonin and IGF-I in Juvenile Barramundi (*Lates Calcarifer*) (Bloch) under 12L:12D and 24L:0D

K. L. Worrall[a], C. G. Carter[b], M. J.R. Porter[c], R. J. Wilkinson[a]

[a] National Centre for Marine Conservation and Resource Sustainability, University of Tasmania, Locked Bay 1370, Launceston, TAS, 7250, Australia

[b] Tasmanian Aquaculture and Fisheries Institute, University of Tasmania, Private Bag 49, Hobart, TAS 7001, Australia

[c] Ridley Aquafeed Pty Ltd, 12 Neon Street, Narangba Brisbane, QLD, 4504, Australia

Corresponding author. Tel.: +61 3 6324 3800; fax: +61 3 6324 3804

E-mail address: kworrall@utas.edu.au

Keywords: Asian sea bass; Barramundi; Growth; *Lates calcarifer*; Melatonin; Photoperiod, IGF-I

5.1 Abstract

The current study analysed the effects of photoperiod on diurnal plasma hormone profiles (melatonin and IGF-I) and growth of barramundi. Barramundi (11.74 ± 0.33 g and 10.31 ± 0.10 cm) were reared under either 12L:12D or 24L:0D and held at 30°C in brackish water (10‰) while fed a commercial pelleted feed twice daily to apparent satiation for a period of 56 days. Diurnal profiles of plasma melatonin and IGF-I in barramundi were analysed at day 20, 40 and 56. Wet weight, total length, SGR, condition factor and feed intake were measured to assess growth performance of barramundi.

Final wet weight (126.53 ± 2.12 g), total length (22.17 ± 0.12 cm) and specific growth rate (SGR weight = 4.25 ± 0.02 % $bw.d^{-1}$; SGR length = 1.38 ± 0.01 % $lt.d^{-1}$) were significantly higher in fish reared under 24L:0D compared to 12L:12D (119.25 ± 1.79 g; 21.52 ± 0.10 cm; 4.15 ± 0.02 $\%.d^{-1}$; 1.32 ± 0.01 $\%.d^{-1}$). Feed intake did not significantly differ between 12L:12D (1.55 ± 0.04 $g.d^{-1}$) and 24L:0D (1.61 ± 0.05 $g.d^{-1}$). Feed conversion efficiencies were significantly improved in fish reared under 24L:0D (133.24 ± 2.05 %) compared to 12L:12D (122.53 ± 1.53 %). In addition, HSI did not significantly different between 12L:12D and 24L:0D. Total lipid content in livers did not significantly differ between treatments, indicating enhanced growth of fish reared under 24L:0D is not due to fish utilizing stored lipids in the liver to mobilise energy to compensate for a greater energy demand for growth and an elevated metabolic rate. This indicates photoperiod is influencing fish's ability to utilize feed more efficiently. Photoperiod is possibly indirectly impacting on fish's ability to utilize feed more efficiently through hormonal stimulation.

In both 12L:12D and 24L:0D, the duration of plasma melatonin levels was consistent with other studies, demonstrating base levels of melatonin during the photophase and peaks occurring during the scotophase (or what would normally be the scotophase). Although melatonin concentrations in 24L:0D followed the same duration as 12L:12D (peaks occurring during the scotophase), these concentrations were reduced (amplitude) at a number of sample times throughout the scotophase. At day 20, plasma melatonin concentrations were significantly reduced in fish reared under 24L:0D at 2100 (2100 = t-test; $P<0.05$; t = 2.592; df = 3), at 2100 and 0000 on day 40 (2100 = t-test; $P<0.05$; t = 2.592; df = 3; 0000 = t-test; $P<0.05$; t = 3.017; df = 3), at 0000 and 0300 at day 56 (0000 = t-test; $P<0.05$; t = 3.407; df = 3; 0300 = t-test; $P<0.05$; t = -2.694; df = 4) in comparison to 12L:12D.

Reduced amplitude of melatonin during the scotophase in fish exposed to 24L:0D may directly or indirectly alter fish growth via influencing the GH/IGF-I axis. In the current study, diurnal plasma IGF-I concentrations varied greatly over a 24 hour period, with general trends showing higher peaks around feeding times. At day 56, peaks of plasma IGF-I concentrations occurred at different times in fish reared at 24L:0D, with significantly lower concentrations at 0600 (43.03 ± 6.79 $ng.mL^{-1}$) and significantly higher concentration at 0900 (86.59 ± 2.70 $ng.mL^{-1}$) in comparison to 12L:12D (101.51 ± 6.34 $ng.mL^{-1}$; 36.24 ± 9.48 $ng.mL^{-1}$). A shift in peak IGF-I concentrations around time of feeding may indicate an altered endogenous rhythm in fish reared under 24L:0D after 56 days. This could suggest photoperiod influences fish growth indirectly as reduced melatonin concentrations may alter circadian rhythms which in turn alter fishes perception of time of feeding, consequently observing a shift in IGF-I peaks around time

of feeding. Altered endogenous rhythms and/or IGF-I concentrations may enable improved feed utilization, prompting growth increases when fish are reared under 24L:0D. Shifts in IGF-I peaks around feeding times, largest reductions in melatonin levels during the scotophase and significant increases in growth were only observed at day 56. As significant growth increases were not observed until day 56, this may suggest the amplitude of melatonin requires to be reduced to a certain threshold to alter circadian rhythms and IGF-I concentrations. Understanding endocrine mechanisms involved with photoperiod perception and growth in barramundi will enable the development of photoperiod manipulation techniques that optimal artificial lighting regime to improve farming techniques.

5.2 Introduction

The current study compares the diurnal endocrine profiles of plasma melatonin and IGF-I in barramundi when reared under 12L:12D and 24L:0D. In previous research, growth of juvenile barramundi was significantly enhanced when fish were reared under 24L:0D, without observing significant increases in feed intake. Photoperiod manipulation affects feed conversion efficiency in fish (Boeuf and Le Bail, 1999; Biswas et al., 2005) although the interaction between the environmental cues and metabolic pathway are not fully understood (Volkoff et al., 2010). The aim of this study was to ascertain whether 24L:0D affected endocrine parameters involved with photoperiod perception (melatonin) and fish growth (insulin-like growth factor –I (IGF-I). Additionally, to clarify whether improved feed utilization in barramundi reared under 24L:0D is related to modifications of (IGF-I) which is one possible pathway involved with growth of barramundi.

Effectiveness of artificial lighting on fish depends on the species as well as light wavelength, intensity and duration of photoperiod (Porter et al., 1998; Boeuf and Le Bail, 1999; Vera et al., 2005; Migaud et al., 2007). Artificial lighting regimes are aimed at altering/suppressing rhythmic secretions of melatonin in order to manipulate fish's endogenous rhythms, simulating a summer photoperiod which is favourable for growth (Porter et al., 2001). Photoperiod is detected in fish via the pineal gland and retina which in turn synthesizes and releases the hormone melatonin (Gern and Greenhouse, 1988). Synthesis and release of melatonin reflects the photophase/scotophase (light/dark) cycle, with base levels occurring during the photophase while high levels peak during the scotophase (Iigo et al., 1991; Falcon, 1999; Migaud et al., 2006). Many tropical and temperate fish are under control of intra-pineal oscillators based within the photoreceptor

cells that are capable of self-sustaining melatonin rhythms that will also continue in the absence of light stimuli (Bolliet et al., 2006; Migaud et al., 2006; Takemura et al., 2006; Falcon et al., 2007). A photoperiodic circadian system comprises of light entering fish and being transformed into a timed neural and hormonal signal (Falcon et al., 2010). Melatonin is one major output of the intra-pineal oscillators, which conveys rhythmic photoperiodic information to the organism. However, a number of species have evolved different circadian systems involved with photoperiod perception. In species such as European sea bass (*Dicentrarchus labrax*) and Atlantic cod (*Gadus morhus*), full amplitude of melatonin production has been shown to rely on both the pineal gland and eyes to perceive photic information whereas in Nile tilapia and African catfish, melatonin production is solely reliant on the eyes (Bayarri et al., 2003; Migaud et al., 2007). Additionally, no endogenous circadian systems have been shown to exist in salmonids (Gern and Greenhouse, 1988; Migaud et al., 2006; Iigo et al., 2007). This demonstrates not all species react to photoperiod manipulation and artificial lighting in the same manner. In addition, for artificial lighting to be effective plasma melatonin levels are required to be reduced below a "critical" threshold level (Porter et al., 1999), thus melatonin analysis provides a valuable tool for assessing fish's perception of light as well as the effectiveness of artificial lighting systems. As far as I am aware, this is the first study to ascertain melatonin synthesis in response to the light/dark cycle in barramundi, in addition to investigating melatonin synthesis in barramundi exposed to continuous light.

The diurnal rhythm of elevated plasma melatonin concentrations occurring during the night with base levels occurring during the day, it allows fish to perceive the time of day.

Additionally, the duration of nocturnal elevation in melatonin provides information concerning the time of year since photoperiod length varies with the season (Reiter, 1993; Porter et al., 2000, 2001; Falcon et al., 2010). These melatonin rhythms act on neuro-endocrine regulation of key physiological processes such as growth and development (Falcon et al., 2003). For example, melatonin acting through MT2 receptors regulates cell proliferation rate in the zebrafish (*Danio rerio)* embryo and accelerates fish development (Danilova et al., 2004). But like most biological processes, growth is influenced by many external environmental cues such as temperature and nutrition – not only photoperiod.

Several growth performance studies have demonstrated increased photoperiod results in increases in growth (Davie et al., 2003), feed intake (Biswas et al., 2006, 2010), GH production (Boeuf and Le Bail, 1999; McCormick et al., 2007) and IGF-I levels (Taylor et al., 2005; Davie et al., 2007). The main endocrine pathway for growth in fish involves the growth hormone (GH) and insulin-like growth factor-I (IGF-I) axis. In this pathway, GH is secreted by the pituitary gland which induces the liver to secrete IGF-I (Duan et al., 1997). IGF-I induces growth-related cellular processes like cell proliferation and differentiation that ultimately results in overall growth of fish (Duan et al., 1997; Duan, 1998). Photoperiod may be impacting on growth via direct simulation of hormones involved with the GH/IGF-I growth axis as melatonin has been observed to directly affect GH concentrations (Falcon et al., 2003, 2010) or via altering endogenous rhythms such as circadian systems (Porter et al., 1998; Endal et al., 2000; Falcon et al., 2010).

To gain knowledge regarding photoperiodic physiological effects on barramundi, this study investigated circadian endogenous melatonin rhythms as well as part of the main endocrine pathway in growth, plasma IGF-I levels. Ascertaining diurnal concentrations will help towards determining whether photoperiod affects growth directly via the GH–IGF axis, or whether its action may be indirectly mediated by melatonin.

Growth was also examined because it has been suggested that increases in IGF-I levels is a component of the photoperiodically entrained seasonal drive of growth and the increase in food intake is a response to satisfy the increased energy demand for this maintaining growth (Webster et al., 2001). Growth differences between 12L:12D and 24L:0D treatments were indicated using wet weight, total length, SGR, feed intake and FCE as well as total lipid content of livers giving an approximate indication of nutritional status in barramundi. This study was the first to ascertain and compare diurnal profiles of plasma melatonin and IGF-I in juvenile barramundi held under 12L: 12D and 24L: 0D to assess how continuous light alters photoperiod recognition and growth stimulation over a period of 56 days

5.3 Material and Methods

5.3.1. Experimental Design

Juvenile barramundi from WBA Hatcheries, Adelaide (South Australia) were acclimated for a period of 3 days in 180 L aquaria at 30^{o}C in 10 ‰ seawater and held under 12L: 12D. Fish were not fed during the acclimation period. Following acclimation, 30 fish were randomly allocated into each of eighteen 80 L tanks maintained at 30^{o}C with 10 ‰ seawater (initial mean stocking density 4.40 kg/m^{3}). Groups of six tanks were organised

in three recirculation systems each with a 500 L reservoir and 500 L biological filter (Katersky and Carter 2007). Water was delivered at a rate of 2.8 $L.min^{-1}$ and oxygen levels maintained above 90% saturation. Water parameters (Appendix 1) was monitored daily and water changes made as necessary to keep water quality within the limits for barramundi (Tucker et al., 2002). Water quality parameters were consistent across all treatment tanks for the duration of the experiments. Particulate dacron filters were cleaned daily and water exchange was less than 10% per day (to replace water discarded during cleaning and siphoning uneaten feed).

Control over the water temperature was achieved using submersible heaters in each reservoir, each controlled with an individual thermostat. Temperature was recorded every half hour with StowAway Tidbit Temperature Loggers (Onset Computer Company, Bourne, MA, USA) as well as each system being measured daily with a thermometer. Diurnal variation in water temperature in each re-circulation system was ± 0.5°C of the intended temperature.

Experimental treatments were partitioned into photoperiods by using individual tank covers which incorporated individual halogen (white light) waterproof lights (20 watt) with timers set to turn on lights, without a dimming effect, at 0700 and turn off at 1900 for the 12L: 12D treatments. Average light intensity throughout the water column was 540 lux (~9 $\mu mol\ s^{-1}\ m^{-2}$) which was measured using a Li-COR Underwater Quantum sensor (LI-192SA) throughout several points within experimental tanks. Fish were maintained on two experimental photoperiods of twelve hours light and twelve hours dark (12L:12D) and continuous light (24L:0D) for a period of 56 days. Both

experimental treatments were hand fed a commercial pelleted feed (Marine Start 1-4 mm, Marine Float 54/10 4mm, crude protein 52% and crude Fat 16%, Ridley Aquafeed, Australia) to satiation twice a day (0900 and 1700). Feed intake was recorded for each tank after each feed with any uneaten pellets being siphoned out, counted and the number converted to an equivalent weight of uneaten feed. Nine tanks were allocated to each photoperiod (12L:12D and 24L:0D) to cover nine sampling time points over a 24 hour time frame (0600, 0900, 1200, 1500, 1800, 2100, 0000, 0300, 0600(2)). This design eliminated the need to repeat sample from tanks. On day 20, 40 and 56 a total of 10 fish were sampled from each tank at the corresponding time point in the 24 h cycle.

5.3.2 Sampling Procedures

Sampling involved netting fish from experimental tanks and transferring fish to a 20 L tank containing iso-eugenol at 40 $mg.L^{-1}$ (AQUI-S, New Zealand Ltd). An initial sample of 20 fish from the acclimation tanks (Day 0) as well as 10 fish at Day 20, 40 and 56 were anaesthetized and measured for individual wet weight (g), total length (cm) and blood sampled for hormone analysis before being euthanized and dissected for analysis of liver total lipid. Blood sampling of fish involved blood being drawn from the caudal vein of fish for analysis of insulin-like growth factor-I (IGF-I) and the photoreceptive hormone, melatonin. Blood was collected using heparinised (ammonium heparin, Sigma; 4mg/ml) syringes (1ml Terumo syringes, 25G Terumo hypodermic needles) then centrifuged at 3500 rpm at 4^{o}C, for 15 mins and stored at -20°C until assayed. Plasma IGF-I levels were analysed using a commercially available RIA kit as mentioned previously described in Wilkinson et al., (2006) (GroPep, Adelaide, Australia). Plasma melatonin concentrations were assayed using total Melatonin kit (IBL International,

Hamburg, Germany) as detailed in section 3.3. Standard AOAC methods were used for nutritional tissue analysis; tissue was freeze dried to a constant weight and analysed for total lipid (Bligh and Dyer, 1959).

5.3.3. Melatonin Radioimmunoassay (RIA)

Plasma levels of melatonin were analysed using a commercially available melatonin direct RIA (Serum/Plasma) kit from IBL International GMBH (Hamburg, Germany), with serial dilutions of enzyme treated barramundi plasma being parallel to the standard curve supplied within RIA kit. Melatonin standards and fish samples in duplicate were pre-treated with 50 µL of enzyme solution and left to incubate for 3 hours at room temperature. 100 µL of assay buffer, 50 µL of Melatonin ^{125}I-Tracer Total Activity (67 274 cpm) and 50 µL of antiserum (rabbit polycolonal) were added standard and samples and centrifuged for 1 minute at 500 x g then left to incubate at room temperature for 24 hours. The following day, bound and free tracer were separated by adding 500 µL of precipitating antiserum (anti-rabbit IgG (goat), PEG, phosphate buffer) was added to each tube and incubated for 15 minutes at room temperature. The precipitate was then centrifuged at 3000 x g for 15 minutes at 20°C. The supernatant was removed by decanting and bound radioactivity determined using a gamma counter. Serial dilutions of enzyme treated barramundi plasma were parallel to the standard curve. The minimum detectable limit of the assay was 0.9 pg/mL. Inter-assay variation was 6.2 - 16% and intra-assay variation was 3.9 – 6.9 %.

5.3.4. Calculations

The following equations were used to calculate feed intake (FI), feed conversion efficiency (FCE), specific growth rate of weight and length (SGR weight and SGR length), condition factor (K) and hepatosomatic index (HSI) for each replicate tank (n = 3) on day 20 and 40 and 56.

FI ($g.d^{-1}$) = total dry feed intake/time (days)

FCE (%) = 100 x (wet weight gain/total dry feed intake).

SGR weight ($\%.bw.d^{-1}$) = $100x (\ln W_2 - \ln W_1)$/time (days)

Where, W_1 and W_2 indicate the initial and final wet weight (g) respectively.

SGR length ($\%.lt.d^{-1}$) = 100 x (TL_2-TL_1)/time (days)

Where, TL_1 and TL_2 indicate the initial and final total length (cm) respectively.

$K = 100 \times (W / L^3)$

Where, W = wet body weight (g) and L = total body length (cm)

HSI (%) = 100 x (wet weight of liver (g)/wet body weight (g))

5.3.5 Statistical Analysis

Statistical analysis was carried out using SPSS 16.0 for windows (SPSS Inc.). For each treatment (12L:12D vs 24L:0D) mean ± standard error was calculated from 10 fish from each tanks over all time points at each sample date. Growth data and feed intake were analysed using one way ANOVA and students t-test as well as two way nested ANOVA

with tank replicates nested within photoperiod and time. Hormone levels were compared between 12L: 12D and 24L: 0D using student's t-test at each time point over the diurnal profile. Differences were considered to be significant if $P<0.05$. Values are presented as means ± standard error (SEM).

5.4 Results

Total feed intake ($g.day^{-1}$) over the course of the experiment (56 days) did not significantly differ between 12L:12D and 24L:0D (t-test; $P>0.05$; t = 0.891; df = 16) (Table 5.1). Feed conversion efficiency was significantly greater in fish exposed to 24L:0D compared to 12L:12D (t-test; $P<0.05$; t = -4.232; df = 13) (Table 5.1).

Initial weights and lengths of barramundi did not significantly differ between treatments (one-way ANOVA; $P>0.05$; F= 0.474; df = 17) (one-way ANOVA; $P>0.05$; F = 0.877; df = 17). At day 20 and 40, weight and length of fish did not significantly differ between 24L:0D and 12L:12D. At day 56, a significant 6 % increase in weight and 3 % increase in length was observed in barramundi exposed to 24L:0D compared to 12L:12D (weight = two-way ANOVA; $P<0.05$; F = 2198.63; df = 7; length = two-way ANOVA; $P<0.05$; F =1638.01; df = 7) (Figure 5.1 A and B) (Table 5.1).

Significant differences in SGR weight and length between treatments only occurred towards the end of the experiment from day 40 to day 56 as well as SGR weight and lengths significantly decreasing over time. Fish reared under 24L:0D demonstrated significantly greater SGR weight and lengths (4.25 ± 0.02 % $bw.d^{-1}$; 1.38 ± 0.02 % $lt.d^{-1}$) on day 56 in comparison to 12L:12D (4.15 ± 0.02 % $bw.d^{-1}$; 1.32 ± 0.01 % $lt.d^{-1}$) (weight

= one-way ANOVA; $P<0.05$; t = -3.110; df = 16; length = one-way ANOVA; $P<0.01$; F = 35.90; df = 5) (Figure 5.2A and B).

Condition factor (K) did not significantly differ between treatments at day 0 and 20. At day 40 and 56, condition factor was significantly lower in fish exposed to 24L:0D compared to 12L:12D (two-way ANOVA; $P<0.05$; F = 23.61; df = 7) (Figure 5.3A). HSI did not significantly differ between treatments at day 0, 20, 40 or 56. At day 0, HSI was significantly lower compared to day 20, 40 and 56 (two-way ANOVA; $P<0.05$; F = 8.783; df = 7) (Figure 5.3B). Total lipid levels did not significantly differ in whole livers of barramundi exposed to either 12L:12D or 24L:0D at day 56 (t-test; $P>0.05$; t = -0.639; df = 15) (Figure 5.4).

Table 5.1. Summary of growth performance parameters in barramundi reared under 12L:12D or 24L:0D photoperiods for 56 days. (Mean + SE, n = 9) Asterisk denotes a significant difference (P<0.05). (Mean + SE, n = 9)

	12L:12D	**24L:0D**
Mean wet weight $_{initial}$ (g)	11.74 ± 0.33	10.31 ± 0.10
Mean wet weight $_{Day\ 56}$ (g)	119.25 ± 1.79	126.53 ± 2.12*
Mean total length $_{initial}$ (cm)	10.31 ± 0.10	10.26 ± 0.10
Mean total length $_{Day\ 56}$ (cm)	21.52 ± 0.12	22.17 ± 0.12*
Feed intake $_{Day\ 56}$ ($g.d^{-1}$)	1.61 ± 0.05	1.55 ± 0.04
FCE(%)	122.53 ± 1.53	133.21 ± 2.05*

FCE = Food conversion efficiency

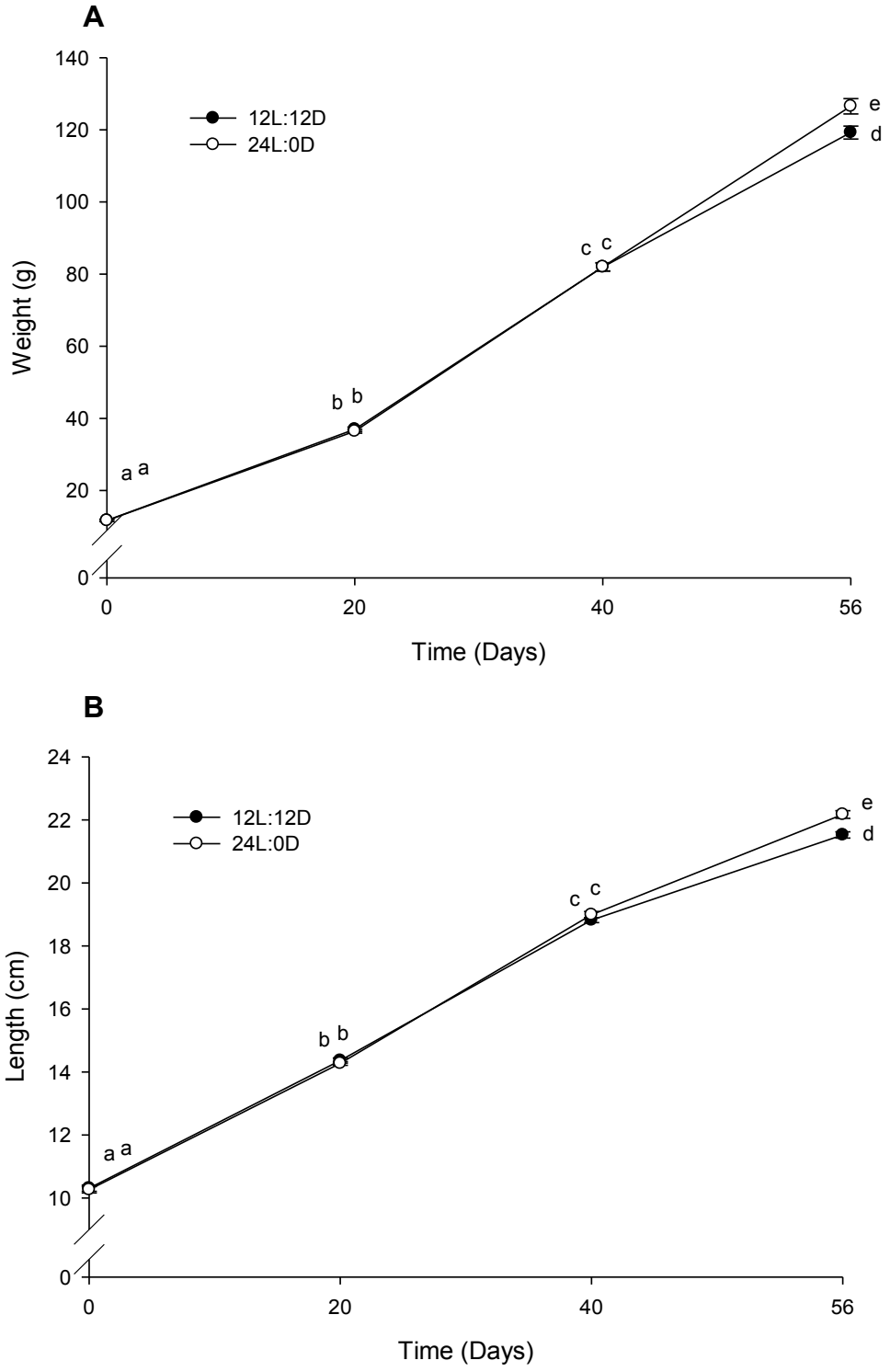
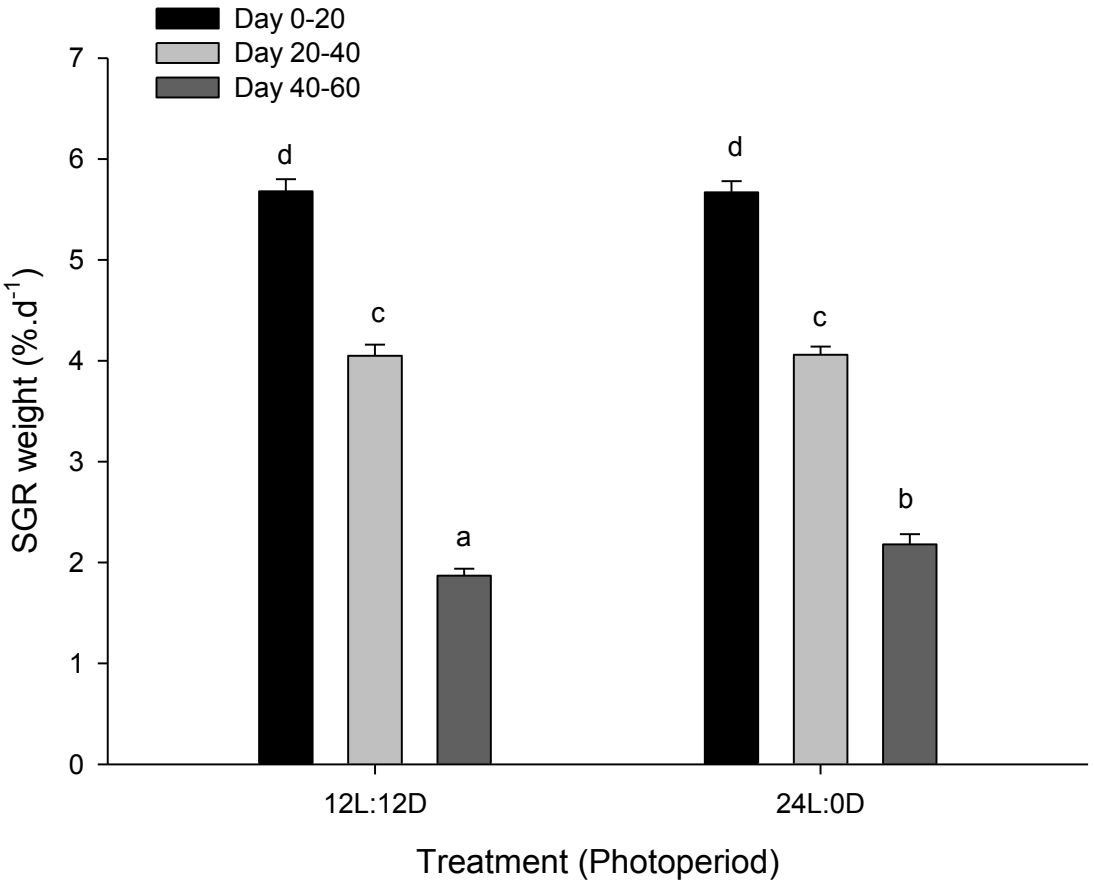


Figure 5.1. Mean wet weight (A) (g ± SEM) and mean total length (B) (cm ± SEM) of juvenile barramundi at day 0, 20, 40 and 56 held under 12L: 12D and 24L: 0D. Different letters denote significant differences (P<0.05) (n = ?).


Day 0-20
Day 20-40
Day 40-60
SGR weight (%.d-1)
0
1
2
3
4
5
6
7
d
c
a
d
c
b
12L:12D
24L:0D
Treatment (Photoperiod)

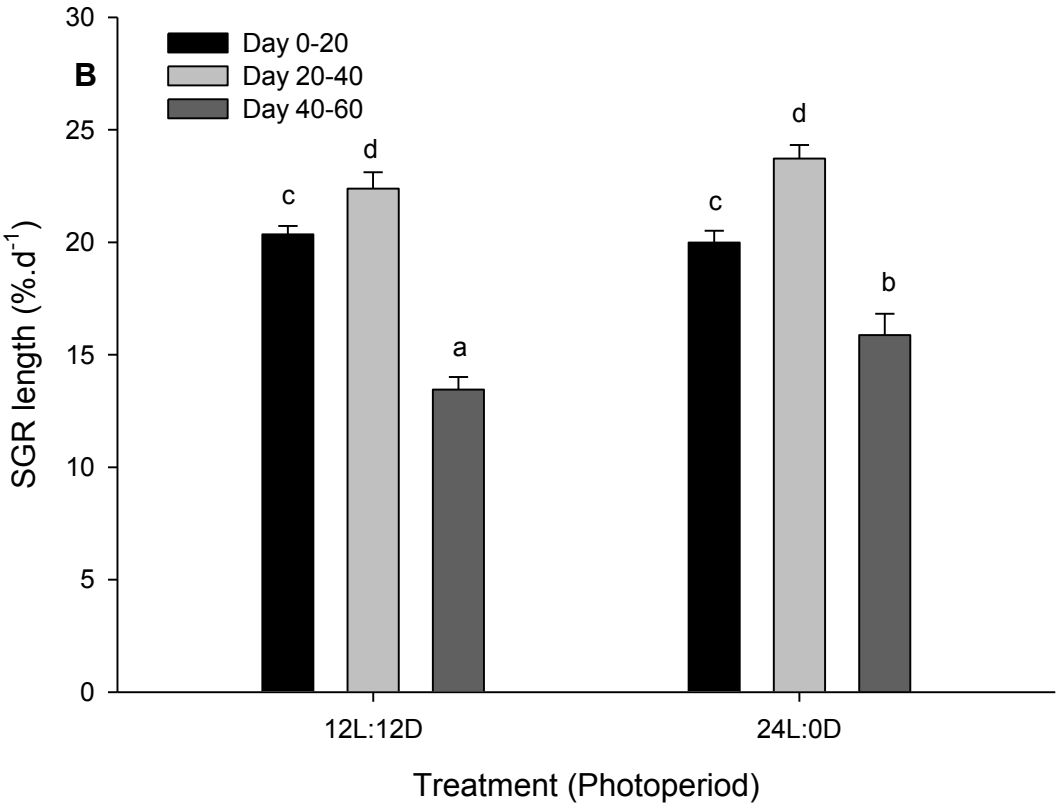


Figure 5.2. Mean specific growth rate for weight (SGR % bw.d^{-1}) (A) and length (SGR % lt.d^{-1}) (B) of juvenile barramundi, at day 20, 40 and 56 held under 24L:0D and 12L:12D. Different letters denote significant differences (P<0.05).

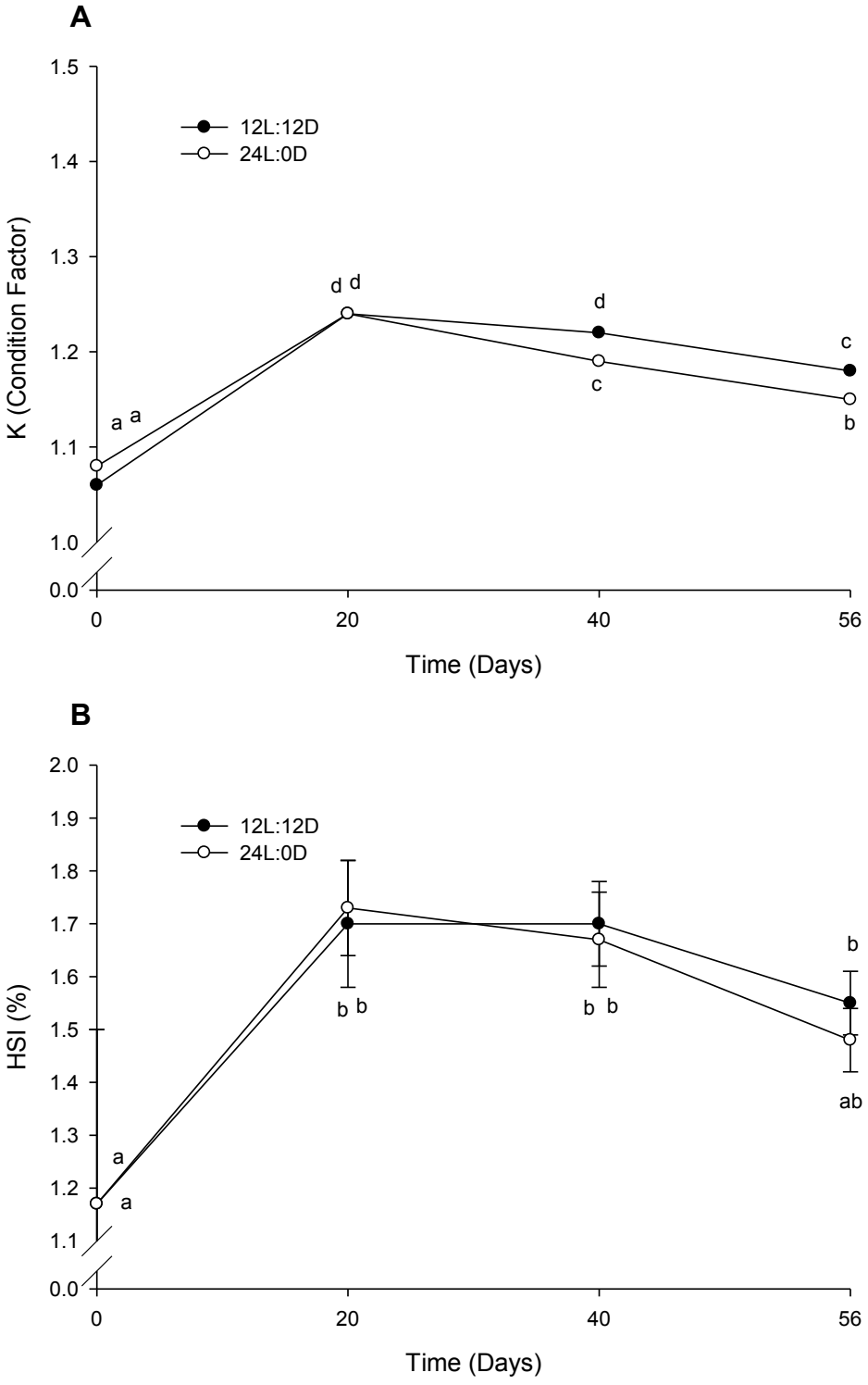


Figure 5.3. Mean condition factor (K) ± SEM (A) and mean HSI ± SEM (%) (B) of juvenile barramundi at Day 0, 20, 40 and 56 held under 12L: 12D and 24L: 0D. Different letters denote significant differences (P<0.05).

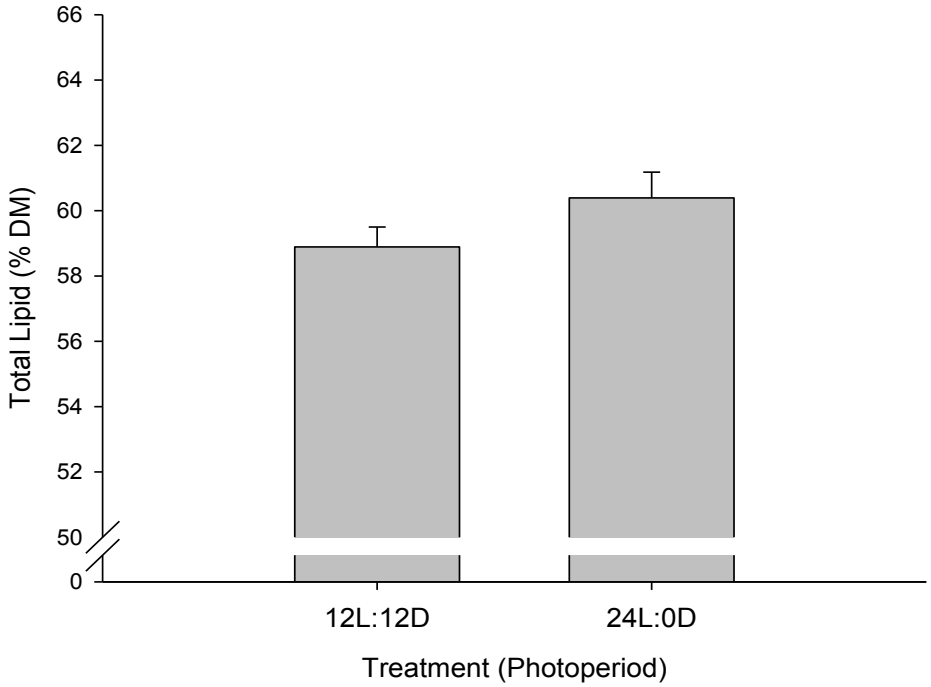
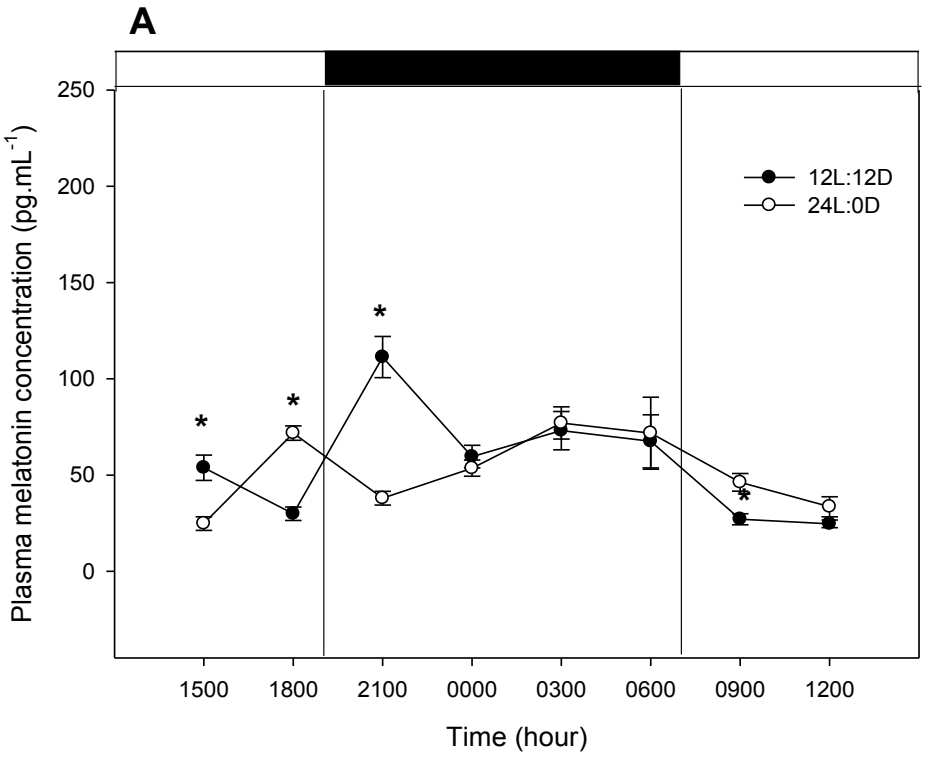


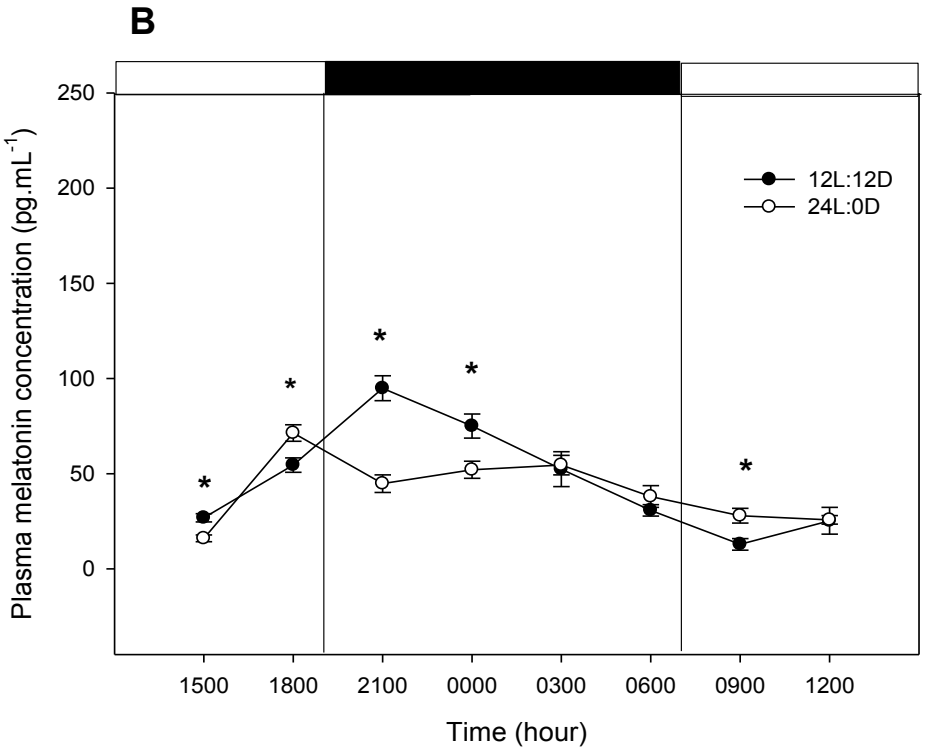
Figure 5.4. Mean total lipid content ± SEM (% DM) in liver of juvenile barramundi held under 12L: 12D and 24L: 0D at day 56.

Average plasma melatonin concentrations taken during the scotophase (from 1900 to 0700) in barramundi exposed to 12L:12D were significantly raised compared to the photophase (from 0700 to 1900) at day 20 (77.86 ± 11.47 $pg.mL^{-1}$; 34.45 ± 5.32 $pg.mL^{-1}$) (t-test; $P<0.05$; t = -3.693; df = 7), day 40 (29.87 ± 8.78 $pg.mL^{-1}$; 56.96 ± 12.45 $pg.mL^{-1}$) (t-test; $P<0.05$; t = -2.069; df = 6) and day 56 (15.15 ± 1.37 $pg.mL^{-1}$; 101.05 ± 34.88 $pg.mL^{-1}$) (t-test; $P<0.05$; t = -2.729; df = 6) (Figure 5.5A, B and C). When reared under 24L:0D, plasma melatonin concentrations did not significantly differ between the photophase and what would normally be the scotophase. At day 20, increased levels of plasma melatonin were observed in barramundi exposed to 24L:0D immediately prior to the scotophase (1800) (108.77 ± 33.22 $pg.mL^{-1}$) and 2 hours after sunrise (0900) (46.21 ± 4.55 $pg.mL^{-1}$) compared to 12L:12D (28.94 ± 3.53 $pg.mL^{-1}$; 27.04 ± 2.89 $pg.mL^{-1}$) (1800 = t-test; $P<0.05$; t = -3.709; df = 4; 0900 = t-test; $P<0.05$; t = -3.243; df = 5) (Figure 5A). This trend continued on day 40 (Figure 5.5B). During the scotophase, plasma melatonin concentrations were significantly depressed in fish reared under 24L:0D at 2100 on day 20 (2100 = t-test; $P<0.05$; t = 2.592; df = 3), at 2100 and 0000 on day 40 (2100 = t-test; $P<0.05$; t = 2.592; df = 3; 0000 = t-test; $P<0.05$; t = 3.017; df = 3), at 0000 and 0300 at day 56 (0000 = t-test; $P<0.05$; t = 3.407; df = 3; 0300 = t-test; $P<0.05$; t = -2.694; df = 4) (Figure 5.5C) in comparison to 12L:12D.

Diurnal plasma IGF-I concentrations varied greatly over a 24 hour period, with general trends showing higher concentrations around feeding times (Figure 5.6A, B and C). At day 20, increases in plasma IGF-I concentrations during times of feeding (0600 and 1800) in both 12L:12D and 24L:0D treatments were observed (Figure 5.6A). At day 56, a shift in peak plasma IGF-I concentrations was observed in fish reared at 24L:0D, with

significantly reduced concentrations at 0600 (43.03 ± 6.79 ng.mL^{-1}) and significantly raised concentration at 0900 (86.59 ± 2.70 ng.mL^{-1}) in comparison to 12L:12D (101.51 ± 6.34 ng.mL^{-1}; 36.24 ± 9.48 ng.mL^{-1}). Additionally at day 56, plasma IGF-I concentrations in fish reared under 24L:0D were significantly reduced at 0000 (1.61 ± 0.18 ng.mL^{-1}) and 0300 (1.56 ± 0.02 ng.mL^{-1}) when compared to 12L:12D (40.56 ± 5.6 ng.mL^{-1}; 39.35 ± 4.63 ng.mL^{-1}. Average daily plasma IGF-I concentrations did not significantly differ between 12L:12D and 24L:0D at day 20, 40 or 56 (Figure 5.7).


A
Plasma melatonin concentration (pg.mL-1)
250
200
150
100
50
0
12L:12D
24L:0D
1500
1800
2100
0000
0300
0600
0900
1200
Time (hour)


B
Plasma melatonin concentration (pg.mL-1)
250
200
150
100
50
0
12L:12D
24L:0D
1500
1800
2100
0000
0300
0600
0900
1200
Time (hour)

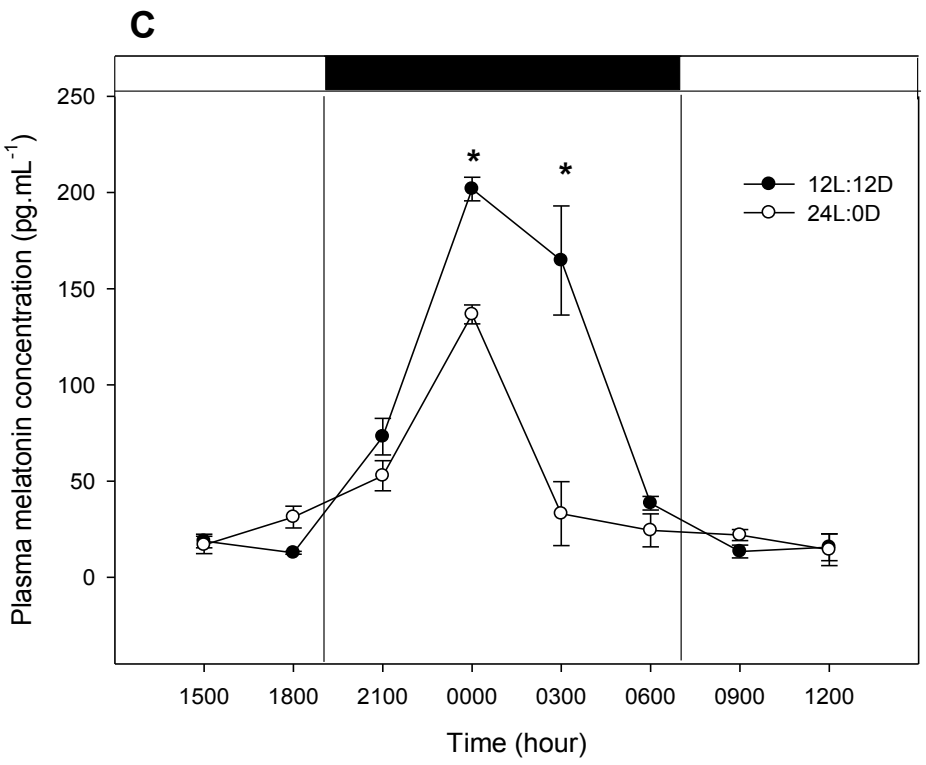
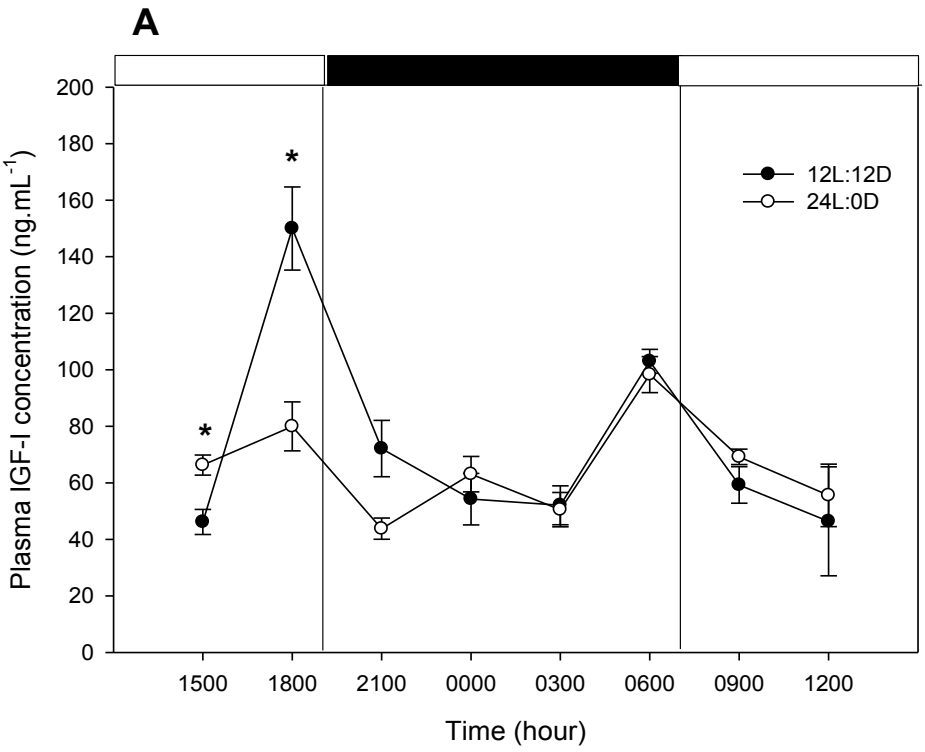
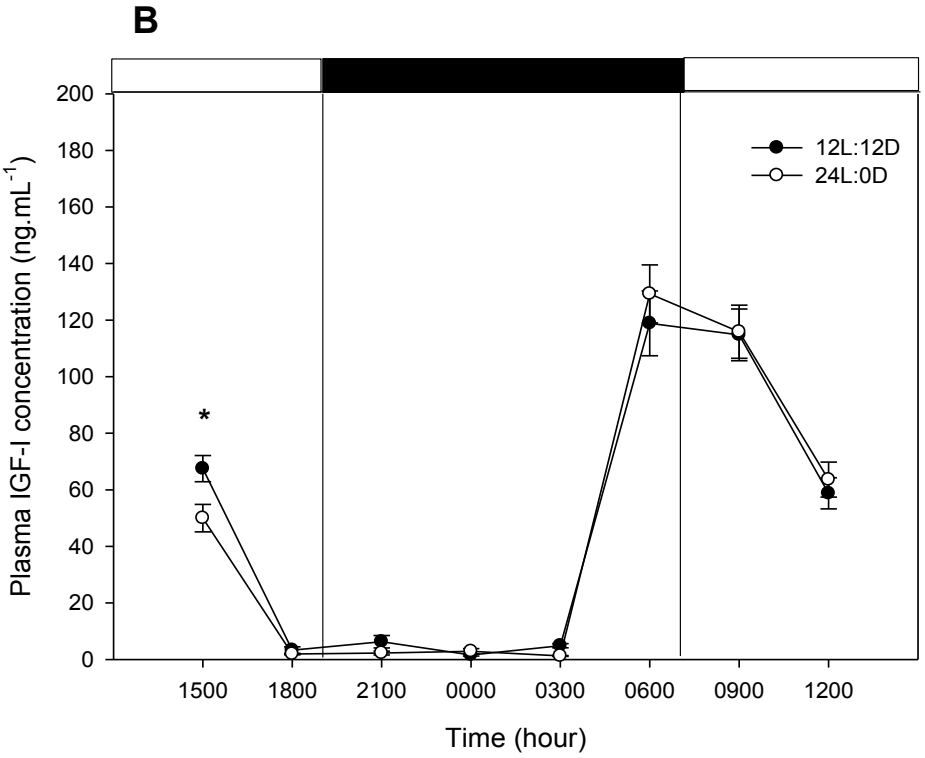


Figure 5.5. Mean plasma melatonin concentration ($pg.mL^{-1}$ ± SEM) of juvenile barramundi, taken at Day 20 (A), Day 40 (B) and Day 56 (C) held under 12L: 12D and 24L: 0D. Black horizontal bar indicates scotophase and white bars the photophase. Asterisks denote significant differences ($P<0.05$).


A
Plasma IGF-I concentration (ng.mL-1)
200
180
160
140
120
100
80
60
40
20
0
*
*
12L:12D
24L:0D
1500
1800
2100
0000
0300
0600
0900
1200
Time (hour)


B
Plasma IGF-I concentration (ng.mL-1)
200
180
160
140
120
100
80
60
40
20
0
*
12L:12D
24L:0D
1500
1800
2100
0000
0300
0600
0900
1200
Time (hour)

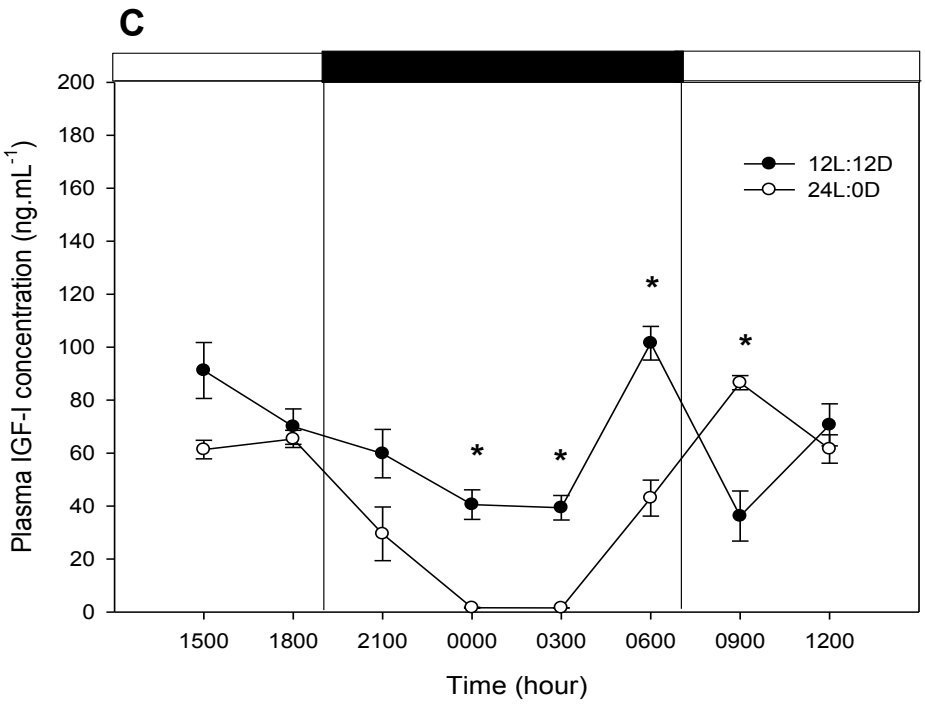


Figure 5.6. Diurnal changes in mean plasma IGF-I concentration ($ng.mL^{-1}$ ± SEM) of juvenile barramundi, taken at Day 20 (A), Day 40 (B) and Day 56 (C) held under 12L:12D and 24L:0D. Black horizontal bar indicates scotophase and white bars the photophase. Asterisks denote significant differences ($P<0.05$).

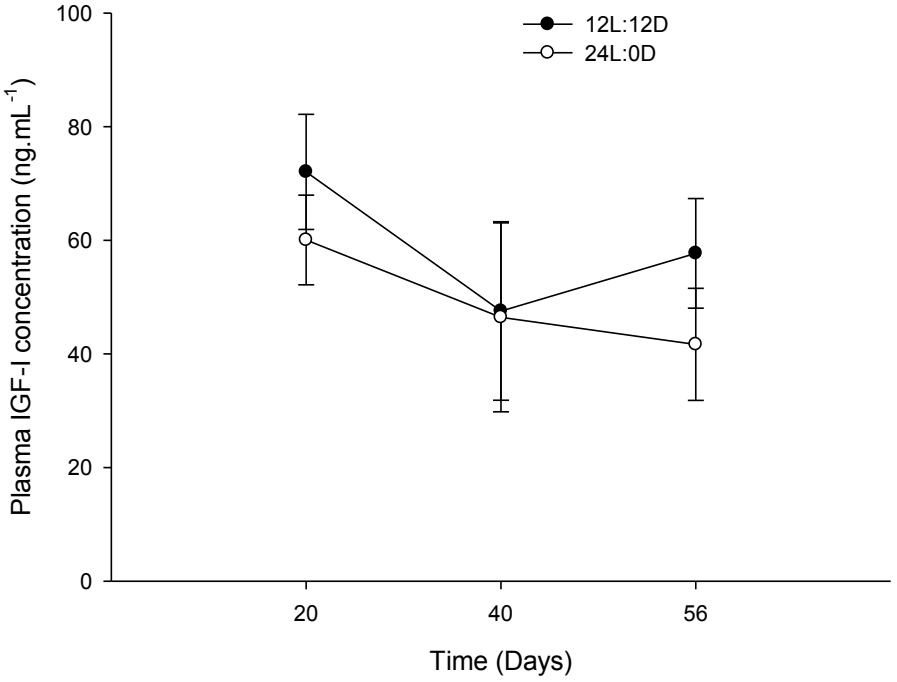


Figure 5.7 Average daily plasma IGF-I concentration ($ng.mL^{-1}$ ± SEM) of juvenile barramundi, taken at Day 20, Day 40 and Day 56 held under 12L:12D and 24L:0D. Asterisks denote significant differences ($P<0.05$).

5.5 Discussion

The present study investigated the effects of 24L:0D on diurnal endocrine profiles of melatonin and IGF-I in comparison to 12L:12D as well as growth parameters of barramundi. Diurnal profiles of plasma melatonin and IGF-I in barramundi were analysed as these hormones are central to fish's photoperiod recognition and growth stimulation. In the previous three chapters, enhanced growth performances of barramundi were observed without increases in feed intake when reared under continuous light. Significant growth increases without an increased feed intake suggested improved feed utilization when reared under 24L:0D. Improved feed utilization may occur from direct mechanisms such as longer time frames enabling enhanced digestion and utilization of feed or alternatively alteration of enzymes causing feed to be metabolised more efficiently. Alternatively extended photoperiod may indirectly influence the endocrine/growth axis. The pathways involved with increased growth rates under extended photoperiods are currently not understood. Therefore the aim of this study was to ascertain whether 24L:0D affected endocrine parameters involved with photoperiod perception (melatonin) and fish growth (IGF-I).

5.5.1 Growth

The current results parallels previous experiments (Chapter 2, 3 and 4), in which significant increases in wet weight, total length and SGR weight and SGR length without significant increases in feed intake occurred in fish when reared under 24L:0D compared to 12L:12D. These results indicate improved utilization of feed is the cause for enhanced growth in fish reared under 24L:0D. Gross et al., (1965) were the first to specify growth

might be influenced by light through better food conversion efficiency and not just stimulated food intake.

Improved feed utilization occurs from a greater efficiency in to retaining nutrients for growth which may occur at any level in the use of nutrients from digestion, absorption as well as how nutrients are used or retained, with photoperiod either directly or indirectly impacting on metabolism (Buddington and Krogdahl, 2004; Nelson and Sheridan, 2006; Klein and Sheridan, 2008). In the current experiment, HSI and total lipid content in the liver of barramundi did not significantly differ between 12L:12D and 24L:0D. This suggests higher growth rates observed in fish reared under 24L:0D are not due to fish using stored energy supplied of lipid in the liver to mobilize energy to compensate for a greater energy demand for growth and an elevated metabolic rate. This suggests other ways in which barramundi reared under 24L:0D retain nutrients as growth. Herbinger and Friars (1991) found strong and significantly positive correlations of condition factor and total lipid content in immature Atlantic salmon, indicating condition factor can be used as an indicator of energy reserve status. Although in this experiment condition factor was significantly higher in fish reared under 12L:12D compared to 24L:0D.

Extended photoperiod such as 24L:0D is suggested to directly alter fishes metabolism due to the extended day length permitting slower and more efficient digestive process which may improve overall digestion and retention of nutrients (Biswas et al., 2006). Alternatively, extended photoperiod may indirectly alter fish growth by direct endocrine stimulation of growth or by altering endogenous control of growth rhythms which in turn

may influence utilization of feed (Krakenes et al., 1991; Taylor et al., 2005; Falcon et al., 2010).

5.5.2 Endocrine Mechanisms Involved with Photoperiod and Growth

Photoperiod is perceived by fish via the hormone melatonin as it is produced and secreted in response to the duration, intensity and spectral composition of light (Reiter, 1993; Eckstrom and Meissl, 1997). As melatonin is released in response to seasonal changes in photoperiod, it thereby affects time regulated functions including locomotor activity, thermal preference, rest, food intake, vertical migration and shoaling, skin pigmentation, osmoregulation and metabolism (including control of hypothalamic monoamines, hepatic lipids and glucose and plasma steroid levels) and growth (Zachman et al., 1992; Reebs et al., 2002; Falcón et al., 2007, 2010).

The current results are not conclusive to allocate barramundi to a particular diurnal melatonin rhythm as mentioned at Falcon et al., (2010). At day 20 and 40, barramundi exposed to 12L:12D demonstrate diurnal melatonin rhythms which observed a discrete peak early mid dark phase. Whereas at day 56, a "Type A" diurnal melatonin rhythms in barramundi exposed to 12L:12D observed a discrete peak in late dark phase as also demonstrated with Atlantic cod and haddock (Falcon et al., 2010). In addition, the duration of diurnal melatonin rhythms was found to continue to occur in barramundi exposed to 24L:0D, albeit with depressed amplitude of melatonin, being significantly reduced during the scotophase at day 20, 40 and 56.

In the current study, the duration of melatonin level elevation was not affected but rather the amplitude of this elevation was significantly reduced in fish exposed to continuous light. Usually the duration of raised melatonin synthesis is dictated by the prevailing photoperiod whereas the amplitude is thought to be influenced by other environmental factors such as temperature and light intensity (Randall et al., 1995; Thrush et al., 1995; Porter et al., 1998). These results suggest barramundi have entrained intra-pineal oscillators within the photoreceptor cells and even in the absence of a defined light/dark cycle can maintain an endogenous melatonin rhythm. This has also been observed in Atlantic cod where fish posses a diel rhythm of melatonin production which continued under endogenous control in the absence of external cues (Porter et al., 2001).

Entrainment of rhythmic circadian production and secretion of melatonin in fish is highly conserved among fish (Falcon et al., 2010). In tropical areas, the phase of the rhythm in fish is locked to the 12L:12D cycle and displays remarkable stability (Martinez-Chavez et al., 2008). Stability of melatonin rhythms were observed in barramundi reared under 24L:0D concentrations of melatonin were being significantly depressed at 2100 during the scotophase on day 20. At day 40 and 56 further depressions during the scotophase (2100 and 0000 at day 40, 0000 and 0300 at day 56) were observed in fish reared under 24L:0D. This suggests entrainment of endogenous circadian systems pertaining to photoperiod in barramundi may be dampened with time. Depression in the amplitude of melatonin over time may be reflected in growth parameters, as significant increases in growth of barramundi reared under 24L:0D was not observed until completion of the trial, day 56. It is apparent that the entrainment of circadian rhythms of melatonin in juvenile barramundi took almost 50 days after which the influence on other parameters

such as IGF and growth became apparent. Duston and Bromage, (1988) found entrainment to long day photoperiods in rainbow trout did not occur for 6-8 weeks. From earlier results in this thesis it is suggested that entrainment may require less time if applied at an earlier stage of development, possibly as the inhibition of melatonin is observed sooner and thereby may begin to influence growth parameters within less than the 56 days observed here.

This has implications for the commercial application of additional light in barramundi as the specific growth rates observed in juvenile fish are significantly greater than later stages of development (Davis and Kirkwood, 1984), with growth of barramundi usually doubling the size every 7 days.

It is therefore likely that barramundi have a very stable entrainment of melatonin rhythms, needing duration of time to alter entrained rhythms (longer than 56 days) or rather, perhaps only amplitude of melatonin secretion can be dampened with extended photoperiods, not duration of elevation. This could suggest there is a lag/adjustment phase before photoperiod is capable of inducing growth, which has also been observed in Atlantic salmon (Oppedal et al., 1997; Taranger et al., 1999). This is further suggested by Taylor et al., (2005), finding significantly greater weight gains in rainbow trout approximately 12-14 weeks after the onset of long days or continuous light.

A lag or adjustment phase before photoperiod is capable of inducing growth would have important implications regarding initial commencement of photoperiod manipulation. Additionally, growth performances may vary with initial commencement of photoperiod

manipulation as fish's sensitivity to light varies with developmental stages (Biswas et al., 2006; Falcon et al., 2010). Smaller fish have been observed to be more sensitive to light compared to older fish due to the greater transmission of light through the pineal window, as well as light transmittance differences being observed between species (Simensen et al., 2000; Migaud et al., 2007).

The exact mechanisms conveying photoperiodic information to the GH/IGF-I growth axis are still unclear; however, extended photoperiod may cause direct photostimulation of growth through an up-regulation of the GH-IGF growth axis (Boeuf and Falcon, 2001; Taylor et al., 2005; Falcon et al., 2007). Melatonin may prove be an intermediary in the up-regulation of the GH-IGF growth axis. Melatonin is known to directly influence growth hormone (GH) (Falcon et al., 2003), which in turn is the primary stimulus for the synthesis and release of plasma IGF-I from the liver (Reineke et al., 2010). Additionally, IGF-I is synthesised and released in stimulus to GH, whereas there is increasing evidence that IGF-I is also GH independent as well as being a negative feedback to GH, further increasing the complexity of this system (Beckman et al., 1998, 2004; Pierce et al., 2001). GH levels were not measured in the present study, but would prove insightful for future studies to correlate GH with melatonin and IGF-I concentrations.

Numerous studies have observed positive correlations between extended photoperiod with increased appetite, growth, GH production and plasma IGF-I levels (Ditchkoff et al., 2001; Taylor et al., 2005, 2008; Imsland et al., 2008). The current results indicate it is hard to compare IGF-I results between studies as diurnal profiles of IGF-I significantly fluctuate throughout a 24 hour period as well as with time and photoperiod. A number of

studies have suggested circulating IGF-I levels may be an effective tool to rapidly assess growth whereas the current results suggest caution should be applied when using circulating IGF-I levels of this purpose. For example, significant growth increases were observed in fish reared under 24L:0D at day 56, and if samples for IGF-I were taken at either 0600 or 0900, contradicting results would be observed following completion of the analysis.

Taylor et al., (2005) suggests melatonin does not act directly on the GH-IGF axis to control growth and similar to the current results, depressed amplitude of melatonin observed during the scotophase under 24L:0D did not seem to directly influence IGF-I concentrations. It should be noted however that the current study used radioimmunoassay methods to detect total IGF-I concentrations which does not necessarily provide the entire picture of IGF's due to the complexity of the IGF system. Circulating levels of IGF-I are affected by a number of factors that determine "free" IGF-I, including IGF-I binding protein as well as the expression of IGF-I receptors in tissues (Shimizu et al., 2000; Reinecke et al., 2005). Most IGF in circulation bind to proteins (IGF binding proteins – IGFBP's) which prolong the half life of IGF, preventing their insulin-like activity and control their availability to target tissues. The remaining "free" IGF, not bound to binding proteins, is biologically active and is believed to be a more sensitive indicator of short-term metabolic changes than total IGF (Frystyk et al., 1994; Zapf, 1997; Shimizu et al., 1999). Possibly 24L:0D may alter IGF-binding proteins or receptors which may improve the biological activity of circulating IGF-I concentrations which may impact on metabolism. Future studies could investigate not only total IGF-I concentrations but "free" IGF-I, IGFBP's, IGF-I receptors as well as IGF-I mRNA

concentrations to achieve a better indication of how the IGF system is being altered when exposed to extended day length.

IGF-I concentrations peaked generally around feeding times, showing reduced levels during the scotophase and peaking just before the beginning on the photophase, especially at day 40. This was also found to be the case in salmonids and other marine species, where plasma IGF-I levels were associated with feeding regimes (Duan, 1998; Pierce et al., 2001; Pérez-Sánches, 2000; Shimizu et al., 2009).This may indicate the use of circulating IGF-I as a tool in fine-tuning feeding regimes in barramundi. Interestingly, at day 56, this peak had shifted in fish reared under 24L:0D.

Although plasma melatonin concentrations did not seem to directly influence IGF-I concentrations, an indirect mechanism of altering circadian rhythms may influence the GH/IGF-I axis. Peaks of IGF-I concentrations occurring at different times around feeding may indicate an altered endogenous rhythm in fish reared under 24L:0D after 56 days. This could suggest photoperiod influences fish growth indirectly as reduced melatonin concentrations may alter circadian rhythms which in turn alter fishes perception of time of feeding, consequently observing a shift in IGF-I peaks around time of feeding. Altered endogenous rhythms and/or IGF-I concentrations may enable improved feed utilization, prompting growth increases when fish are reared under 24L:0D. Only by day 56 were significant increase in growth observed in fish under 24L:0D which was concomitant with the largest reductions in melatonin levels as well as shifts in IGF-I peaks around feeding times. This may suggest the amplitude of melatonin

requires to be reduced to a certain threshold to alter circadian rhythms and IGF-I concentrations.

5.6 Conclusions

Continuous photoperiod (24L:0D) significantly increased wet weight, total length, SGR weight and length of juvenile barramundi without significant increased feed intake compared to 12L:12D. In addition, HSI and total lipid level in the liver of barramundi did not significantly differ between 12L:12D and 24L:0D. This suggests enhanced growth of fish exposed to 24L:0D is due to better utilization of feed rather than increased feed intake and/or fish being unable to maintain the liver as a nutrient reservoir.

It is proposed 24L:0D affected endocrine parameters involved with photoperiod perception (melatonin) and fish growth (IGF-I) which impacted on the capacity to utilize feed more efficiently and ultimately influenced growth and growth efficiency. Depressed amplitude of melatonin, during the scotophase in fish exposed to 24L:0D, may directly or indirectly alter fish growth via the GH/IGF-I axis. In the current study no clear effect of photoperiod on circulating IGF-I levels was observed, although after 56 days, increased growth in fish reared under 24L:0D was concomitant with depressed amplitude of melatonin concentrations during the scotophase as well as IGF-I peaks at feeding time being altered. This could suggest photoperiod influences growth within a threshold, with increased growth not occurring until amplitude of melatonin is depressed below a certain point (as achieved in the current study after 56 days). In turn, depressed melatonin concentrations may influence endogenous rhythms in fish as peaks in IGF-I concentration shifted after 56 days of subjection to 24L:0D which may enable improved feed

utilization, as observed with concomitant growth increases when fish are reared under 24L:0D. Altogether, this suggests melatonin has indirect influence on the GH/IGF-I growth axis although investigating the IGF system to its full extent (IGF-I, IGFBP's, IGF-I receptors) will enable a better understanding of endocrine mechanisms involved with photoperiod perception and growth in barramundi. Ultimately this knowledge will enable the development of optimal artificial lighting regime to improve farming techniques.

5.7 References

Bayarri, M. J., de Lama, M. A. R., Madrid, J. A., Sanchez-Vazquez, F. J. 2003. Both pineal and lateral eyes are needed to sustain daily circulating melatonin rhythms in sea bass. Brain Research 969 (1-2), 175-182.

Beckman, B. R. and W. W. Dickhoff, 1998. Plasticity of smolting in spring chinook salmon: relation to growth and insulin-like growth factor-I. Journal of Fish Biology 53(4): 808-826.

Beckman, B. R., M. Shimizu, 2004. The effect of temperature change on the relations among plasma IGF-1, 41-kDa IGFBP, and growth rate in postsmolt coho salmon. Aquaculture 241(1-4): 601-619

Biswas, A. K., Seoka, M., Inove, Y., Takii, K., Kumai, H. 2005. Photoperiod influences the growth, food intake, feed efficiency and digestibility of red sea bream (*Pagrus major*) Aquaculture 250, 666-673.

Biswas, A. K., Seoka, M., Tanaka, Y., Takki, K., Kumai, H. 2006. Effect of photoperiod manipulation on the growth performance and stress response of juvenile red sea bream (*Pagrus major*). Aquaculture 258 (1-4), 350-356.

Biswas, A., Seoka, M., Inagaki, H., Takii, K. 2010. Reproduction, growth and stress response in adult sea bream, *Pagrus major*, exposed to different photoperiods at spawning season. Aquaculture Research 41 (4), 519-527

Boeuf, G., Falcon, J., 2001. Photoperiod and Growth in Fish. Vie Milieu 51, 247-266.

Boeuf, G., Le Bail, P.Y., 1999. Does light have an influence on fish growth? Aquaculture 177, 129-152.

Bolliet, V., Ali, M.A., Lapointe, F.J., Falcon, J., 1996. Rhythmic melatonin secretion in different teleost species: An *in vitro* study. Journal of Comparative Physiology 165B, 677-683.

Buddington, R.K., Krogdahl, A. 2004. Hormonal regulation of the fish gastrointestinal tract. Comparative Biochemistry and Physiology (A) Molecular and Integrated Physiology 139 (3), 261 – 271.

Danilova, N., Krupnik, V. E., Sugden, D., Zhadanova, I.V. 2004. Melatonin stimulates cell proliferation in zebrafish embryo and accelerates its development. Faseb Journal 18 (2), 75.

Davie, A., Porter, M.J.R., Bromage, N.R. 2003. Photoperiod manipulation of maturation and growth of Atlantic cod (*Gadus morhua*). Fish Physiology and Biochemistry 28, 399-401.

Davie, A., Porter, M.J.R., Bromage, N.R., Migaud, H. 2007. The role of seasonally altering photoperiod in regulating physiology in Atlantic cod (*Gadus morhua*) Part II. Somatic growth. Canadian Journal of Fisheries and Aquatic Science 64 (1), 98-112.

Davis, T.L., Kirkwood, G.P. 1994. Age and growth studies on barramundi, Lates calcarifer (Bloch) in Northern Australia. Australian Journal of Marine and Freshwater Research 35 (6), 673 - 689.

Ditchkoff,S.S., Spicer, L.J., Masters, R.E., Lochmiller, R.I., 2001. Concentrations of insulin-like growth factor-I in adult male white-tailed deer (*Odocoileus virginianus*): associations with serum testosterone, morphometrics and age during and after the breeding season. Journal of Comparative Biochemistry and Physiology, A: Molecular Integrated Physiology 129, 887 – 895.

Duan, C. 1997 The Insulin-like Growth Factor System and Its Biological Actions in Fish. American Zoology 37, 491-503.

Duan, C., 1998. Nutritional and Developmental regulation of insulin-like growth factors in fish. American Society for Nutritional Sciences 128: 306S-314S.

Duston, J., Bromage, Niall. 1988. The entrainment and gating of teh endogenous circannual rhythms of reproduction in the female rainbow trout (Salmo gairdneri). Journal of Comparative Physiology A: Neuroethology, Sensory, Neural and Behavioural Physiology 164 (2), 259 – 268.

Endal, H.P., Taranger, G.L., Stefansson, S.O., Hansen, T., 2000. Effects of continuous additional light on growth and sexual maturity in Atlantic salmon, *Salmo salar*, reared in sea cages. Aquaculture 191, 337-349.

Ekstrom, P., and Meissl, H. 1997. The pineal organ of teleosts fishes. Review in Fish Biology and Fisheries 7, 199 – 284.

Falcon, J. 1999. Cellular circadian clocks in the pineal. Progress in neurobiology 58 (2), 121-162.

Falcon, J., Besseau, L., Fazzari, D., Attia, J., Gaildrat, P., Beauchaud, M., Boeuf, G. 2003. Melatonin modulates secretion of growth hormone and prolactin by trout pituitary glands and cells in culture. Endocrinology 144 (10), 4648-4658.

Falcon, J., Besseau, L., Sauzet, S., Boeuf, G. 2007. Melatonin effects on the hypothalamo-pituitary axis in fish. Trends in Endocrinology and Metabolism 18 (2), 81-88.

Falcon, J., Migaud, H., Munoz-Cueto, J.A., Carrillo, M. 2010. Current knowledge on the melatonin system in teleosts fish. General and Comparative Endocrinology 165, 469-482.

Frystyk, J., Skjaerbaek, C., Dinesen,B., Orskov, H. 1994. Free Insulin-like growth factors (IGF-I and IGF-II) in human serum. FEBS Letters 348 (2), 185 – 191

Gern, W.A., and Greenhouse, S.S. 1988. Examination of invitro melatonin secretion from superfused trout (*salmo-gairdneri*) pineal organs maintained under diel illumination or continuous darkness. General and Comparative Endocrinology 71 (1), 163-174.

Gross, W.L., Roelofs, E.W., Fromm, P.O. 1965. Influence of photoperiod on growth of green sunfish, Lepomis cyanellus. Journal of the Fisheries Research Board of Canada 22 (6), 1379 - 1386.

Herbinger, C.M., Friars, G.W. 1991. Correlation between condition factor and total lipid content in Atlantic salmon, *Salmo salar* L. parr. Aquaculture Fisheries Management 22, 527 – 529.

Iigo, M., Kezuka, H., Aida, K., Hanya, I. 1991. Circadian-Rhythms of Melatonin Secrtion from Superfused Goldfish (*Carassius-Auratus*) Pineal Glands Invitro. General and Comparative Endocrinology 83 (1), 152 – 158.

Iigo, M., Abe, T., Kambayashi, S., Oikawa, K., Masuda, T., Mizusawa, K., Kitamura, S., Azuma, T., Takagi,Y. 2007. Lack of circadian regulation of in vitro melatonin release from the pineal organ of salmonid teleosts. General and Comparative Endocrinology 154, 91 – 97.

Imland, A.K., Foss, A., Koedijk, R., Folkvord, A., Stefansson, S.O., Jonassen, T.M. 2008. Persistent growth effects of temperature and photoperiod in Atlantic cod (*Gadus morhua*). Journal of Fish Biology 71 (5), 1371 - 1382

Katersky, R.S. & Carter, C.G. 2007. High growth efficiency occurs over a wide temperature range for juvenile barramundi, *Lates calcarifer*, fed a balanced diet. Aquaculture, 272**,** 444-450.

Klein, S.E., Sheridan, M.A. 2008. Somatostatin signalling and the regulation of growth and metabolism in fish. Molecular and Cellular Endocrinology 286 (1-2) 148 – 154.

Krakenes, R., Hansen, T., Stefansson, S. O., Taranger, G.L. 1991. Continuous light increases growth rate of Atlantic salmon (*Salmo salar* L.) postsmolts in sea cages. Aquaculture 95 (3-4), 281-287.

Martinez-Chavez, C.C., Al-Khamees, S., Campos-Mendoza,A., Penman, D.J., Migaud, H. 2008. Clock controlled endogenous melatonin rhythms in Nile tilapia (*Oreochromis niloticus niloticus*) and African catfish (*Clarias gariepinus*). Chronobiological International 25, 31-49.

Migaud, H., Wang, N., Gardeur, J., Fontaine, P. 2006 Influence of photoperiod on reproductive performances in Eurasian perch *Perca fluviatilis*. Aquaculture 252 (2-4), 385-393.

Migaud, H., Davie, A., Chavez, C.C.M., Al-Khamees, S. 2007. Evidence for differential photic regulation of pineal melatonin synthesis in teleosts. Journal of Pineal Research 43 (4), 327-335.

McCormick, S.D., Hrimpton, J.M., Moriyama, S., Bjornsson, B.T. 2007. Differential hormonal responses to Atlantic salmon parr and smolt to increased daylength: A possible developmental basis for smolting. Aquaculture 273 (2-3), 337-344.

Nelson, L.W., Sheridan,M.A. 2006. Gastroenteropancreatic hormones and metabolism in fish. General and Comparative Endocrinology 148, 116 – 124.

Oppedal, F., Taranger, G.L., Juell, J.E., Fosseidengen, J.E., Hansen, T. 1997. Light intensity affects growth and sexual maturation of Atlantic salmon (*Salmo salar*) postsmolts in sea cages. Aquatic Living Resources 10, 351 – 357.

Perez-Sanchez, J. 2000. The involvement of growth hormone in growth regulation, energy homeostatis and immune function in the gilthead sea bream (*Sparus aurat*a): a short review. Fish Physiology and Biochemistry 22 (2), 135 – 144

Pierce, A.L., Beckman, B.R., Shearer, K.D., Larsen, D.A., Dickhoff, W.W. 2001. Effects of ration on somatotrophic hormones and growth in coho salmon. Comparative Biochemistry and Physiology B 128, 255-264.

Porter, M.J.R., Randall, C.F., Bromage, N.R., Thorpe, J.E. 1998. The role of melatonin and the pineal gland on development and smoltification of Atlantic salmon (*Salmo salar*) parr. Aquaculture 168 (1-4), 139 - 155

Porter, M.J.R., Duncan, N.J., Mitchell, D., Bromage, N.R., 1999. The use of cage lighting to reduce plasma melatonin in Atlantic salmon (*Salmo salar*) and its effects on the inhibition of grilsing. Aquaculture 176, 237-244.

Porter, M.J.R, Stefansson, S.O., Nyhammer, G., Karlsen, O., Norberg, B. and Bromage, N.R. 2000. Environmental influences on melatonin secretion in Atlantic cod (*Gadus morhua L.*) and their relevance to commercial culture. Fish Physiology and Biochemistry 3, 191-200.

Porter, M.J.R., Duncan, N., Handeland, S.O., Stefansson, S.O., Bromage, N.R., 2001. Temperature, light intensity and plasma melatonin levels in juvenile Atlantic salmon. Journal of Fish Biology 58, 431-438.

Randall, C.F., Bromage, N.R., Thorpe, J.E., Miles, M.S., Muir, J.S., 1995. Melatonin Rhythms in Atlantic Salmon (*Salmo-Salar*) Maintained under Natural and out-of-Phase Photoperiods. General and Comparative Endocrinology 98, 73-86.

Reebs, S.G. 2002. Plasticity of diel and circadian activity rhythms in fishes. Reviews in Fish Biology and Fisheries 12, 349 – 371.

Reiter, R.J. 1993. Melatonin: clinical relevance. Best practice and research clinical endocrinology and metabolism 17 (2), 273-285.

Reinecke, M., Bjornsson, B.T., Dickhoff, W.W., McCormick, S.D., Navarro, I., Power, D.M., Guitierrez, J. 2005. Growth hormone and insulin-like growth factors in fish: Where we are and where to go. General and Comparative Endocrinology 142, 20-24.

Reinecke, M. 2010. Influences of the environment on the endocrine and paracrine fish growth hormone – insulin-like growth factor-I system. Journal of Fish Biology 76, 1233 – 1254.

Shimizu, M., Swanson,P., Dickhoff, W.W. 1999. Free and protein-bound insulin-like growth factor – I (IGF-I) and IGF-binding proteins in plasma of coho salmon, *Oncorhynchus kisutch*. General and Comparative Endocrinology 115 (3), 398 – 405.

Shimizu, M., Swanson, P., Fukada, H., Hara, A., Dickhoff, W.W. 2000. Comparison of extraction methods and assay validation for salmon insulin-like growth factor-I using commercially available components. General and Comparative Endocrinology 119, 26 – 36.

Shimizu, M., Cooper, K.A., Dickhoff, W.W., Beckman, B.R. 2009. Postprandial changes in plasma growth hormone, insulin, insulin-like growth factor (IGF)-I, and IGF-binding proteins in coho salmon fasted for varying periods. American Journal of Physiology and Regulatory Integrated Comparative Physiology 297, R352 – R361.

Takemura, A., Ueda,S., Hiyakawa, N., Nikaido,Y. 2006. A direct influence of moonlight intensity on changes in melatonin production by cultured pineal glands of the golden rabbitfish, *Siganus guttatus*. Journal of Pineal Research 40 (3), 236-241.

Taranger, G.L., Haux, C., Hansen, T., Stefansson, S.O., Bjornsson, B.T., Walther, B.T., Kryyi, H. 1999. Mechanisms underlying photoperiodic effects on age at sexual maturity in Atlantic salmon, *Salmo salar*. Aquaculture 177 (1-4), 47 – 60.

Taylor, J.F., Migaud, H., Porter, M.J.R., Bromage, N.R. 2005. Photoperiod influences growth rate and plasma insulin-like growth factor-I levels in juvenile rainbow trout, *Oncorhynchus mykiss*. General and Comparative Endocrinology 142, 169 – 185.

Taylor, J.F., Porter, M.J.R., Bromage, N.R., Migaud, H. 2008. Relationships between environmental changes, maturity, growth rate and plasma insulin-like growth factor-I (IGF-I) in female rainbow trout. General and Comparative Endocrinology 155 (2) 257 - 270.

Thrush, M.A., Duncan, N.J., Bromage, N.R. 1994. The use of photoperiod in the production of out of season Atlantic salmon (*Salmo salar*) smolts. Aquaculture 121, 29 – 44.

Tucker, J.W., Russell, D.J., Rimmer, M.A. 2002. Barramundi culture: A success story for aquaculture in Asia and Australia. World Aquaculture 33 (3), 53-59.

Vera, L.M., Lopez-Olmeda, J.F., Bayarri, M.J., Madrid, J.A., Sanchez-Vazques, F.J. 2005. Influence of light intensity on plasma melatonin and locomotor activity rhythms in tench. Chronobiology International 22 (1), 67-78.

Volkoff, H., Hoskins, L.J., Tuziak, S.M. 2010. Influence of intrinsic signals and environmental cues on the endocrine control of feeding in fish: Potential application in aquaculture. General and Comparative Endocrinology 167, 352 – 359.

Webster, J.R., Corson, I.D., Littlejohn, R.P., Martin, S.K., Suttie, J.M. 2001. The roles of photoperiod and nutrition in the seasonal increases in growth and insulin-like growth factor-I secretion in male red deer. Animal Science 73, 305 – 311.

Wilkinson, R.J., Porter, M., Woolcott, H., Longland, R., Carragher, J.F. 2006. Effects of aquaculture related stressors and nutritional restriction on circulating growth factors (GH, IGF-I and IGF-II) in Atlantic salmon and rainbow trout. Comparative Biochemistry and Physiology, Part A 145, 214 – 224.

Zachman, A., Ali, M.A., Falcon, J. 1992. Melatonin and its effects in fishes: an overview. In: Ali, M.A. (Ed.), Rhythms in Fishes. Plenun Press, New York, pp. 149 – 165.

Zapf, J. Total and free IGF serum levels. European Journal of Endocrinology 136 (2), 146 – 147.

CHAPTER 6

GENERAL DISCUSSION

6.1 Discussion Overview

Photoperiod manipulation is a commonly used tool in the aquaculture industry to influence endogenous rhythms in fish and thereby alter developmental events such as reproductive timing, maturation and growth. Controlling these events through additional artificial lighting can maximise commercial production through enhanced growth rates to attain market sizes in as short a time period as possible and hence improve farming efficiency (Hansen et al., 1999). The majority of photoperiod manipulation research has been applied to temperate species, whereas recently these techniques have also been successful in sub-tropical and tropical species.

This research was designed to ascertain whether photoperiod manipulation techniques of extended day length would influence growth of a tropical species, barramundi, which is a rapidly expanding and highly valued aquaculture species in Australia. This thesis aimed to investigate the effects of photoperiod manipulation on the somatic growth of juvenile barramundi. Specifically, the effects of varied temperatures on photoperiod manipulation, the transference of photoperiod manipulation techniques from indoor recirculation systems to inland freshwater ponds, synergistic effects of feeding regimes under different photoperiod regimes and lastly, changes in endocrine parameters under different photoperiod/temperature/feeding regimes and their possible involvement with fish growth.

This chapter discusses four key areas of research and their commercial implications, with the transfer of photoperiod manipulation techniques from tanks to ponds combined and discussed within the first key area:-

1. Effects of Photoperiod on Growth in Juvenile Barramundi (Chapter 6.2)
2. Effects of Photoperiod and Temperature on Growth in Juvenile Barramundi (Chapter 6.3)
3. Effects of Photoperiod and Feeding Regimes on Growth in Juvenile Barramundi (Chapter 6.4)
4. Effects of Photoperiod on Endocrine Mechanisms Involved with Growth in Juvenile Barramundi (Chapter 6.5)

6.2 Effects Photoperiod on Growth of Juvenile Barramundi

Growth of juvenile barramundi was significantly increased by long day photoperiod manipulation. In 5 out of 6 experiments, continuous light (24L:0D) significantly increased wet weight, total length, SGR weight and SGR length. These results parallel with a number of studies observing increased growth parameters in sub – tropical and tropical fish species, (Biswas et al., 2005; Tucker et al., 2006; Almazan-Rueda, 2004; El-sayed and Kawanna, 2004) and numerous studies in temperate species (reviewed in Bromage and Porter, 2001).

In these experiments, increased growth of barramundi under 24L:0D occurred without significant differences in feed intake, HSI or total lipid levels in the liver of barramundi when compared to 12L:12D. No significant differences in lipid content of livers or HSI suggest fish growth is not an immediate short term growth event with the fish drawing on energy reserves. Additionally, in these experiments, condition factor did not significantly differ between treatments. This serves as an indicator of energy reserve status with strong positive correlations of condition factor and total lipid content observed previously

(Herbinger and Friars, 1991). Implications that increased growth under 24L:0D is a temporary situation is further unsubstantiated as barramundi have demonstrated significant increases in growth throughout extended time periods from 40 – 56 days (Chapter 2, 3 and 5) to 120 days (Chapter 4). Similarly, Imsland et al., (2007) also saw persistent growth increases when harvesting Atlantic cod, 30 months after exposing juveniles to a defined 3 month continuous light treatment.

Increases in growth without increases in feed intake seen in the current study, is in accordance with other tropical species reared under extended day length (Gines et al., 2004; Elsayed and Kawanna, 2007; Petit et al., 2003). This finding indicates growth increases under 24L:0D are due to improved feed utilization rather than simply increased feed intake (Boeuf and Le Bail, 1999). Davidson (1997) demonstrated similar improved growth and FCE by light treatments as a result of prolonged period of exercise. This Improved feed utilization occurs from a greater efficiency in the use of nutrients from digestion, absorption as well as how nutrients are used or retained, with photoperiod either directly or indirectly impacting on metabolism (Buddington and Krogdahl, 2004; Nelson and Sheridan, 2006; Klein and Sheridan, 2008). Photoperiod may directly impact on the somatic uptake of nutrients by providing longer day lengths for digestion to occur, as suggested by Biswas et al., (2006), alternatively 24L:0D may indirectly influence fish by altering endocrine and/or nutrient retention or assimilation (Rungruangsak-Torrissen et al., 2006; Cruz and Brown, 2009; Volkoff et al., 2010). The current research points to the latter, as endocrine parameters of IGF-I and melatonin were altered in fish reared under 24L:0D (to be discussed in Chapter 6.5).

The observed growth increase under 24L:0D varied greatly depending on initial size of juvenile barramundi. A 44% increase in final weight after 40 days was observed in juveniles with an initial weight of 2.7 g (Chapter 1). In comparison, a 6.5% to 9.5% increase in final weight was observed after 50 and 56 days in juveniles with an initial weight of 11.74 g to 35.63 g respectively. It is suggested that larger growth increments could be attained if the 24L:0D photoperiod is applied at an earlier stage of development due to entrained circadian rhythms being retained in barramundi for a certain period of time. Duston and Bromage (1988) found the effective entrainment to long day photoperiods in rainbow trout did not occur for 6 – 8 weeks. Larger growth increments seen at an earlier age in this study may involve the more rapid entrainment of circadian rhythms at earlier stages of development in barramundi, a concept which will be discussed in more depth in Chapter 6.5. It is apparent that the entrainment of circadian rhythms of various hormones in juvenile barramundi took between 40 and 50 days after which improved growth became apparent. This suggests early application of photoperiod manipulation is required to ensure maximised benefits.

As the efficacy of photoperiod manipulation is dependent on rearing conditions (Barlow et al., 1995; Worrall et al., 2004), it was necessary to investigate whether photoperiod manipulation techniques could be transferred from indoor re-circulation systems to a commercial setting such as inland freshwater ponds, being the most common farm scenario in Queensland. The results from both studies in Chapter 4 paralleled the observations in previous experiments (Chapters 2, 3 and 5), demonstrating significant increases in growth without significant increases in feed intake when reared under 24L:0D supplied by artificial lighting. However, the efficacy of 24L:0D to enhance

barramundi, is significantly impaired by reduced water temperature and rationed feed intake as discussed in Chapter 6.3 and 6.4.

6.3 Photoperiod and Temperature Effects on Growth of Juvenile Barramundi.

Temperature has a marked effect on many key physiological processes in fish (Brett and Groves, 1979). In the current study temperature significantly influenced growth rates and feed intake of juvenile barramundi. Decreased growth, feed intake and feed conversion efficiencies occurred with decreased water temperatures, irrespective of photoperiod regimes. Katersky and Carter, (2005) demonstrated optimal growth for small barramundi occurs at water temperatures around 26 - 36°C. Therefore, reduced growth and feed intake that occurred at low water temperatures were to be expected and are in line with other studies (Jobling, 1994; Imsland et al., 2001).

Reduced growth parameters observed at lower water temperatures have been attributed to a direct retardation of the biochemical reactions in ectotherms through reduced metabolic processes such as digestive and absorptive capabilities (Ibarz et al., 2005, 2007). As temperature increases, feed intake will increase to a maximum and then decrease rapidly prior to the upper limit for thermal tolerance (Jobling, 1994). Metabolic rate increases exponentially as the temperature increases and, at any given temperature, the difference between feed intake and metabolic rate will determine the energy available for growth (Brett and Groves, 1979; Jobling, 1994). Any temperatures below 22°C are sub-optimal

for barramundi growth and anything less than 18°C is at the lower limit of their thermal tolerance (Barlow et al., 1996).

In respect to the effects of temperature on photoperiod manipulation, at low water temperatures of 20/22 °C and 24/25°C, photoperiod manipulation was ineffective. Only at 29°C and 30°C did photoperiod manipulation become effective. This parallels McCormick et al., (2000) findings of low temperature limiting physiological responses to increased day length in Atlantic salmon. This was also observed with barramundi reared in a commercial saltwater raceway farming, where extended day length of 18L:6D did not improve growth of juvenile barramundi during winter temperatures of ~ 19°C (Hovette, 2005).

As mentioned in Chapter 6.2, photoperiod is most likely indirectly influencing growth of barramundi via endocrine responses to 24L:0D. Low temperature may limit endocrine responses to 24L:0D by reducing the rate or capacity of physiological changes to occur/respond (Lin and Somero, 1998), for example the binding capabilities of IGFBP's at low temperature, which will be discussed further in Chapter 6.5. Additionally, temperature has been demonstrated to directly affect the hormone involved with photoperiod perception, melatonin, with higher temperatures increasing the amplitude of the melatonin cycle and increase the sensitivity of the pineal to light (Ekstrom and Meissl, 1997; Porter et al., 2001). In this regard, it is possible that at low temperatures the sensitivity of the pineal to light is hindered resulting in no significant differences in growth when fish are reared under 24L:0D.

This finding is of significant value for the application of photoperiod manipulation in commercial applications. This suggests the application of continuous light to improve growth rates of barramundi will be ineffective at water temperatures 25°C and below, although further investigations over a greater range of temperatures is suggested to ascertain specific temperatures allowing photoperiod manipulation to be effective.

6.4 Photoperiod and Feeding Regimes on Growth of Juvenile Barramundi.

Feed ration under varying photoperiods had a significant impact on growth in barramundi. Significant reductions in growth were observed in barramundi fed a 3% $bw.d^{-1}$ compared to being fed to satiation. When barramundi were fed to satiation in Chapter 4, growth equated to an approximate 70% increase compared to barramundi fed a 3% $bw.d^{-1}$ ration over the entire 50 days. To compare, feed intake in barramundi fed to satiation ranged from 8% $bw.d^{-1}$ at the beginning of the experiment to 4% $bw.d^{-1}$ by day 50. A rationed feed of 3% $bw.d^{-1}$ is considered low for barramundi (18.6 g) as optimal rations for growth of barramundi better reflect those observed in fish fed to satiation. Optimal ration ranges for barramundi growth are suggested to range from 9 % $bw.day^{-1}$ for 10 g juveniles to 3.8% $bw.day^{-1}$ for 50 g juveniles when held at 29°C and fed a 15MJ digestible energy diet kg^{-1} diet (Williams and Barlow, 1999; Glencross et al., 2006). Harpaz et al., (2005) found no significant growth benefits in juvenile barramundi (20 g) when increasing feed rations from 4% biomass per day to 6% biomass per day and observed poor growth in barramundi (20 g) fed 2% biomass per day in comparison to 4% biomass per day. In the current research, juveniles were allocated a lower than optimal

ration to ensure all feed was consumed, allowing determination of growth influences of photoperiod to be separated from effects of potential differential feed intake.

Reduced growth rates observed at 3% bw.d^{-1} in this study suggests barramundi could not meet the nutrient and energy requirements for their maintenance and somatic development. This suggests the enhancing growth effect of continuous light is dependent on receiving adequate feed to utilize this feed more efficiently. Further investigations replicating this study with increased rations would provide a better understanding towards the affects of feed ration on photoperiod manipulation.

In addition to feeding ration, feeding frequency can also affect growth rates (Reddy and Leatherland, 2003). Feeding frequency significantly affected growth in barramundi when fed to satiation but not when fed a ration of 3% bw.d^{-1}. When barramundi are fed at 3% bw.d^{-1} there is no benefit in feeding this ration in smaller meals over a longer time frame (photophase and scotophase) compared to being fed during the photophase when held under 12L:12D and 24L:0D. As mentioned previously, at 3% bw.d^{-1}, fish may only be receiving enough feed for basic maintenance. Further investigations into feeding frequency and photoperiod interactions when barramundi are fed closer to optimal rations are needed. This being said, from a commercial point of view, there were also no negative impacts on growth when meals were spread over 24 hours, which may prove to be an alternate feeding practice, beneficial for re-circulation systems. Harpaz et al., (2005) suggests changing the distribution of waste product load exposed to biological filters to a constant influx of organic material rather than peaks during the day, allowing higher filtration efficiency and better performance of the biological filter.

Barramundi reared under 24L:0D and fed to satiation with additional feeds over what would normally be the scotophase, continued to feed well over the 24 hour period and demonstrate further growth increases. These results are in parallel with a number of previous studies in temperate, sub-tropical and tropical species capable of feeding well over extended photoperiods (Burke et al., 2005; Tucker et al., 2006; Biswas and Takeuchi, 2003). However, in the current study, significant growth increases were concomitant with significantly lowered FCE. This could simply be a case of overfeeding, with fish being fed another meal before efficiently assimilating the previous feed, causing gastric overloading to occur and therefore a decrease in FCE (Booth et al., 2008). Alternatively, if feed is being utilized more efficiently by direct mechanisms, this may indicate ingestion, digestion and assimilation does not occur as efficiently over the scotophase compared to the photophase. For example, Harpaz et al., (2005) observed the activity of digestive enzymes (brush border proteolytic enzyme) was higher in barramundi fed during the day time compared to night. Whether feed utilization is being improved by either direct or indirect mechanisms will have ramification on timing of feeds.

This opens the possibility of fine tuning feeding practices over a 24 hour period to increase FCE's while still attaining larger growth increases. As feeding frequency is strongly correlated with gastric evacuation time (Lee et al., 2000; Riche et al., 2004), research investigating gastric evacuation times of juvenile barramundi is needed to optimise feeding frequency regimes over 24L:0D. In addition, the possibility of continually feeding barramundi over 24 hours, apart from increases growth rates, may

also reduce formation of hierarchies and reduce cannibalism which is a significant problem in farming barramundi. This is a topic worthy of further investigation.

6.5 Photoperiod Effects on Endocrine Mechanisms Involved with Fish Growth.

It is suggested that growth increases in barramundi reared under 24L:0D are a consequence of improved feed utilization. In addition, barramundi demonstrated altered concentrations of hormones related to photoperiod perception, melatonin, and significant increases in the growth related hormone, IGF-I. While it is clear that melatonin facilitates the transduction of photic information to other endocrine systems, the actual mechanism for this is not fully understood. Reviews on endocrine mechanisms involved with photoperiod and growth by Falcon et al., (2010) and Migaud et al., (2010) provide up to date information regarding current knowledge, however, the exact pathways in which photoperiod affects growth is still unclear.

Diurnal plasma melatonin concentrations in barramundi were secreted in a similar manner to other temperate and tropical fish species, with higher concentrations being released during the scotophase and returning to base concentrations during the photophase (Porter et al., 2001; Taylor et al., 2005; Falcon et al., 2007; Martinez-Chavez et al., 2008). When barramundi are subjected to 24L:0D, circadian rhythms of melatonin remain, indicating entrainment of these rhythms even in the absence of light/dark cues as observed in other tropical species, Nile tilapia and African catfish (Martinez-Chavez et al., 2008). Strong entrainment of melatonin rhythms were maintained for a period of 18

days in Nile tilapia and 4 days in African catfish when exposed to constant darkness (Martinez-Chavez et al., 2008). A strong entrainment of melatonin rhythms in tropical species may a reflection of their evolution from a steady photic environment found in tropical regions (Martinez-Chavez et al., 2008). Or alternatively, the level of light intensity occurring during experiments may not have been sufficient to reach the minimum threshold required to supress melatonin. As Bayarri et al., 2002 demonstrated, European sea bass required 6.0uW/cm2 before suppression of melatonin occurred. It would be informative to gain melatonin profiles of different light intensities to determine whether suppression of melatonin under 24L:0D is a matter of higher light intensity or further dampening of entrainment – requiring more time to reach lower levels of suppression. Or whether entrainment is so entrenched in barramundi that the current results represent the maximum suppression of melatonin.

The synthesis of melatonin may also be influenced by temperature as the pineal gland has been shown to be affected by temperature (Eckstrom and Meissel, 1997; Porter et al., 2001). As demonstrated in Chapter 2, at low temperatures, 24L:0D did not provide significant growth benefits when reared under a higher temperature of 30°C. High temperatures have been observed to increase the amplitude of melatonin (Zachmann et al., 1992; Bolliet et al., 1994), therefore possibly sub-optimal temperature may reduce melatonin levels of a tropical species in a way that overrides the enhancing growth effects of 24L:0D. Alternatively, at low temperatures, enzyme activity is altered which may influence binding proteins and receptors which would ultimately affect circulating levels of hormones (Falcon and Collin, 1989; Thibault et al., 1993).

Interestingly, barramundi exposed to 24L:0D showed reductions in melatonin levels occurring during the scotophase, being apparent from day 20 onwards. This parallels results found in an earlier experiment with barramundi reared under similar conditions (Worrall et al., 2004). Similarly, Porter et al., (1999) found the amplitude of melatonin during the scotophase was significantly reduced in Atlantic salmon when exposed to additional artificial night-time illumination. In the current study, further reductions in melatonin levels during the scotophase were observed by day 40 and 56. This suggests the entrainment of endogenous circadian systems in barramundi may be dampened with time.

The entrainment of circadian rhythms of melatonin and IGF-I in juvenile barramundi took between 40 and 50 days in this study after which the specific growth rate increased. This concurs with previous studies which demonstrated a period of entrainment before an altered photoperiod is capable of enhancing growth in Atlantic salmon (Oppedal et al., 1997; Taranger et al., 1999) and in rainbow trout (Bromage et al., 2001), where the effective entrainment to long day photoperiods did not occur for 6 – 8 weeks. Similarly, Rungruangsak-Torrissen et al., (2009) demonstrated Atlantic salmon took 70 days to adjust to a new environment of 24L:0D, as demonstrated by altered enzyme activity of trypsin and chymotrypsin. In this regard, by subjecting barramundi to manipulated photoperiods at an earlier stage of development may enable entrainment of circadian rhythms to be altered sooner and thereby observe earlier growth increases. In addition, the size of barramundi subjected to 24L:0D may influence the production of melatonin, an effect which has been observed in other fish species (Porter et al., 2003).

The partial inhibition of melatonin synthesis during the scotophase in barramundi reared under 24L:0D may stimulate growth through an up-regulation of the GH-IGF growth axis as reported in other species (Boeuf and Falcon, 2001; Taylor et al., 2005; Falcon et al., 2007). In the current study no clear effect of photoperiod on circulating IGF-I levels was observed, although after 56 days, IGF-I peaks (occurring around feeding times) altered. This may suggest melatonin is an intermediary in the up-regulation of the GH-IGF growth axis by indirectly influencing IGF-I via other endocrine pathways such as GH. Melatonin is known to directly influence growth hormone (GH) (Falcon et al., 2003), which in turn is the primary stimulus for the synthesis and release of plasma IGF-I from the liver (Reineke et al., 2010). Additionally, IGF-I is synthesised and released in stimulus to GH, whereas there is increasing evidence that IGF-I is also GH independent as well as being a negative feedback to GH, further increasing the complexity of this system (Beckman et al., 1998, 2004; Pierce et al., 2001).

In the majority of experiments, when single, day-time measurements of circulating IGF-I were made, levels were typically higher in fish reared under 24L:0D (fish which also demonstrated increased growth). Similarly, Dyer et al., (2004) also observed a positively correlation between plasma IGF-I levels and growth rates in Atlantic salmon and barramundi. Interestingly, in chapter 5 diurnal profiles of IGF-I were found to be highly variable on each sampling occasion, with a general trend of IGF-I peaks occurring around meal times. This was also found to be the case in salmonids and other marine species, where plasma IGF-I levels were associated with feeding regimes (Duan, 1998; Pierce et al., 2001; Pérez-Sánchez, 2000; Shimizu et al., 2009).

Taylor et al., (2005) demonstrated exposure to 18L:6D induced higher IGF-I levels, irrespective of water temperature and/or feed rate and with subsequent increases in weight of rainbow trout, suggests evidence for direct endocrine stimulation of growth by photoperiod rather than a change in growth rhythms. In contrast, in the same study, Taylor et al., (2005) demonstrating supra-physiological levels of implanted melatonin inhibited growth rates of rainbow trout without observing differences in plasma IGF-I levels, suggesting melatonin does not act directly on the GH/IGF axis to control growth. Results from the current research, point more toward the latter, with barramundi under 24L:0D observing reductions in the amplitude of plasma melatonin and subsequent altered IGF-I peaks around feeding times as well as significant growth increases becoming apparent after 56 days.

In summary, it is suggested that melatonin has indirect influence on the GH/IGF-I growth axis of barramundi via the transduction of environmental information through modified endogenous circadian rhythms.

6.6 Future Research/Implications for Commercial Industry

6.6.1 Temperature

As optimal temperatures for growth usually decrease with fish size (Bjornsson et al., 2001; Jonassen et al., 1999) ascertaining optimal temperatures for barramundi at varying stages of development would be useful. Optimal temperatures for barramundi growth has been attained for small fish (26 - 36°C) (Katersky and Carter, 2005) although inter-relationships between temperature and photoperiod may be different as it was demonstrated 24L:0D did not influence growth at 24/25°C. Investigating the effectiveness of 24L:0D to improve growth rates at water temperatures between 25 and

29 will enable a better understanding of temperature interactions with photoperiod. Additionally, it would be useful to investigate nutritional proximate analyses on barramundi reared under 12L:12D and 24L:0D to ascertain retention, utilization and storage of nutrients/energy when subjected to low water temperature.

6.6.2 Feeding Regime

Feed utilisation obviously plays an important role in the ability of 24L:0D to increase growth of barramundi (Glencross et al., 2010). As hormone pathways are complex with positive and negative feedback, it may prove beneficial to start from the opposite end and investigate how feed is being utilized more efficiently which may enable determination of endocrine parameters involved. One approach would be to investigate feed utilisation based on bioenergetic principles and measure all components of the energy and protein budgets and establish where there were quantitatively important differences in digestion, respiration and or nitrogenous excretion (Brafield, 1985).

Knowing barramundi will continue to feed and grow well when reared under 24L:0D and fed throughout a 24 hour period, expands investigations into determining optimal feeding regimes under continuous light. Digestion efficiency through protein digestion has been suggested to be the most important criteria for growth efficiency of fish (Rungruangsak-Torrissen et al., 2006, 2009). Rungruangsak-Torrissen et al., (2009) found continuous light to increase digestion, absorption and transport of free amino acids for protein synthesis in the white muscle of Atlantic salmon. Investigating ingestion, digestion and assimilation of feed during what would normally be the scotophase is needed to ascertain optimal FCE's of feeds fed over the "scotophase" period. Harpaz et al., (2005) also

demonstrated brush border enzyme activity was higher during the day compared to night in barramundi although fish were reared under natural photoperiod – not extended. To achieve better FCE's over a 24 hour period, investigation of enzyme activities over this period is needed. Additionally, investigating the effects of sustained exercise on metabolism when exposed to different photoperiods may provide information regarding light providing indirect effects (GH-IGF-1 axis) on growth and FCE. This could attained by videotaping barramundi under different photoperiod to ascertain swimming activity as well as behavioural changes such as chasing and dominance behaviours.

The current research also suggests a lag/adjustment phase of between 40 – 56 days, therefore investigations into adjustment phases when barramundi are exposed to 24L:0D is needed and if these phases can be altered ie. depending on initial commencement of 24L:0D or size of barramundi or temperature.

Determining gastric evacuation rates of barramundi at various life stages will help to ascertain time frames for fish to optimally assimilate and digest feed to avoid wastage of feed (Jobling, 1981, 1987). This will enable determination of optimal feeding frequencies when feeding fish over a 24 hour period when reared under 24L:0D. Associated with this, studies should also investigate feeding behaviour of barramundi, specifically with the use of auto- or self feeders to allow ease of feeding (should feeding throughout the night prove beneficial).

Aggressive behaviour and cannibalism is a significant production problem within the culture of barramundi (Schipp et al., 2007). Enhanced growth of barramundi under

24L:0D may be negated due to increased aggressive behaviour and/or extra swimming which would consume extra energy of fish reared under continuous light. Almaza-Rueda et al., (2004) demonstrated juvenile African catfish reared under 24L:0D resulted in 41.6% more scars and wounds compared to 12L:12D in addition to spending more time swimming under continuous light. In this respect, investigating a resting phase where the lights are turned off may prove beneficial by fish using less energy in swimming and engaging in aggressive interactions.

6.6.3 Endocrine Mechanisms

Photoperiod manipulation has been shown to positively affect growth of barramundi; therefore, further research to optimise artificial lighting regimes can be investigated. As demonstrated in other fish species, optimal light intensity, wavelengths as well as intermittence of photoperiod has proved to be different from species to species (Boeuf and Falcon, 2001). In this regard, to optimise artificial lighting used on barramundi, the minimum threshold of light intensity to suppress melatonin needs to be ascertained as well as barramundi's response to various wave lengths would prove beneficial

Obtaining a better understanding of melatonin rhythms in barramundi and the positive/negative pathways involved within the GH/IGF-I growth axis will provide knowledge into how continuous light enhances somatic growth in barramundi. For example, melatonin has been suggested to influence the hormone ghrelin which is involved in regulating appetite, modulating gastrointestinal, cardiovascular and pancreatic functions (Kojima and Kangawa, 2005). Correlations between melatonin, ghrelin, GH and IGF-I levels and growth performance have not yet been investigated in fish and would be helpful in determining pathways involved with fish growth.

Unfortunately technical problems prevented the analysis of ghrelin and GH in the current study.

Possibly 24L:0D may alter IGF-binding proteins or receptors which may improve the biological activity of circulating IGF-I concentrations which may impact on metabolism. Future studies could investigate not only total IGF-I concentrations but "free" IGF-I, IGFBP's, IGF-I receptors as well as IGF-I mRNA concentrations to achieve a better indication of how the IGF system is being altered when exposed to extended day length. Future research could also investigate tissue level expression of key hormones.

6.7 Conclusions

This research provides an initial guide to the application of artificial lighting to increase somatic growth of barramundi. This thesis confirms that photoperiod manipulation affects growth of juvenile barramundi as well as providing novel research into effects of photoperiod on barramundi growth under varied rearing conditions.

Results from this thesis demonstrate that photoperiod manipulation techniques of extended day length of 24L:0D allow juvenile barramundi to utilize feed more efficiently and thereby increase growth without increasing feed intake. In addition, it is proposed growth increases observed under 24L:0D are due to an involvement of endocrine mechanisms associated with melatonin and IGF-I.

For Australian conditions, Johnston (1998) has shown that maximizing growth rate of fish is more important than feed costs in determining overall profitability of barramundi

farming. As the Australian barramundi industry currently only uses photoperiod manipulation to manipulate spawning events, the application of this technique would prove beneficial to the barramundi industry. The current experiments have assessed photoperiod manipulation techniques on indoor recirculation systems and inland freshwater ponds whereas due to the nature of different farm conditions, individual farm scenarios would need to be investigated to determine optimal artificial lighting as well as feasibility studies to determine growth increments needed to justify the expense of artificial lighting. The findings from this research are now currently applied on a variety of culture conditions within the Australian barramundi aquaculture. Ultimately this knowledge will enable the development of optimal artificial lighting regimes to improve farming techniques for barramundi.

6.8 References

Almazan-Rueda, P., Schrama, J.W., Verreth, J.A.J., 2004. Behavioural responses under different feeding methods and light regimes of the African catfish (*Clarias gariepinus*) juveniles. Aquaculture 231, 347-359

Barlow, C.G., Pearce, M.G., Rodgers, L.J., Clayton, P., 1995. Effects of photoperiod on growth, survival and feeding periodicity of larval and juvenile barramundi *Lates calcarifer* (Bloch). Aquaculture 138, 159-168.

Barlow, C., Williams, K., Rimmer, M. 1996. Sea bass culture in Australia. Infofish International 2/98, 26 – 29, 31-33.

Beckman, B. R. and W. W. Dickhoff, 1998. Plasticity of smolting in spring chinook salmon: relation to growth and insulin-like growth factor-I. Journal of Fish Biology 53: 808-826.

Beckman, B. R., M. Shimizu, 2004. The effect of temperature change on the relations among plasma IGF-1, 41-kDa IGFBP, and growth rate in postsmolt coho salmon. Aquaculture 241(1-4): 601-619.

Biswas, A.K. and Takeuchi, T. 2003. Effects of photoperiod and feeding interval on food intake and growth rate of Nile tilapia, *Oreochromis niloticus* L. Fisheries Science 69 (5), 1010-1016.

Biswas, A. K., Seoka, M., Inove, Y., Takii, K., Kumai, H. 2005. Photoperiod influences the growth, food intake, feed efficiency and digestibility of red sea bream (*Pagrus major*) Aquaculture 250, 666-673.

Bjornsson, B., Steinarsson, A., Oddgeirsson, M. 2001. Optimal temperature for growth and feed conversion of immature cod (Gadus morhua L). ICES Journal of Marine Science 58, 29 – 38.

Boeuf, G., Le Bail, P.Y., 1999. Does light have an influence on fish growth? Aquaculture 177, 129-152.

Boeuf, G., Falcon, J., 2001. Photoperiod and Growth in Fish. Vie Milieu 51, 247-266.

Bolliet, V., Ali, M.A., Lapointe, F.J., Falcon, J., 1996. Rhythmic melatonin secretion in different teleost species: An *in vitro* study. Journal of Comparative Physiology 165B, 677-683.

Booth, M.A., Tucker, B.J., Allan, G.L., Fielder, S. 2008. Effect of feeding regime and fish size on weight gain, feed intake and gastric evacuation in juvenile Australian snapper, *Pagrus auratus*. Aquaculture 282 (1-4), 104 – 110.

Brafield, A.E. 1985. Laboratory studies of energy budgets. In: *Fish Energetics, New Perspectives* (Editors – P.Tytler and P.Calow), pp. 257 – 281. Croom Helm, London and Sydney.

Bromage, N., Porter, M., Randall,C. 2001. The environmental regulation of maturation in farmed finfish with special reference to the role of photoperiod and melatonin. Aquaculture 197, 63 – 98.

Buddington, R.K., Krogdahl, A. 2004. Hormonal regulation of the fish gastrointestinal tract. Comparative Biochemistry and Physiology (A) Molecular and Integrated Physiology 139 (3), 261 – 271.

Bureau, D.P., Kaushik, S.J., Cho, C.Y. 2002. Bioenergetics. In: *Fish Nutrition, Third Edition* (Editors J.E. Halver and R.W. Hardy), pp. 2-59. Academic Press, San Diego.

Burke, M.G., Kirk, M.R., Macbeth, A., Bevan, D.J., Moccia, R.D. 2005. Influence of photoperiod and feed delivery on growth and survival of first feeding Artic Charr. North Americal Journal of Aquaculture 67, 344 – 350.

Cruz, E.M. and Brown, C.L. 2009. Influence of the photoperiod on growth rate and insulin-like growth factor-I gene expression in Nile tilapia *Oreochromis niloticus*. Journal of Fish Biology 75, 130-141.

Duston, J., Bromage, Niall. 1988. The entrainment and gating of teh endogenous circannual rhythms of reproduction in the female rainbow trout (Salmo gairdneri). Journal of Comparative Physiology A: Neuroethology, Sensory, Neural and Behavioural Physiology 164 (2), 259 – 268.

Duan, C. 1998. Nutritional and Developmental regulation of insulin-like growth factors in fish. American Society for Nutritional Sciences 128, 306S - 314S.

Dyer, A.R., Barlow, C.G, Bransden, M.P., Carter, C.G., Glencross, B.D., Richardson, N., Thomas, P.M., Williams, K.C., Carragher, J.F. 2004. Correlation of plasma IGF-I concentrations and growth rate in aquacultured finfish: a tool for assessing the potential of new diets. Aquaculture 236, 583-592.

Ekstrom, P., Meissl, H., 1997. The pineal organ of teleost fishes. Reviews in Fish Biology and Fisheries 7, 199-284.

El-Sayed, A.M., Kawanna, M., 2004. Effects of photoperiod on the performance of farmed Nile tilapia *Oreochromis niloticus*: I. Growth, feed utilization efficiency and survival of fry and fingerlings. Aquaculture 231, 393-402.

Falcon, J., Collin, J.P. 1989. Photopreceptors in the pineal of lower vertebrates: Functional aspects. Cellular and Molecular Life Sciences 45 (10) 909 - 913.

Falcon, J., Besseau, L., Sauzet, S., Boeuf, G. 2007. Melatonin effects on the hypothalamo-pituitary axis in fish. Trends in Endocrinology and Metabolism 18 (2), 81-88.

Falcon, J., Migaud, H., Munoz-Cueto, J.A., Carrillo, M. 2010. Current knowledge on the melatonin system in teleosts fish. General and Comparative Endocrinology 165, 469-482.

Gines, R., Afonso, J. M., Arguello, A. 2004. The effects of long-day photoperiod on growth, body composition and skin colour in immature gilthead sea bream (*Sparus aurata*). Aquaculture Research 35 (13), 1207-1212.

Glencross, B. 2006. The nutritional management of barramundi, *Lates calcarifer* – a review. Aquaculture Nutrition 12 (4), 291 – 309.

Glencross, B. 2010. A factorial growth and feed utilization model for barramundi, Lates calcarifer, based on Australian production conditions. Aquaculture Nutrition 14 (4), 360 – 373.

Hansen, T., Stefansson, S., Taranger, G.L., 1992. Growth and sexual maturation in Atlantic salmon, *Salmo salar* L., reared in sea cages at two different light regimes. Aquaculture Fish Management 23, 275-280.

Harpaz, S., Hakim, Y., Barki, A., Karplus, I., Slosman, T., Eroldogan, O.T. 2005. Effects of difference feeding levels during day and/or night on growth and brush border enzyme activity in juvenile *Lates calcarifer* reared in freshwater re-circulating tanks. Aquaculture 248 (1 – 4), 325 – 335.

Herbinger, C.M., Friars, G.W. 1991. Correlation between condition factor and total lipid content in Atlantic salmon, *Salmo salar* L. parr. Aquaculture Fisheries Management 22, 527 – 529.

Hovette, E., 2005. Influence of photoperiod and light intensity on growth and survival of *Lates calcarifer*. Honours Thesis, Good Fortune Bay, Queensland.

Ibarz, A., Blasco, J., Beltrain, M., Gallardo, M.A., Sanchez, J., Sala, R., Fernandez-Borras, J. 2005. Cold induced alterations on proximate composition and fatty acid profiles of several tissues in gilthead sea bream (*Sparus aurata*). Aquaculture 249, 477 - 486.

Ibarz, A., Beltran, M., Fernandez-Borras, J., Gallardo, M.A., Sanchez, J., Blasco, J. 2007. Alterations in lipid metablism and use of energy depots of gilthead seabream (*Sparus aurata*) at low temperatures. Aquaculture 262, 470-480.

Imsland, A.K., Foss, A., Gunnarsson, S., Berntssen, M.H.G., Fitzgerald, R., Bonga, S.W., Ham, E.V., Naevdal, G., Stefansson, S.O. 2001. The interaction of temperature and salinity on growth and food conversion in juvenile turbot (*Scophthalmus maximus*) Aquaculture 198 (3-4), 353 - 367

Imsland, A.K., Foss,A., Koedijk,R., Folkvord, A., Stefansson, S.O., Jonassen, T.M. 2007. Persistent growth effects of termperature and photoperiod in Atlantic cod *Gadus morhua.* Journal of Fish Biology 71 (5), 1371 – 1382.

Jobling, M. 1981. Mathematical models of gastric emptying and the estimation of daily rates of food consumption for fish. Journal of Fish Biology 19, 245 – 257.

Jobling, M. 1987. Influences of food particle size and dietary energy content on patterns of gastric evacuation in fish: test of a physiological model of gastric emptying. Journal of Fish Biology 30, 299 – 314.

Jobling, M. 1994. Fish Bioenergetics. Fish and Fisheries Series 13. Chapman and Hall, London.

Jobling, M. 2010. Aquaculture International 18, 501 – 510.

Johnston, W.L. 1998. Commercial barramundi farming – estimating profitability, Queensland Department of Primary Industries, Brisbane, Australia. 16pp.

Jonassen, T.M., Imsland, A.K., Stefansson, S.O. 1999. The interaction of temperature and fish size on growth of juvenile halibut. Journal of Fish Biology 54 (3), 556 – 572.

Katersky, R.S. & Carter, C.G. 2005. Growth efficiency of juvenile barramundi, *Lates calcarifer*, at high temperatures. Aquaculture 250, 775-780.

Klein, S.E., Sheridan, M.A. 2008. Somatostatin signalling and the regulation of growth and metabolism in fish. Molecular and Cellular Endocrinology 286 (1-2) 148 – 154.McCormick, S.D., Moriyama, S., Bjornsson, B.T. 2000. Low temperature limits

photoperiod control of smolting in Atlantic salmon through endocrine mechanisms. American Journal of Physiology – Regulatory Integrative and Comparative Physiology 278 (5), R1352-R1361.

Kojima, M., Kangawa, K. 2005. Ghrelin: structure and function. Physiological Reviews 85, 495 – 522.

Lee, S., Hwang, U., Cho, S. 2000. Effects of feeding frequency and dietary moisture content on growth, body composition and gastric evacuation of juvenile Korean rockfish (Sebastes schlegeli). Aquaculture 187 (3-4), 399 – 409.

McCormick, S.D., Moriyama, S., Bjornsson, B.T. 2000. Low temperature limits photoperiod control of smelting in Atlantic salmon through endocrine mechanisms. American Journal of Physiology, Regulatory and Integrated Comparative Physiology 278, 1352 – 1361.

Martinez-Chavez, C.C., Al-Khamees, S., Campos-Mendoza,A., Penman, D.J., Migaud, H. 2008. Clock controlled endogenous melatonin rhythms in Nile tilapia (*Oreochromis niloticus niloticus*) and African catfish (*Clarias gariepinus*). Chronobiological International 25, 31-49.

Migaud, H., Davie, A., Taylor, J.F. 2010. Current knowledge on the photoneuroendocrine regulation of reproduction in temperate fish species. Journal of Fish Biology 76 (1), 27 – 68.

Nelson, L.W., Sheridan,M.A. 2006. Gastroenteropancreatic hormones and metabolism in fish. General and Comparative Endocrinology 148, 116 – 124.

Oppedal, F., Taranger, G.L., Juell, J.E., Fosseidengen, J.E., Hansen, T. 1997. Light intensity affects growth and sexual maturation of Atlantic salmon (*Salmo salar*) postsmolts in sea cages. Aquatic Living Resources 10, 351 – 357.

Perez-Sanchez, J. 2000. The involvement of growth hormone in growth regulation, energy homeostatis and immune function in the gilthead sea bream (*Sparus aurat*a): a short review. Fish Physiology and Biochemistry 22 (2), 135 – 144

Petit, G., Beauchaud, M., Attia, J., Buisson, B. 2003. Food intake and growth of largemouth bass (*Micropterus salmonides*) held under alternated light/dark cycle (12L:12D or exposed to continuous light. Aquaculture 228 (1-4), 397-401

Pierce, A.L., Beckman, B.R., Shearer, K.D., Larsen, D.A., Dickhoff, W.W. 2001. Effects of ration on somatotrophic hormones and growth in coho salmon. Comparative Biochemistry and Physiology B 128, 255-264.

Porter, M.J.R., Randall, C.F., Bromage, N.R., Thorpe, J.E. 1998. The role of melatonin and the pineal gland on development and smoltification of Atlantic salmon (*Salmo salar*) parr. Aquaculture 168 (1-4), 139 - 155.

Porter, M.J.R., Duncan, N.J., Mitchell, D., Bromage, N.R., 1999. The use of cage lighting to reduce plasma melatonin in Atlantic salmon (*Salmo salar*) and its effects on the inhibition of grilsing. Aquaculture 176, 237-244.

Porter, M.J.R., Duncan, N., Handeland, S.O., Stefansson, S.O., Bromage, N.R. 2001. Temperature, light intensity and plasma melatonin levels in juvenile Atlantic salmon. Journal of Fish Biology 58 (2), 431 – 438.

Porter, M.J.R, Woolcott, H.M, Pankhurst, N.W. 2003. The use of additional lighting and artificial photoperiods to recondition early maturing Atlantic salmon (*Salmo salar*) in Tasmania. Fish Physiology and Biochemistry 28, 391-393

Reddy, P.K., Leatherland, J.F. 1995. Influence combination of time of feeding and ration level on the diurnal hormone rhythms in rainbow trout. Fish Physiology and Biochemistry 14, 25-36.

Reinecke, M. 2010. Influences of the environment on the endocrine and paracrine fish growth hormone – insulin-like growth factor-I system. Journal of Fish Biology 76, 1233 – 1254.

Riche, M., Haley, D.I., Oetker, M., Garbrecht, S., Garling, D.L. 2004. Effect of feeding frequency on gastric evacuation and the return of appetite in tilapia Oreochromis niloticus L. Aquaculture 234 (1-4), 657 – 673.

Rungruangsak-Torrissen, K., Moss, R., Andresen, L.H., Berg,A., Waagbo, R. 2006. Different expressions of trypsin and chymotripsin in relation to growth in Atlantic salmon (*Salmo salar* L.). Fish Physiology and Biochemistry 32, 7 – 2.

Rungruangsak-Torrissen, K., Sunde, J., Berg, A.E., Nordarden, U., Fjelldal., G., Oppedal, F. 2009. Digestive efficiency, free amino acid pools and quality of growth performance in Atlantic salmon (*Salmo salar L.*) affect by light regimes and vaccine types. Fish Physiology and Biochemistry 35, 255 – 272.

Shimizu, M., Cooper, K.A., Dickhoff, W.W., Beckman, B.R. 2009. Postprandial changes in plasma growth hormone, insulin, insulin-like growth factor (IGF)-I, and IGF-binding proteins in coho salmon fasted for varying periods. American Journal of Physiology and Regulatory Integrated Comparative Physiology 297, R352 – R361.

Taranger, G.L., Haux, C., Hansen, T., Stefansson, S.O., Bjornsson, B.T., Walther, B.T., Kryyi, H. 1999. Mechanisms underlying photoperiodic effects on age at sexual maturity in Atlantic salmon, *Salmo salar*. Aquaculture 177 (1-4), 47 – 60.

Taylor, J.F., Migaud, H., Porter, M.J.R., Bromage, N.R. 2005. Photoperiod influences growth rate and plasma insulin-like growth factor-I levels in juvenile rainbow trout, *Oncorhynchus mykiss*. General and Comparative Endocrinology 142, 169 – 185.

Tucker, B.J., Booth, M.A., Allan, G.L., Booth, D., Fielder, D.S. 2006. Effects of photoperiod and feeding frequency on performance of newly weaned Australian snapper *Pagrus auratus*. Aquaculture 258 (1-4), 514-520

Volkoff, H., Hoskins, L.J., Tuziak, S.M. 2010. Influence of intrinsic signals and environmental cues on the endocrine control of feeding in fish: Potential application in aquaculture. General and Comparative Endocrinology 167, 352 – 359.

Williams, K., Barlow, C.G. 1999. Dietary requirements and optimal feeding practices for barramundi (*Lates calcarifer*). Queensland Department of Primary Industires, Queensland, Australia.

Worrall, K., Carter, C., Porter, M.J.R. 2004. Effects of Photoperiod on the Growth of Juvenile Barramundi (*Lates calcarifer*). Hons Thesis, University of Tasmania.

Zachman, A., Ali, M.A., Falcon, J. 1992. Melatonin and its effects in fishes: an overview. In: Ali, M.A. (Ed.), Rhythms in Fishes. Plenun Press, New York, pp. 149 – 165.