Cannibalism in Barramundi *Lates calcarifer*: Understanding Functional Mechanisms and Implication to Aquaculture

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Summary

Cannibalism is a major issue in the culture of piscivorous fish and severe cannibalism can cause great production loss especially during the larval and juvenile period. This thesis uses barramundi *Lates calcarifer* as a representative of typical carnivores to understand the mechanisms behind fish cannibalism and to develop protocols for fish cannibalism control in aquaculture. Four studies were performed to investigate (1) the morphological limitation of cannibalism, (2) the prey size selection and cannibalistic behaviour, (3) the bioenergetics and growth advantage of cannibalism, and (4) the effects of environmental and biotic factors on barramundi cannibalism. In study 1, it was found that barramundi would become cannibals when conspecifics were smaller than 50% of the cannibal size. Once an individual became cannibal, it would progressively develop its predatory capacity to ingest a conspecific prey up to 78-72% of its total length (25-131 mm total length, respectively). The maximum ingestible prey size relative to the cannibals decreased as cannibals grew, as a result of allometric growth of body parts. In study 2, cannibalistic barramundi (40–130 mm total length) showed a consistent preference for smaller prey when the prey size was in the range of 30-65% cannibal size. A behaviour-energetic analysis showed that smaller prey would result in more energetic gain in cannibals. Cannibalistic profitability was inversely correlated to cannibal size as illustrated by the reduction of prey vulnerability as fish grew. In study 3, cannibalistic barramundi (77 mm total length) showed a better growth performance by preying on conspecifics than siblings fed on formulated diets. The better growth performance by preying conspecifics was achieved by higher feed conversion efficiency and more energy allocation to growth than in those fish fed solely on formulated diet. Therefore, the fast-growing cannibals would continuously find slow-growing and smaller victims to prey on, leading to a long-lasting cannibalism. In study 4, the effects of stocking density (1, 5 and 10 fish L^{-1}) and feeding frequency (once and three times per day) on barramundi cannibalism were tested in a 40-day trial starting with homogeneous fish size of 20 mm total length in average. Low feeding frequency and high stocking density were more likely to provoke cannibalism. The increase in fish size heterogeneity under the regime of low feeding frequency led to the emergence of cannibals, resulting in long-lasting cannibalism. In contrast, feeding frequency at three times per day suppressed cannibalism though could not exclude mortalities owing to wounds and suffocation. In summary, aggressive or predatory behaviour is inherent to barramundi, but cannibalistic impact on fish survival in aquaculture can be reduced by keeping a fish size difference under 50% through size grading, high feeding frequency and low stocking density. When the size of smaller fish is <50% of larger ones, cannibalism will prevail in the barramundi population during the nursery period of fish culture.

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.

Flavio F. Ribeiro

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Chapter 1

General Introduction

Cannibalism or intraspecific predation is defined as the process of killing and consuming the whole or the major part of an individual belonging to the same species (Fox, 1975, Polis, 1981, Elgar and Crespi, 1992a, Baras, 2013). Originally regarded as a laboratory artefact (Colinvaux, 1973), rarely observed in nature (Lorenz, 1966, Smith and Price, 1973), cannibalism is now considered as a common phenomenon with adaptive value, observed in diverse taxa, living in different habitats and sharing different social and feeding habits (Fox, 1975, Polis, 1981). At the individual level, cannibalism confers nutritional advantages, expands the range of food availability and decreases the intraspecific competition for resources, then contributing more genes to the next generation (Polis, 1981, Fox, 1975, Elgar and Crespi, 1992b). At the population level, cannibalism increases stability and resilience and decreases the probability of extinction by self-regulating the population below the carrying capacity of the environment. This process is known as a “lifeboat strategy”, where individuals in a cannibalistic population survive periods of food restriction by eating each other whereas individuals in non-cannibalistic population may starve to death (Polis, 1981).

In fish, cannibalism has been documented in over 200 fish species, but its occurrence must have been even more widespread, and its absence should be considered exceptional in fish communities (Smith and Reay, 1991). Fish cannibalism can be classified based on three main criteria (Smith and Reay, 1991): (1) prey developmental stage (egg or post-hatching); (2) genetic relationship between cannibal and prey (filial, sibling and non-kin); (3) and age relationship between cannibal and prey (intracohort or intercohort). These criteria are interrelated, resulting in distinct classes of cannibalism, possibly occurring more than once in a single species. In spite of the occurrence of different classes of cannibalism among fish families, hereafter, this thesis will focus on the sibling intracohort cannibalism of post-hatching larvae and juveniles, since this is the type of cannibalism hindering the production efficiency in fish culture (Hecht and Pienaar, 1993, Baras and Jobling, 2002, Baras, 2013). For the sake of simplicity, “sibling intracohort cannibalism of post-hatching stages” will be mention hereafter as “intracohort cannibalism” or solely “cannibalism”.

1.1. Cannibalism in Fish Culture

Intracohort cannibalism can produce high commercial losses in piscivorous fish culture especially at the early juvenile stage (Hecht and Pienaar, 1993, Ruzzante, 1994, Hseu et al., 2007b). Aquaculture selects for fish species based on their high fecundity, feeding behaviour during early life stages, robustness to the environment, fast development and growth rate, traits that are in a close relationship with boldness, aggressive and feeding behaviour (Hecht and Pienaar, 1993, Baras and Jobling, 2002). Therefore, aquaculture may be inadvertently selecting cannibalism, once the “shooters”, individuals growing at a faster rate and greater feeding tendency, are usually selected as broodstock (Hecht and Pienaar, 1993). Moreover, the captivity conditions in aquaculture may enhance the propensity of individuals to become a cannibal due to restriction of dispersing, high stocking densities and inappropriate food supply (Smith and Reay, 1991, Hecht and Pienaar, 1993, Baras and Jobling, 2002), leading to the development of cannibalistic polyphenism (Elgar and Crespi, 1992a). Since the aquaculture industrial sector relies on a balanced commercial cost to profit ratio, hatchery management should be designed to promote satisfactory outcomes, reducing mortality rates and improving fish growth rates. Breeding programs could be an efficient tool to reduce cannibalism impact in fish hatcheries, but the selection of less cannibalistic traits while maintaining fast growth rates is a high challenge. Therefore, the knowledge of the functional mechanisms behind fish cannibalism and the effects of biotic factors on its dynamics are essential to develop protocols for cannibalism control in hatcheries.

The onset of cannibalism may occur at same time of the start of exogenous feeding, when the mouth structures required for suction feeding and prey holding are developed (Baras et al., 2000, Baras and Jobling, 2002, Baras, 2013). At the early developmental stage, size variation in a fish population is low and putative cannibals are unlikely to encounter conspecifics small enough to be swallowed as whole (Baras and Jobling, 2002). However, piscivorous fish have a larger mouth gape size than the caudal peduncle and a slight size difference allows potential cannibals to catch preys tail-first and swallow them up to the head (Cuff, 1980, Hecht and Appelbaum, 1988, Baras and Jobling, 2002, Baras, 2013). This type of intraspecific predation is termed type I or incomplete cannibalism (Baras, 2013, Hecht and Appelbaum, 1988) (Fig. 1.1A). Incomplete cannibalism can lead to growth heterogeneity since conspecific prey are more nutritious than live food prey such as rotifers and *Artemia* commonly used in larviculture (Kubitza and Lovshin, 1999). Besides, some other factors also promote size heterogeneity during larval fish rearing, such as genetic traits, resource competition, variation on developmental ontogeny and morphological deformities (Baras and Jobling, 2002, Kestemont et al., 2003, Baras, 2013). The weaning process coinciding with the metamorphosis from larvae to juvenile can further expedite size heterogeneity since individuals accepting formulated diets with high nutrition usually exhibit faster growth than their siblings solely fed on zooplankton (Kestemont et al., 2003). Therefore, once the fish size heterogeneity is attained in larviculture, incomplete cannibalism is usually replaced by complete cannibalism where cannibals ingest the prey whole and head-first (Baras and Jobling, 2002, Hseu et al., 2007b, Baras, 2013) (Fig. 1.1B).

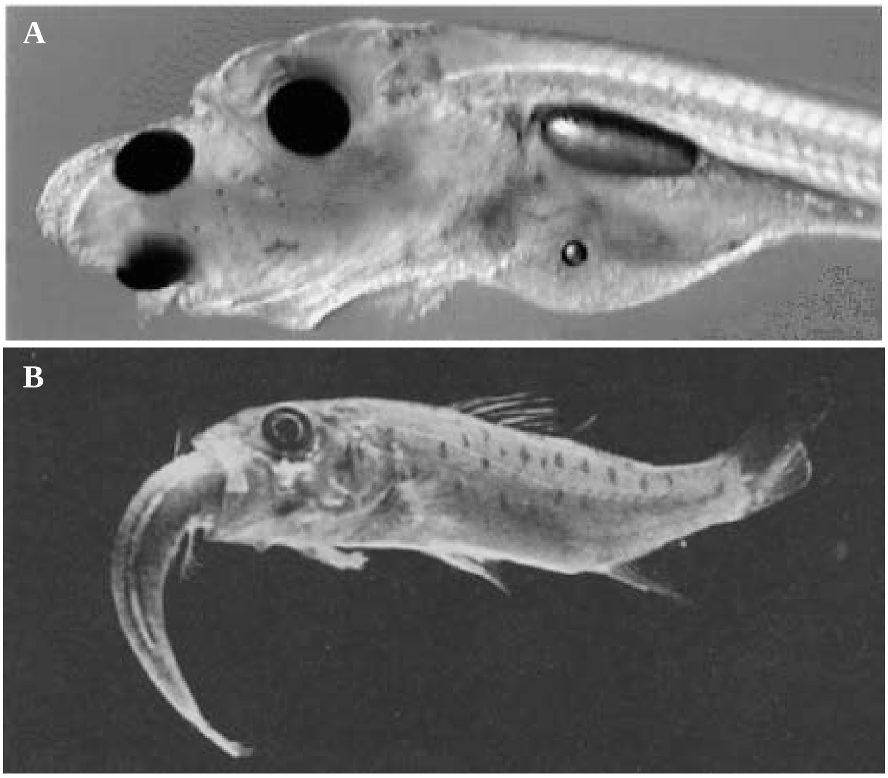

Fig. 1.1. Two types of intracohort cannibalism in fish. Panel (A): Type I or incomplete cannibalism occurs in the larvae of dorada *Brycon moorei* (Baras et al., 2000), when a cannibal catches the prey tail-first and swallows it up to the head; Panel (B): type II or complete cannibalism occurs in juvenile sea bass *Dicentrarchus labrax* (Katavić et al., 1989), when a cannibal catches the prey head-first and ingests the whole body of the prey.

Once complete cannibalism starts, its occurrence and impact would further depend on the dynamics of growth – size heterogeneity – cannibalism (Baras, 2013). Several environmental factors have been reported to affect the dynamics of cannibalism in fish culture (Hecht and Pienaar, 1993, Baras and Jobling, 2002, Baras, 2013), but fish size grading has been the most common method to control cannibalism in fish culture. Nevertheless, the adequacy and efficiency of size grading are dependent on the dynamics of cannibalism which is ultimately governed by the morphological limitation of complete cannibalism, the prey size selection of cannibals preying on conspecifics and the cannibal’s growth advantage over siblings feeding formulated diets (Baras and Jobling, 2002, Baras, 2013). However, our understanding on the role of these functional mechanisms in regulating the cannibalism outcomes is inadequate. Therefore, there is a need to investigate the main components of the dynamics of cannibalism and also understand the effects of biotic factors on the event of cannibalism in predatory fish species.

1.2. Morphological Constraints in Complete Cannibalism

Complete cannibalism is characterised by the cannibal ingesting the whole body of a prey. Consequently, the size difference between cannibal and prey is essential for the occurrence of complete cannibalism (Hecht and Appelbaum, 1988, Baras and Jobling, 2002, Baras, 2013). The identification of the maximum prey size that a cannibal can ingest is fundamental to determine the allowable size difference in a fish population before next size grading is made (Hseu et al., 2007b, Qin and Fast, 1996, Johnson and Post, 1996). In cannibalism control, morphological characteristics of both prey and cannibal are used to estimate the upper physical limits for complete cannibalism (Dabrowski and Bardega, 1984). In previous studies, species-specific models have been built to predict the maximum prey size that a cannibal is morphologically capable to ingesting (Table 1.1). These models are based on the assumption that a given size of cannibal can swallow a prey size if the prey’s largest cross-sectional dimension is equal to or smaller than the cannibal mouth size (Johnson and Post, 1996, Qin and Fast, 1996, Hseu et al., 2003, Hseu et al., 2004). In most cases, the impact of complete cannibalism is directly proportional to the maximum ingestible prey size (Baras and Jobling, 2002). The lower the maximum ingestible prey size (% cannibal size), the lower the cannibalistic tendencies (Table 1.1), since a substantial size difference between prey and cannibals must be attained to allow cannibals finding suitable conspecific sizes to prey on. However, in most cannibalistic fish species, the maximum ingestible prey size reduces as fish grow larger, as a result of the allometric growth of the mouth and body parts (Qin and Fast, 1996, Baras, 2013). Generally in piscivorous species, the ratio between gape size and body length is high in the larval and early juvenile stages and decreases as fish grows (Baras and Jobling, 2002), leading to high cannibalism propensity in younger fish than in older ones (Qin and Fast, 1996). Nevertheless, this situation is not observed in some species such as in groupers, where the maximum prey size is predicted to increase with the fish size (Table 1.1). Therefore, the knowledge on the ontogeny of the morphological constraint of cannibalism, during the stages when cannibalism highly occurs, is crucial to understand the dynamics of cannibalism and develop species-specific size grading protocols for cannibalism control in aquaculture.

Table 1.1. Morphological limitation of complete cannibalism in some cultured fish species. TL_{can} and TL_{prey} are total length of cannibal and prey, respectively.

Species	TL_{can} (mm)	Model TL_{prey} (mm) =	Max. ingestible prey size (% TL_{can})	Reference
Channa striatus	60 - 120	$0.41TL_{can} + 25.81$	84 - 62	Qin and Fast (1996)
Clarias gariepinus	60 - 110	$0.40TL_{can} + 3.31$	46 - 43	Hecht and Appelbaum (1988)
Engraulis capensis	10 - 35	$0.58TL_{can} – 2.90$	29 - 49	Brownell (1985)
Epinephelus coioides	15 - 70	$0.80TL_{can} – 1.50$	70 - 77	Hseu et al. (2003)
E. fuscoguttatus	24 - 64	$0.71TL_{can} – 1.75$	63 - 68	Hseu et al. (2007b)
E. lanceolatus	25 - 70	$0.83TL_{can} – 2.48$	73 - 79	Hseu et al. (2004)
Gadus morhua	40 - 160	$0.87TL_{can} – 0.003TL_{can}^{2} – 1.12$	70 - 44	Otterå and Folkvord (1993)
Micropterus salmonides	50 - 100	$0.31TL_{can}^{1.01}$	32 - 32	Johnson and Post (1996)
Seriola lalandi	20 - 120	$0.54TL_{can} – 2.54$	41 - 52	Ebisu and Tachiara (1993)
S. quinqueradiata	15 - 40	$0.49TL_{can} + 0.30$	51 - 49	Sakakura and Tsukamoto (1996)
Theragra chalcogramma	85 - 174	$0.67TL_{can} – 5.98$	60 - 63	Sogard and Olla (1994)

1.3. Conspecific Prey Size Selection

Prey size selection is defined as any difference in prey size composition in the predator diet compared to the composition of available prey sizes in the environment (Ivlev, 1961, Chesson, 1978, Baras et al., 2010). Similar to other piscivorous fish species (Juanes, 1994), some cannibalistic species show a tendency of selection for small conspecifics when given a choice of size range, as observed in giant grouper *Epinephelus lanceolatus* (Hseu et al., 2007a), orange-spotted grouper *E. coioides* (Hseu and Huang, 2014), Atlantic cod *Gadus morhua* (Blom and Folkvord, 1997), yellow perch *Perca flavescens* (Post and Evans, 1989), Arctic char *Salvelinus alpinus* (Amundsen et al., 1995), catfish *Pangasius djambal* (Baras et al., 2010), dorada *Brycon moorei* (Baras et al., 2000) and Amazonian catfish *Pseudoplatystoma punctifer* (Baras et al., 2011). The reasons for selecting smaller prey may be explained by the concept of energy gain maximization. According to the optimal foraging theory (OFT), predators select prey that gives high energy gain per unit of effort (Stephens and Krebs, 1986, Sih and Moore, 1990, Mittelbach, 2002, Gill, 2003). Although predators may yield higher energy by ingesting larger prey, the costs associate with the predation acts, such as handling time and unsuccessful capture attempts, may be higher than ingesting smaller prey (Juanes, 1994) and the smaller prey may become the most profitable food item. The size selection may also vary with the life stage of a predator, since prey vulnerability, as a function of prey escape skills, is inversely related to the absolute fish size (Baras, 2013), leading to the reduction of feeding profitability as a predator grows. Therefore, the reduction in prey vulnerability together with the increase of the predator’s morphological constraint makes cannibalism difficult for large cannibals (Baras, 2013). Furthermore, once all smaller prey individuals have been succumbed to cannibalism, cannibals are forced to move up to consume larger prey despite the reduction of foraging profitability. Consequently, the population becomes more homogeneous in size and the propensity of cannibalism is progressively reduced. Therefore, the knowledge of the prey size selection and size dependent predation profitability is crucial to understand the dynamics of cannibalism and to develop appropriate protocols for cannibalism control in fish culture.

1.4. Growth Advantage of Cannibals

Once complete cannibalism has emerged in a captive fish population, its continuation and potential impact depend on how rapidly cannibals grow in comparison to their siblings, or on the growth advantage of cannibals over their siblings feeding on formulated diets in an aquaculture context (Baras and Jobling, 2002). The growth advantage of cannibals can be achieved by a higher assimilation of the consumed energy into growth when compared with siblings feeding alternative food (Baras 2013). If a cannibal does not gain growth advantage over non-cannibalistic siblings, as observed in the catfish *P. djambal* (Baras et al., 2010), cannibalism should disappear at some stage since cannibals would remove all potential small prey from the population (Baras, 2013). However, if a cannibal does possess growth advantage over their siblings ingesting a formulated diet, as observed in the Amazonian catfish *P. punctifer* (Baras et al., 2011), fast growing cannibals would always find slow-growing and highly profitable conspecifics to prey on. In this scenario, the emergence of large and fast growing cannibals would potentially overcompensate any size-dependent constraints upon growth and ingestion capacity, leading to even greater size heterogeneity and long-lasting cannibalism in the population (Baras, 2013). Therefore, the comprehension of the energy budget and the growth trajectories of cannibals in comparison with non-cannibalistic siblings are essential to understand the developmental ontogeny of cannibalism over time, which would ultimately contribute to developing management strategies to control cannibalism in fish culture.

1.5. Effects of Biotic Factors on Cannibalism

Food abundance is one of the major factors influencing cannibalism in many reared fish species during the early life stage (Polis, 1981, Hecht and Appelbaum, 1988, Smith and Reay, 1991, Hecht and Pienaar, 1993, Dou et al., 2000, Baras and Jobling, 2002, Baras, 2013). Any restriction on food availability (quantitatively, temporally and spatially) may trigger cannibalism simply by means of hunger motivation (Baras and Jobling, 2002, Baras, 2013). In fact, the increase in daily feeding ration can successfully suppress intracohort cannibalism in the African catfish *Clarias gariepinus* (Hecht and Appelbaum, 1988, Al-Hafedh and Ali, 2004) and juvenile snakehead *Channa striatus* (Qin and Fast, 1996). Similarly, the increase in feeding frequency can reduce the impact of cannibalism in juvenile European sea bass *Dicentrarchus labrax* (Katavic et al. 1989). Once fish are fed in excess during nursery, determination of the influence of feeding frequency on cannibalism is crucial towards working out a feeding regime for cannibalism control (Katavic et al. 1989).

The increase in fish stocking density can also enhance cannibalism due to a high predator-prey encounter frequency (Sogard and Olla, 1994, Baras, 2013). High rates of cannibalism are generally associated with high fish density, as observed in fat snook, *Centropomus parallelus* (Corrêa and Cerqueira, 2007), carp *Cyprinus carpio* (van Damme et al., 1989), European sea bass *D. labrax* (Hatziathanasiou et al., 2002) and European perch *Perca fluviatilis* (Kestemont et al., 2003). Cannibalism may be worsened under high stocking density simply because the probability of prey encounter is increased (Smith and Reay, 1991, Baras and Jobling, 2002, Baras et al., 2000, Hecht and Appelbaum, 1988). Conversely, high stocking density may cause perceptual-confusion for prey capture, as observed in the catfish *Heterobranchus longifilis* (Baras et al., 1999) or suppress territorial behaviour, as observed in perch *Perca fluviatilis* (Mélard et al., 1996, Baras et al., 2003). Nevertheless, high fish density coupled with food restriction can promote food competition, inter-individual growth variability, size heterogeneity and consequently the emergence of new cannibals in the population (Baras, 2013, Baras and Jobling, 2002). Maintaining a suboptimal feeding will further exacerbate fish size heterogeneity and consequently encourage cannibalism, since cannibalistic individuals would grow substantially faster than non-cannibalistic siblings under restricted feeding, as observed in European sea bass *D. labrax* and European perch *P. fluviatilis* (Katavić et al., 1989, Baras et al., 2003). Therefore, stocking density along with the frequency of feed provision is a key parameter that needs to manage to reduce cannibalism in fish under captivity.

1.6. Barramundi *Lates calcarifer* the Model Species

Barramundi *Lates calcarifer*, commonly known as Asian seabass, is geographically distributed through the northern Indian and tropical western Indian Ocean, from Iran to northern Australia, including China, Taiwan and Papua New Guinea (Tucker, 2002). As a member of the family Latidae, the barramundi is a catadromous species, spawning and going through hatching and early larval development in estuary. At the early juvenile stage, barramundi enter swamps then move to upstream at later juvenile stages where they live until maturation of 3-4 years old and then migrate downstream to spawn (Grey, 1987). Barramundi is an ambush and opportunistic predator, mainly preying upon crustaceans and fish in nature, though cannibalism on conspecifics also occurs depending on the environmental circumstances (Davis, 1985, Moore, 1982, Russel, 1987). Barramundi supports extensive commercial and recreational fisheries in Australia and Papua New Guinea and it is also an important fish species for aquaculture in Australasia (Tucker, 2002). Barramundi has been increasingly cultured in Australia since 1980s due to its popular demanding market, fast growth, and adaptation to either freshwater or marine habitat for aquaculture (Schipp et al., 2007). In Australia, barramundi is mainly cultured in Queensland, North Territory and Western Australia, with an annual production of 3500 tonnes, generating $30 million of profitability for the aquaculture sector in 2012 (Skirtun et al., 2013). The seed supply for barramundi aquaculture in Australia relies on the hatchery supply, but strong cannibalism in hatchery is a main problem hindering the production of barramundi fingerling for grow-out (Tucker, 2002). During nursery, cannibalism can cause a great loss after newly metamorphosed juveniles are weaned from live food onto formulated diets. Cannibalism can start as early as 15 mm total length (TL) and may last during the whole nursery period until fish reach 150 mm TL (Schipp et al., 2007). Frequent size grading is a common method to control cannibalism in the barramundi nursery, but it is highly labour intensive and can cause damage to fish (Schipp et al., 2007, Tucker, 2002). Therefore, the present thesis uses the highly cannibalistic barramundi as model species to gain knowledge on the functional mechanism behind the dynamics of cannibalism in barramundi culture. The outcomes of the thesis research provides more understanding on the ontogeny and nature of fish cannibalism and develops practical strategies towards management of cannibalism in fish farming especially during the nursery phase.

1.7. Study Objectives

The main aim of the present thesis is to understand the biological mechanisms of fish cannibalism and to provide fundamental knowledge to develop management protocols for cannibalism control in the hatchery phase of fingerling production. Specifically, this thesis explores the functional mechanisms of cannibalism through the studies on (1) the morphological limitation of cannibalism, (2) the prey size selection of cannibal upon conspecific prey and (3) the growth advantage of cannibals over their sibling feeding on formulated diets in small replicated aquaria. Furthermore, the present thesis also addresses the effects of biotic factors on the dynamics of growth – size heterogeneity – fish cannibalism in a large system to validate the findings derived from small aquaria. Therefore, four studies were conducted using barramundi *Lates calcarifer* as a model fish species to address the above thesis objectives:

1.7.1. Study 1: Modelling Size-Dependent Cannibalism in Barramundi: Cannibalistic Polyphenism and its Implication to Aquaculture

The aim of this study is to determine the maximum prey size that juvenile barramundi cannibals are morphologically capable to ingesting. The results of this study contribute fundamental information to working out the protocols for size grading practices in barramundi fingerling production. It also provides indication of the existence of cannibalistic polyphenism in barramundi when cannibals are challenged with progressively increasing prey sizes.

1.7.2. Study 2: Prey Size Selection and Cannibalistic Behaviour of Juvenile Barramundi

Based on the understanding of morphological restriction on fish cannibalism, the present thesis further investigates size-dependent prey selection from fish behavioural perspectives. The behaviour-energetic approach provides explanation on prey size selection in the context of the optimal foraging theory. In addition, this study tests the hypothesis of passive or active prey size selection of cannibalism. The outcomes of this study provide insights into our knowledge on piscivory behaviour and feeding strategies of cannibals at different size categories. Practically, this study contributes to the overall understanding of the dynamics of cannibalism in fish culture, aiding the development of protocols for cannibalism control in aquaculture.

1.7.3. Study 3: Bioenergetics of Cannibalism in Juvenile Barramundi: Exploring Growth Advantage of Fish Fed Live Prey and Formulated Diet

With the understanding on morphological and behavioural characteristics of fish cannibalism, the present study assesses the third functional mechanism on the dynamics of fish cannibalism, i.*e.*, the growth advantage between cannibalistic and non-cannibalistic barramundi using a bioenergetics approach. This study particularly compared the energy budget of cannibalistic and non-cannibalistic barramundi and tackled the dilemma if cannibalistic individuals would gain growth advantage through a higher allocation of the consumed energy into growth than that in fish fed formulated diets, leading cannibalism to an ever-lasting event in fish culture. The outcomes of the present study, in conjunction with the results obtained in the previous two studies would closely predict the dynamics of cannibalism in barramundi nursery. These findings led the present thesis to test the dynamics of cannibalism through manipulations of feeding frequency and fish stocking densities in the subsequent study.

1.7.4. Study 4: Dynamics of Intracohort Cannibalism and Size Heterogeneity in Juvenile Barramundi at Different Stocking Densities and Feeding Frequencies

Based on the results of the previous three studies in small aquaria, this study hypothesises that size heterogeneity and cannibalism can be controlled through manipulation of biotic factors in large tanks. Specifically, this study tests the dependent effects of feeding frequency and stocking density on the temporal dynamics of fish growth, size heterogeneity and cannibalism in juvenile barramundi. The outcomes of this study not only contribute to the understanding of the temporal feature of cannibalism under different combinations of feeding frequency and stocking density, but also provide practical management suggestions on cannibalism control of juvenile barramundi during the nursery production period.

1.8. Thesis Organization

The present thesis consists of six chapters to illustrate specific research approaches, methodologies and results ultimately contributing to better understanding of fish cannibalism and development of protocols for cannibalism control in fish culture. Chapter 1 provides the overall research background, identifies knowledge gap and presents the study objectives of the present thesis. Four independent studies form four data chapters (2, 3, 4 and 5) and each one is presented as an independent manuscript suitable for publication in different journals. Consequently, some repetition of background and methods may be found among chapters. Although independent objectives are specified in each chapter, they are all complementary to each other towards the overall thesis objectives stated in this introduction chapter. Within each chapter, the word “study” refers to each respective and sole chapter. All of the studies were performed by the author of the present thesis under the supervision of his principal supervisor. However, the principal supervisor is listed as a co-author on each manuscript for publication in peer reviewed journals. In chapter 5, a third co-author contributed to experiment implementation, data collection and interpretation. Chapter 6 combines all the results obtained in the four independent chapters to discuss the major objectives of the present thesis, and provides the general conclusions and future research.

In summary, the thesis is organized in the following manner:

- Chapter 1: General Introduction.
- Chapter 2: Modelling Size-Dependent Cannibalism in Barramundi: Cannibalistic Polyphenism and its Implication to Aquaculture.
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- Chapter 3: Prey Size Selection and Cannibalistic Behaviour of Juvenile Barramundi.
  This chapter has been accepted for publication by the Journal of Fish Biology after minor revision as: Ribeiro FF, Qin JG. Prey size selection and cannibalistic behaviour of juvenile barramundi *Lates calcarifer*. Journal of Fish Biology (accepted after minor revision on 3/8/2014).
- Chapter 4: Bioenergetics of Cannibalism in Juvenile Barramundi: Exploring Growth Advantage of Fish Fed Live Prey and Formulated Diet.
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calcarifer (Bloch): exploring growth advantage of fish fed live prey and formulated diet. *In press*. doi:10.1111/are.12685.

- Chapter 5: Dynamics of Intracohort Cannibalism and Size Heterogeneity in Juvenile Barramundi at Different Stocking Densities and Feeding Frequencies.

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- Chapter 6: General Discussion.

1.9. References

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Chapter 2

Modelling Size-Dependent Cannibalism in Barramundi: Cannibalistic Polyphenism and its Implication to Aquaculture

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2.1. Abstract

This study quantified size-dependent cannibalism in barramundi *Lates calcarifer* through coupling a range of prey-predator pairs in a different range of fish sizes. Predictive models were developed using morphological traits with the alternative assumption of cannibalistic polyphenism. Predictive models were validated with the data from trials where cannibals were challenged with progressing increments of prey sizes. The experimental observations showed that cannibals of 25-131 mm total length could ingest the conspecific prey of 78-72% cannibal length. In the validation test, all predictive models underestimate the maximum ingestible prey size for cannibals of a similar size range. However, the model based on the maximal mouth width at opening closely matched the empirical observations, suggesting a certain degree of phenotypic plasticity of mouth size among cannibalistic individuals. Mouth size showed allometric growth comparing with body depth, resulting in a decreasing trend on the maximum size of ingestible prey as cannibals grow larger, which in parts explains why cannibalism in barramundi is frequently observed in the early developmental stage. Any barramundi has the potential to become a cannibal when the initial prey size was <50% of the cannibal body length, but fish could never become a cannibal when prey were >58% of their size, suggesting that 50% of size difference can be the threshold to initiate intracohort cannibalism in a barramundi population. Cannibalistic polyphenism was likely to occur in barramundi that had a cannibalistic history. An experienced cannibal would have a greater ability to stretch its mouth size to capture a much larger prey than the models predict. The awareness of cannibalistic polyphenism has important application in fish farming management to reduce cannibalism.

Keywords: Fish, Cannibalism, Aquaculture, Barramundi, Prey, Predator

2.2. Introduction

Polymorphism, the occurrence of discrete intraspecific morphs, is triggered by genetic differences, phenotypic plasticity, or a combination of both (Komiya et al., 2011, Smith and Skulason, 1996). In fish, such as Arctic char *Salvelinus alpinus*, distinct intraspecific morphotypes can be a result of phenotypic plasticity associated with adaption to resources and ecological environments (Andersson, 2003, Hindar and Jonsson, 1993). Polyphenism on the other hand refers to alternative phenotypes in a population that are originated from a single genotype in response to environmental stimuli (West-Eberhard, 1989, Hoffman and Pfennig, 1999, Smith and Skulason, 1996, Closs et al., 2003). If such phenotypic plasticity gives advantages for some individuals to ingest a larger prey and consume their conspecifics, this phenomenon is regarded as cannibalistic polyphenism.

True cannibalistic polyphenic individuals are clearly specialized in an intraspecific diet and have distinctive behaviour, morphology and life history (Polis, 1981), which are not common in fishes, but occur quite frequently in other taxa, such as amphibians (Crump, 1992). Nonetheless, resource polymorphism has been reported in certain fish species (Smith and Skulason, 1996). For example, some individuals of Arctic char exhibit a broader or larger mouth, faster growth rates and more aggressive behaviour than others (Jonsson and Jonsson, 2001). In aquaculture, these traits are selected for, thereby leading to inadvertent selection of cannibalism in a farmed fish population (Hecht and Pienaar, 1993), and causing frequent occurrence of intracohort cannibalism in piscivorous species. Furthermore, aquaculture conditions enhance the propensity of some individuals to become cannibals due to restriction of fish dispersing, overcrowding, and uneven food distribution, leading to size heterogeneity and cannibalism (Smith and Reay, 1991, Hecht and Pienaar, 1993, Baras and Jobling, 2002). As a result, such conditions can promote development of cannibalistic polyphenism.

The onset of intracohort cannibalism may occur shortly after hatch such as in dorada *Brycon moorei* (Baras et al., 2000), or at a later stage as in most marine fish (Baras and Jobling, 2002) depending on the development patterns of the species. Once the cannibalistic process starts, it may persist during the juvenile phase of development as long as enough size heterogeneity enables a cannibal to prey on smaller conspecifics (Baras and Jobling, 2002). The current practice to control intracohort cannibalism in aquaculture is by size grading (Parazo et al., 1991, Hecht and Pienaar, 1993), but such procedure is labour consuming, largely unsatisfactory and stressful to fish (Qin et al., 2004). As in the prey-predator relationship of teleosts, morphological factors determine the maximum prey size that predators can ingest (Dabrowski and Bardega, 1984). Assuming that a cannibal can ingest a prey if the largest body dimension of the prey cross section is equal to or smaller than the maximum mouth dimension of the cannibal, some morphological models have been used to determine the largest size variation that is acceptable so as to make the exercise of complete cannibalism impossible after size sorting (Hseu et al., 2007, Hseu et al., 2003, Hseu et al., 2004, Qin and Fast, 1996, Johnson and Post, 1996). The largest prey cross-sectional dimensions (e.g., head height, body depth or width) are reliable factors for estimating the maximum capacity of a cannibal to ingest its prey. Nevertheless, the maximum mouth dimension may be subjective by researchers' choice (Johnson and Post, 1996). Gape size (Baras et al., 2010), opened mouth height (Otterå and Folkvord, 1993, Parazo et al., 1991), closed mouth width (Sogard and Olla, 1994, Johnson and Post, 1996), and opened mouth width (Hseu et al., 2004, Hseu et al., 2003, Qin and Fast, 1996, Hecht and Appelbaum, 1988, Baras, 1999, Baras et al., 2013) have been used to predict the maximum ingestion capacity of cannibalistic fish species. However, in order to have a reliable prediction, the maximum mouth dimension must be carefully selected according to specific traits of the target species such as using mouth elasticity in snakehead *Channa striatus* (Qin and Fast, 1996) and orientation of the prey on cannibal mouth in orange-spotted grouper *Epinephelus coioides* (Hseu et al., 2003) and giant grouper *E. lanceolatus* (Hseu et al., 2004). Furthermore, cannibalistic polyphenism has never been built into a model to predict size-dependent cannibalism in fish. As some individuals may possess larger jaws and a wider mouth (Polis, 1981), existing models based on the parts of a population average may underestimate the maximum prey size that a cannibal can ingest. Moreover, few models have been validated with an independent dataset, but if done, the maximum size of ingestible prey is underestimated as in snakehead (Qin and Fast, 1996) and largemouth bass *Micropterus salmoides* (Johnson and Post, 1996), or overestimated as in the giant (Hseu et al., 2004) and orange-spotted (Hseu and Huang, 2014) groupers.

The aim of this study was to determine size-dependent cannibalism in a highly cannibalistic fish, the barramundi *Lates calcarifer* (Latidae). Models were developed using the mouth width as the largest mouth dimension and the alternative assumption of polyphenism. Subsequently, the models were validated based on empirical results taken from a series of independent observations from different prey-predator pairs. Barramundi was used as a model species because it is an economically important fish for aquaculture in tropical and subtropical regions (Qin et al., 2004). In a previous model, Parazo et al. (1991) suggested that the total length (TL) of ingestible prey was 67-61% of the cannibal size in barramundi of 10-50 mm TL. However, Parazo’s model was based on an inappropriate measurement of mouth size and the empirical validation might be prejudiced by prey size preference, as it was based on the stomach analysis of cannibals from an undisturbed population of cultured fish. Thus, the present study used a new approach to assess the maximum prey size that cannibalistic barramundi can ingest from direct observations. The new model simulates a more realistic scenario to quantify the size relationship between cannibal and victim individuals in barramundi, with potential application in other cannibalistic fishes.

2.3. Materials and Methods

2.3.1. Ethics Statement

This study was carried out in strict accordance with the recommendations in the Animal Welfare Act 1985 and the Australian Code of Practice for the Care and Use of Animals for Scientific Purpose 7th Edition. The protocol, species, and number of animals used in this study were approved by the Flinders University Animal Welfare Committee (Project Number: E347). In any trial situations, each prey had an opportunity to avoid the predators in their cannibal challenge since we allocated more open space in each aquarium to facilitate prey escape. Euthanasia procedures were performed under overdose (43 mg l^{-1}) of AQUI-S® (New Zealand Ltd). All fish handling were followed by light anesthesia (15 mg l^{-1}) with AQUI-S, and all efforts were made to alleviate fish suffering.

2.3.2. Fish and Rearing Conditions

Hatchery raised barramundi *Lates calcarifer* of 34 days after hatching from the same cohort were obtained from West Beach Hatchery, West Beach, South Australia, and transported to the Animal House, Flinders University. Upon arrival, fish were visually graded into large, medium and small sizes, and stocked into three holding tanks (300 l) filled with freshwater. Each tank was equipped with an external biofilter and kept at 27-28ºC. Fish were divided into three groups and fed at different rates with dry pellets (NRD® range, 400 to 2,000 µm; 55% protein, 9% lipid, INVE Ltd, Thailand). Group 1: 360 large fish (1.2 fish l^{-1}) fed to satiation twice a day in order to produce large individuals to be used as cannibals; Group 2: 950 small fish (3.2 fish l^{-1}) fed once a day at a restricted ration to produce a range of small fish sizes to be used as prey on the cannibal challenge experiment; and Group 3: 650 medium fish (2.2 fish l^{-1}) fed twice a day under moderate feeding restriction in order to promote a range of fish sizes to be used for morphological measurements. Tanks were cleaned twice a day to remove uneaten pellets, faeces and dead fish. Water parameters were daily checked and maintained at 27.8 ± 0.2 °C, 7.7 ± 0.2 mg l^{-1} dissolved oxygen, 7.5 ± 0.1 pH, and < 0.5 mg l^{-1} ammonia and nitrite nitrogen. A photoperiod of 12L:12D was used at a light intensity of 350 Lux during the hours of light with abrupt transition between dark and light periods.

2.3.3. Morphological Models Construction

Periodically, 368 juveniles were sampled from fish in Group 3 for morphological measurements. Fish were collected with a hand net, euthanized with overdosed AQUI-S (43 mg l^{-1}, AQUI-S New Zealand Ltd) and immediately measured for total length (TL, mm), body depth (BD, mm) and mouth width (MW, mm) to the nearest 0.01 mm using a dissecting microscope or a digital caliper. Fish from 15 to 140 mm TL were sampled, as this comprised the size range corresponding to the time interval when intracohort cannibalism was intense in barramundi fingerling culture (Schipp et al., 2007). The selection of morphological parts for measurement was under these two assumptions: (1) cannibalistic barramundi swallow their conspecific prey in whole with head first (Baras and Jobling, 2002); (2) when cannibalistic barramundi ingest their conspecific prey, the maximal prey body depth is positioned laterally from side to side in the cannibal mouth. Such assumptions were used to predict the maximum prey size for barramundi cannibals from 35 to 140 mm TL. Total length (TL) was measured as the distance from the tip of the snout to the end of the caudal fin and body depth (BD) as the distance between the anterior edge of the dorsal fin and the bottom of the abdomen. Two measurements of mouth width were taken: mouth width at the close position (MWc) as the distance between the outer edges of the maxillary bones just beneath the eyes with the mouth closed; and mouth width at the open position (MWo) as the horizontal largest cross-section distance with the mouth fully stretched in an ellipse shape. With both mouth width measurements, an estimate of mouth width extension (MWE) for each fish was calculated as $MWE\ (\%MWc) = [(MWo - MWc)/MWc)] \times 100$.

The morphological predictive models were developed assuming that a $TL_{cannibal}$ can swallow a TL_{prey} if the BD_{prey} is equal to or smaller than the $MW_{cannibal}$. The relationships between $MW_{cannibal}$ *vs*. $TL_{cannibal}$ and BD_{prey} *vs*. TL_{prey} were used to predict the maximum prey length (TL_{prey}) for given sizes of cannibals ($TL_{cannibal}$). Models were developed using four different estimates of mouth size: closed mouth width (MWc); maximum closed mouth width (MWcmax); opened mouth width (MWo); maximum opened mouth width (MWomax).

2.3.4. Cannibal Challenge

A series of pairwise trials were performed to empirically observe the maximum conspecific prey size that a cannibalistic barramundi can ingest. Cannibals from 25 to 131 mm TL were individually challenged with single conspecific prey of known sizes, starting from 45% of cannibal TL. The system consisted of 20 × 6 l aquaria (20 × 20 × 25 cm) connected to a freshwater recirculation system equipped with a communal 200 l biofilter and set in the same experimental room as the holding tanks. Aquaria were cleaned daily to remove faeces. Water quality and physical parameters were kept the same as those in the holding tanks.

Initially, 20 potential cannibals were sampled from fish in Group 1, anesthetized (AQUI-S, 15 mg l^{-1}), measured for TL and individually stocked into each aquarium. Then, potential prey were collected from fish in Group 2, anesthetized (AQUI-S, 15 mg l^{-1}), measured for TL, individually selected and matched their respective cannibal. No food was provided during the trials. Predation was checked twice a day (0900 and 1700 h). In case of predation, the cannibal was re-measured in order to decide the next prey size to be offered, and a new prey larger than the previous one would be selected from Group 2, anesthetized (AQUI-S, 15 mg l^{-1}), measured for TL and individually matched the same cannibal. This procedure was repeated progressively by increasing the prey size at about 5% per change according to prey size availability. As the maximum prey size approached to the maximum ingesting limit for cannibals, the incremental rate of the new prey size was reduced to about 2%. The morphological limit for cannibals was considered maximum when both cannibal and prey coexisted for over 4 days. In that occasion, both fish were measured and the cannibal was replaced by a larger one.

Successful predation events were considered completion when the prey had been fully swallowed and digested by the cannibal. Cannibals in the process of digesting prey were easily identified due to their extended belly. Such consideration avoided significant discrepancies on growth rate between cannibal and prey during the next pairing period. In some circumstances, the prey was dead on the bottom of the aquarium after having been discarded by the cannibals due to unsuccessful capture attempt. In those cases, a new prey of a similar size was paired with the same cannibal. If the cannibal would kill but not ingest the prey again, that prey size was considered the upper limit of the cannibal and the cannibal was replaced by a larger one.

2.3.5. Statistical Analysis

All absolute estimates for body parts were regressed against TL and an analysis of covariance (ANCOVA) was used to test for homogeneity of the regression slopes of the body depth (BD, mm) and mouth width (MW, mm) estimates using total length (TL, mm) as a covariate. Linear regression analysis was used to assess the independence between mouth width extension (MWE as %MWc) and closed (MWc) and opened (MWo) mouth widths (%TL). MWE was regressed against TL to determine the capacities of mouth width extension as fish grew. Pearson's correlation analysis was used to assess the strength of correlations. All predictive models based on morphological measurements for the maximum prey to cannibal size ratio enabling the occurrence of intracohort cannibalism were developed using simple linear regression analysis. The results from the cannibal challenge experiment were used to estimate a revised model for maximum prey size for cannibals based on the empirical data. The size of the first offered prey was compared between the successful versus non-successful cannibalistic pairs with t-test to identify the criteria for the initial prey-predator size ratio that would provoke cannibalism. All statistics were considered significant at $P < 0.05$.

2.4. Results

2.4.1. Morphological Models

During the early juvenile stage (15-30 mm TL), body depth (BD) showed positive allometric growth, attaining its maximum dimension relative to body size (28% TL) when fish were around 35 mm TL (Fig. 2.1). Thereafter, BD slightly decreased and reached 25% TL at the late juvenile stage (140 mm TL; Fig. 2.1). Closed and opened mouth widths (MWc and MWo) presented slightly negative allometric growth as fish grew larger, decreasing from 13% to 9% TL (15- 135 mm TL) and from 17% to 15% TL (35-135 mm TL), respectively (Fig. 2.1).

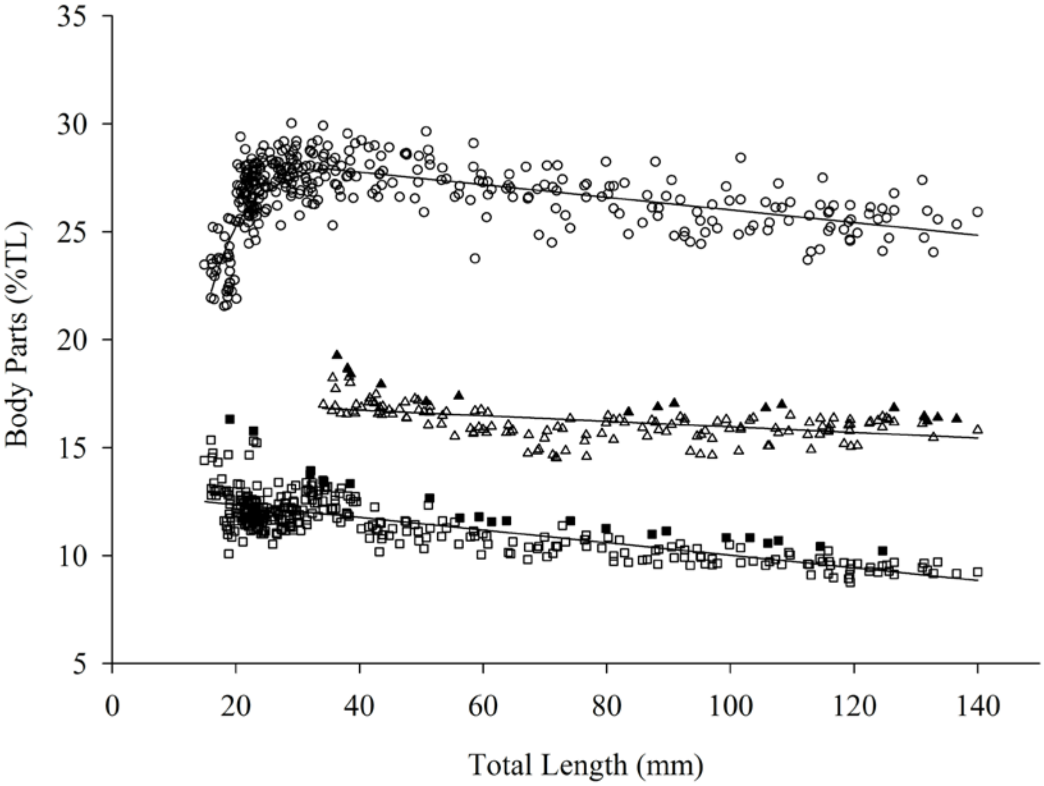

Fig. 2.1. Morphological variation between relative body parts (%TL) and total length (TL, mm) of juvenile barramundi. Body depth (BD in circles, *n* = 368), closed mouth width (MWc in squares, *n* = 360) and opened mouth width (MWo in triangles, *n* = 154) are plotted against total length. Each symbol represents an individual estimate. Filled symbols represent maximum values of mouth width estimates.

The relationship between absolute body depth (BD), mouth width (MW) estimates and total length (TL) fitted on linear regression equations (Table 2.1). ANCOVA analyses showed significant differences between the regression slopes of the body parts ($df = 4$, $F = 4.988$, $P < 0.0001$), suggesting that absolute body depth increases faster than mouth width. The significant differences between the regression slopes of the mouth width estimates were due to the increase in the mouth width extension (MWE) as fish grew larger (Fig. 2.2).

A marked inter-individual variability was observed for all morphological variables. Estimates for both opened and closed mouth widths presented a consistent variability during the juvenile phase (Fig. 2.1). Inter-individual variability was also observed for MWE, varying consistently at about 30% (±15%) for the whole range of fish size (Fig. 2.2). The positive correlation between MWc and MWo ($r = 0.505$, $n = 153$, $P < 0.0001$; Table 2.1) and the non-significant correlation between MWo and MWE ($r = 0.085$, $n = 153$, $P = 0.294$; Table 2.1) indicated that the MWo was more affected by the MWc than by the MWE. In contrast, the negative correlation between MWc and MWE ($r = -0.811$, $n =153$, $P < 0.0001$; Table 2.1) indicated that the highest MWE (Fig. 2.2) were associated with the smallest MWc (Table 2.1). Therefore, the maximum values of MWc and MWo were used to develop specific models to reflect polyphenism in mouth width.

Table 2.1. Relationship of morphological parts of barramundi (15-140 mm TL).

Relationships	**Equations**	r^2	***df***	***F***	***p* intercept**	***p* slope**
Absolute measures (mm)						
MWc *vs* TL	MWc = 0.091 (0.001)^{c} TL + 0.850 (0.034)	0.986	359	25,181	<0.0001	<0.0001
MWcmax *vs* TL	MWcmax = 0.093 (0.001)^{c} TL + 1.508 (0.071)	0.998	21	9,620	<0.0001	<0.0001
MWo *vs* TL	MWo = 0.155 (0.001)^{b} TL + 0.462 (0.117)	0.988	153	12,873	<0.0001	<0.0001
MWomax *vs* TL	MWomax = 0.157 (0.001)^{b} TL + 1.067 (0.134)	0.999	16	11.708	<0.0001	<0.0001
BD *vs* TL	BD = 0.255 (0.001)^{a} TL + 0.483 (0.063)	0.994	367	57,299	<0.0001	<0.0001
Relative measures (% body parts)						
MWc *vs* MWE	MWE = -10.355 (0.608) MWc + 163.997 (6.443)	0.657	152	284.86	<0.0001	<0.0001
MWo *vs* MWE	MWE = 1.259 (1.196) MWo +34.324 (19.439)	0.007	152	1.109	0.079	0.294
MWc *vs* MWo	MWc = 0.583 (0.081) MWo + 1.079 (1.319)	0.255	152	51.711	0.415	<0.0001

Absolute measures (mm). MWc: closed mouth width; MWcmax: maximum closed mouth width; MWo: opened mouth width; MWomax: maximum opened mouth width; BD: body depth; TL: total length;

Relative measures (% body parts). MWc: closed mouth width (% TL); MWo: opened mouth width (% TL): MWE: mouth width extension (% MWc).

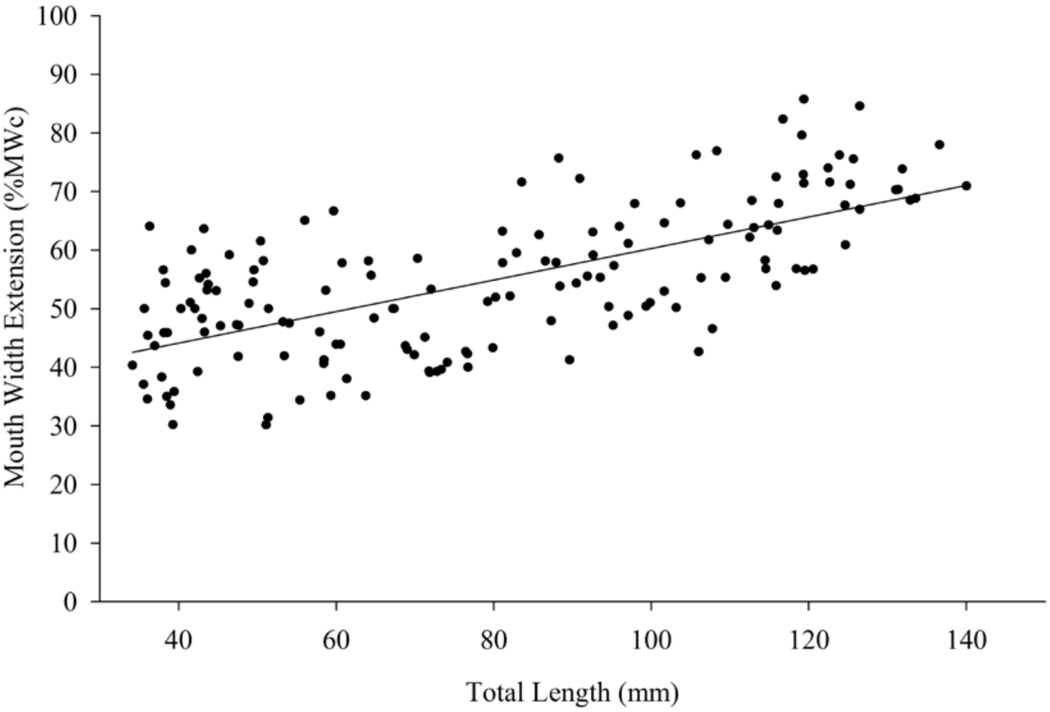

Fig. 2.2. Relationship between mouth width extension (MWE, % MWc) and total length (TL, mm) of juvenile barramundi. MWE and TL are positively correlated (r = 0.660, n = 154, P < 0.0001). The solid line represents the average MWE in fish varying from 34 to 140 mm TL and is expressed as MWE = 0.269 (0.024) TL + 33.328 (2.083) (r^2 = 0.446, df = 152, F = 121, P < 0.0001).

Assuming that a $TL_{cannibal}$ could swallow a TL_{prey} if the BD_{prey} was equal to or smaller than the $MW_{cannibal}$, the maximum conspecific prey size for cannibalistic barramundi was predicted by simple linear regression (Table 2.2). All models predicted that the maximum prey TL increased with increasing cannibal TL (Fig. 2.3A). However, when expressed as a proportion of cannibal TL, the models showed a slightly declining trend in the size of maximum prey as cannibal TL increased (Fig. 2.3B). The closed mouth width (MWc) model predicted that the maximum prey size decreased from 40 to 37% or from 50 to 39 % of cannibal TL considering the maximum values (MWcmax), for cannibals of 30-140 mm TL. The maximum prey size remained constant at 61% of the cannibal TL when the model was based on the opened mouth width (MWo). However, when considering maximum opened mouth width (MWomax) the model predicted a decreasing trend from 68 to 63% of cannibal TL, for a similar size range of cannibals. Such decreasing tendencies as cannibals grew larger were related to the slightly fast increase in body depth comparing with the mouth width (Table 2.1).

Table 2.2. Prediction of prey size (TL_{prey}, mm) from cannibal size ($TL_{cannibal}$, mm) based on different predictive model equations for cannibalistic barramundi (30-140 mm TL).

Models	Equations
MWc	$TL_{prey} = 0.3569\ TL_{cannibal} + 1.4392$
MWcmax	$TL_{prey} = 0.3647\ TL_{cannibal} + 4.0196$
MWo	$TL_{prey} = 0.6078\ TL_{cannibal} - 0.0824$
MWomax	$TL_{prey} = 0.6157\ TL_{cannibal} + 2.2902$
Revised	$TL_{prey} = 0.7090\ TL_{cannibal} + 1.8881$

MWc: closed mouth width; MWcmax: maximum closed mouth width; MWo: opened mouth width; MWomax: maximum opened mouth width.

2.4.2. Cannibal Challenge

In total, 495 prey-cannibal pairs were tested using 102 potential cannibals from 25 to 131 mm TL. There was no substantial variation of the prey size during the pairing periods. In those cases when predation did not occur, the final prey size was -0.50 - 1.40% of the initial prey size as the percent of cannibal TL (*n* = 55). Of all potential cannibals challenged, 75% became true cannibals ingesting at least one conspecific prey. These cannibals consumed 61.6% of the total number of prey while dead prey on the bottom accounted for 20.2% of prey mortality. Four cases of suffocation were observed during the trials where cannibals died with the prey stuck in mouth. In addition, three half-ingestion events were observed in this study, where the cannibals predigested half of the prey and discarded the other half. Interestingly, in all these cannibalistic events, prey sizes were 65% of cannibal TL. When the prey size was firstly offered at 58.36 ± 5.37% cannibal TL, the 25% of the large fish tested did not become cannibals, but the other 75% of the large fish became cannibals when the prey size firstly offered was 50.77 ± 2.57% cannibal TL (unpaired t-test; *df* = 100; $P < 0.0001$).

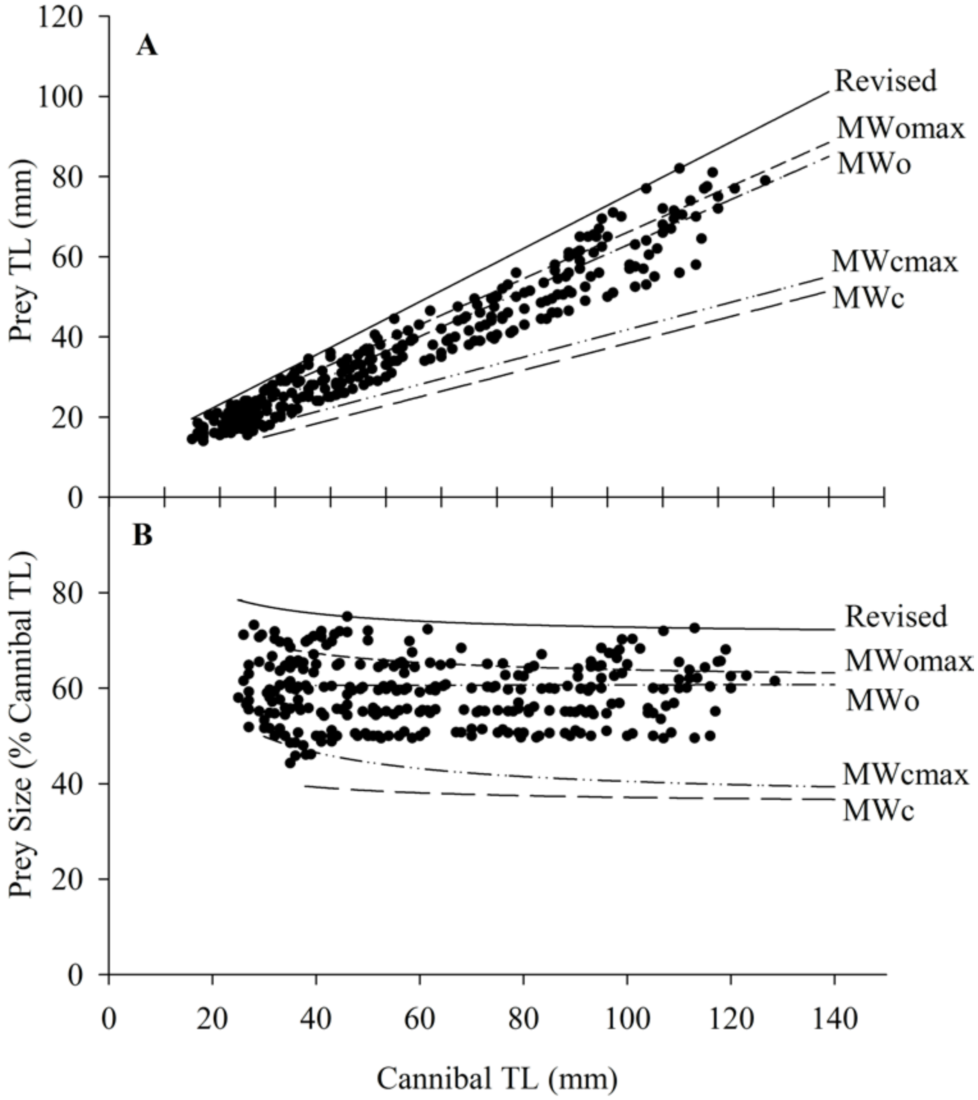

Fig. 2.3. Maximum conspecific prey size for barramundi cannibals. Prey size in panel A is expressed as prey TL, mm, and in panel B expressed as % of cannibal TL. Regression lines include (1) the maximum size of prey ingested (“Revised” in filled circles on the top) and (2) model predictions on prey size based on closed mouth width (MWc), maximum closed mouth width (MWcmax), opened mouth width (MWo), and maximum opened mouth width (MWomax).

The results of the cannibal challenged with prey showed that cannibals were able to ingest a conspecific prey larger than the size that all models could predict (Fig. 2.3A, B). For instance, according to the models based on MWc, MWcmax, MWo or MWomax, a cannibal of 106.50 mm TL could ingest a prey of 39, 43, 65 or 68 mm TL (37, 40, 61 or 64% of cannibal TL), respectively. Results from the cannibal challenge trial showed that identical sized cannibals could ingest a conspecific prey of 77 mm TL (72% of cannibal TL). Thus, according to empirical observations, cannibals of 25-131 mm TL could ingest the prey of 78-72% of cannibal TL, respectively. Such reduction in the maximum prey size is a result of a faster growth of the body depth in relation to the mouth size (Table 2.1). The increase in mouth width extension as fish grew larger (Fig. 2.2) would compensate the part of negative allometric growth of the mouth width.

2.5. Discussion

A model by Parazo et al. (1991) predicted that cannibalistic barramundi of 10-50 mm TL (total length) can ingest a maximum conspecific prey size of 67-61% of cannibal TL, respectively. However, the empirical results in the present study showed that barramundi cannibals (25 - 131 mm TL) could ingest conspecific prey of 78-72% of cannibal TL, respectively. All predictive models using morphological traits considering the alternative assumption of cannibalistic polyphenism underestimate the maximum prey size that a cannibal can possibly ingest.

All successfully cannibalistic events in the present study were orientated by head being sucked in first and cannibals ingesting the whole prey. Moreover, cannibalistic barramundi ingested their prey horizontally, making the size of mouth width become the limiting factor for prey ingestion. Thus, using the closed mouth width (MWc) as an independent factor, the predictive model shows a maximum prey size of 40-37% cannibal TL, for the cannibals of 30-140 mm TL, respectively. Alternatively, when the model was developed with the opened mouth width (MWo), it predicts that a cannibal can ingest a maximum prey of 61% of the cannibal TL. Our model prediction is in accordance with that by Parazo et al. (1991) who predicted a maximum prey size of 67-61% of cannibal TL, when cannibals were 10-50 mm TL, respectively, based on mouth size as the distance from the dorsal to the ventral boundary of the mouth opened. Whatever the case was, when predictive models were compared with the empirical results in this study, the models underestimate the maximum conspecific prey size that cannibals can ingest. Similar conclusions were drawn by Qin and Fast (1996) on snakehead *Channa striatus* and Johnson and Post (1996) on largemouth bass *Micropterus salmoides* when their predictive models were confronted with empirical data.

In the present study, despite the inter-individual variability on mouth width and in the capacity of mouth width extension, the models based on maximum values of the closed (MWcmax) and opened mouth widths (MWomax) underestimate the maximum ingestible capacity of cannibalistic individuals. Nevertheless, the model using MWomax predicts a slightly higher cannibalistic capacity than the model using MWo, which is closer to the empirical observation. The high inter-individual variability on MWE indicates marked polyphenism in the mouth extension capacity. However, as a negative correlation was detected between MWE and MWc, greater mouth extension capacities seemingly compensate for the disadvantage of having smaller mouth dimensions. As a result, the polyphenic trait of the MWo, which obviously represents the maximum predation capacity of cannibalistic barramundi, is rather a result of larger mouth width than higher mouth extension capacity. The polyphenic trait of a mouth provides not only a cannibalistic advantage, but a feeding advantage on other food. Thus, polyphenism should be considered when assessing feeding ecology of piscivorous fish species in general.

Allometric growth of the mouth is common in fish species and together with size heterogeneity it can determine the dynamics of complete cannibalism in fish (Otterå and Folkvord, 1993, Baras and Jobling, 2002). Previous observations on barramundi feeding showed that the onset of complete cannibalism mainly occurs after metamorphosis, when fish are being weaned to inert diets (Kestemont et al., 2003, Baras and Jobling, 2002). In the present study, mouth width showed slower growth than the body depth. As both variables set the morphological boundary for complete cannibalism, both predictive and revised models show a decreasing trend on the maximum ingestible prey size as barramundi grow larger. As a result, cannibalism in barramundi is more likely to occur in early juvenile than during latter stages (Baras, 2013), which agrees with the findings on cannibalism by Otterå and Folkvord (1993) for *G. morhua*, and Qin and Fast (1996) for *C. striatus*.

Morphological constraints are not the only cause of a general reduction trend on cannibalism rate as fish grow larger. Cannibalistic fish usually prefer smaller prey as reported in *P. djambal* (Baras et al., 2010) and *Pseudoplatystoma punctifer* (Baras et al., 2011). Thus, once smaller prey have succumbed to cannibalism, reducing the size heterogeneity of the population (Baras and Jobling, 2002), cannibals are forced to move up to larger prey, which may not be energetically profitable as preying on smaller prey since such a size shift may represent an increase in pursuit and handling time and reduce energy gain per capture attempt (Gill, 2003, Juanes, 1994, Scharf et al., 1998, Juanes and Conover, 1994, Ellis and Gibson, 1997). In aquaculture where plenty of inert food of high energetic content is available, cannibals may choose to abandon a cannibalistic diet because such diet is not profitable anymore. In contrast, if cannibalistic individuals do enjoy growth advantages over siblings feeding on alternative diets, as observed in the Amazonian catfish *Pseudoplatystoma punctifer* (Baras et al., 2011), cannibalism will hardly become to an end as the higher growth rate of cannibals may compensate the morphological constraints as fish get larger. On the other hand, if alternative inert food is supplied accordingly, non-cannibalistic individuals may achieve more competitive growth rates (Baras et al., 2011) and they can grow beyond the prey spectrum of the cannibals (Baras and Jobling, 2002). Further studies should assess the dynamics of intracohort cannibalism in barramundi when alternative inert diet is applied at different developmental stages.

The cannibal challenge experiment was purposely designed in a small scale aiming to maximise the propensity of cannibalism. Small enclosures were used to limit escape ability of small prey and large cannibals were individually stocked, previously acclimated and deprived of alternative food, which is similar to the designs by Sogard and Olla (1994) Johnson and Post (1996), Hseu et al. (2004) and Baras et al. (2010). In this experiment, 75% of the prey available to cannibals were ingested proving that the environment was appropriate for the exercise of cannibalism. Furthermore, the pairing period was defined as four days, a similar period used by Qin and Fast (1996), which was assumed to be short enough to avoid significant behavioural and physiological changes in cannibals and prey, but long enough to promote maximum hunger for cannibals. In addition, cannibalistic events were orientated towards the same prey size (% cannibal TL) offered since prey and cannibals presented similar growth rates during the course of the 4-day paring period. Interestingly, barramundi could become cannibals when the first prey sizes were <50% of their predator body length, but the fish could never become a cannibal when the first prey was >58% of the cannibal size. This may indicate that, once all cannibals are removed from a barramundi population, the size difference of 50% can be a safe margin to avoid the emergence of new cannibals. Furthermore, once a fish had experienced as a cannibal, this fish would use the full morphological capacity to ingest a prey, even though the size of prey may exceed the model prediction. Challenging cannibals with an increasing prey size in the absence of alternative food may have stimulated the phenotypic plasticity in the mouth apparatus, such as hypertrophied jaw musculature (Smith and Skulason, 1996, Polis, 1981) resulting in greater predation capacity when compared with the predictive models based on fish samples taken from a fish population where food was present and cannibalism was not stimulated.

Unsuccessful cannibalistic events, such as suffocation and half-ingested prey were recorded during the cannibal challenge experiment. Previous studies have used suffocation events as a reference of the maximum prey size limit for cannibals (Sakakura and Tsukamoto, 1996, Ebisu and Tachiara, 1993). In the present study, unsuccessful cannibalistic events occurred when the prey size was 65% of cannibal TL, which is below the upper size limit determined by the revised model. However, when compared with the predictive model based on the opened mouth width (MWo), prey sizes were slightly larger than the model predicted. Therefore, it seems that those unsuccessful cannibalistic events were performed by hunger-motivated individuals to cannibalize a larger prey they could possibly handle. In addition, dead prey were occasionally observed on the bottom of the tank. In most of these cases, cannibals resumed predation when a live prey of similar size was re-offered, suggesting that such event did not represent the maximum prey size they can ingest and they are probably associated with cannibal’s difficulties to handle the prey or prey’s abilities to escape from predation acts. Whatever the case, all these events can account for fingerling mortality leading to significant losses in fingerling production.

In summary, this study provides a new approach to predict cannibalistic events in fish under an aquaculture situation and offers recommendation on criteria for size grading practices. In order to reduce the incidence of intracohort cannibalism in a barramundi population, no conspecific prey smaller than 78-72 % of cannibal TL should co-inhabit with cannibals from 30 to 140 mm TL, respectively. Furthermore, once all cannibals were removed from the population through size sorting, a size difference of 50% should be set as a threshold to avoid the emergence of new cannibals. Predictive models based on mouth width and body depth of a population average underestimate the maximum prey size for cannibalistic barramundi. However, when polyphenism was considered on measuring of the opened mouth width, the model became closer to the reality, suggesting that when predicting the upper prey size limit for complete cannibalism, the assumption of cannibalistic polyphenism must be considered to keep a safe margin and avoid significant losses due to cannibalistic mortality.

Some unsettled issues still exist. It is still unclear whether the fish with polyphenic mouth size are always consuming the largest prey and it is also uncertain whether these cannibals make the most of their prey’s energy. Presumably, a large mouth facilitates handling and increases capture success, but energetic benefit of being a cannibal needs further investigation. In aquaculture, we recommend putting aside those fish that are cannibals because they could have broader mouth dimensions than others, and if this trait is heritable, it can complicate rearing in the future.

2.6. Acknowledgements

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Chapter 3

Prey Size Selection and Cannibalistic Behaviour of Juvenile Barramundi

This chapter has been accepted for publication by the Journal of Fish Biology after minor revision as:

Ribeiro FF, Qin JG. Prey size selection and cannibalistic behaviour of juvenile barramundi *Lates calcarifer*. Journal of Fish Biology (accepted after minor revision on 3/8/2014).

3.1. Abstract

This study assessed the cannibalistic behaviour of juvenile barramundi *Lates calcarifer* and examined the relationship between prey size selection and energy gain of cannibals. Prey handling time and capture success by cannibals were used to estimate the ratio of energy gain to energy cost in prey selection. Cannibals selected smaller prey despite its capability of ingesting larger prey individuals. In behavioural analysis, prey handling time significantly increased with prey size, but it was not significantly affected by cannibal size. Conversely, capture success significantly decreased with the increase of both prey and cannibal sizes. The profitability indices showed that the smaller prey provides the most energy return for cannibals of all size classes. These results indicate that *L. calcarifer* cannibals select smaller prey for more profitable return. However, the behavioural analysis indicates that *L. calcarifer* cannibals attack prey of all size at a similar rate but ingest smaller prey more often, suggesting that prey size selection is passively orientated rather than at the predator's choice. The increase of prey escape ability and morphological constraint contribute to the reduction of intracohort cannibalism as fish grow larger. This study contributes to the understanding of intracohort cannibalism and development of strategies to reduce fish cannibalistic mortalities.

Keywords: Piscivory, Optimal foraging theory, Cannibal size, Prey size, Profitability, Passive prey selection

3.2. Introduction

Cannibalism or intraspecific predation is defined as a process of killing and consuming the whole or the major part of an individual of the same species (Fox, 1975, Polis, 1981, Smith and Reay, 1991, Elgar and Crespi, 1992, Hecht and Pienaar, 1993, Baras and Jobling, 2002). Initially considered a laboratory artefact (Colinvaux, 1973), cannibalism is now regarded as a common phenomenon in nature and has been observed in many groups of animals, from protozoa to mammals, living in different habitats sharing a variety of social and feeding habits (Fox, 1975, Polis, 1981). In fish, cannibalism has been confirmed in 36 of 410 families of teleost fish, but its occurrence is suggested to be even more widely spread (Smith and Reay, 1991). In captivity, intracohort cannibalism exists in most piscivorous finfish, especially during the juvenile stage, when food competition, growth and size heterogeneity are exacerbated (Hecht and Pienaar, 1993, Baras and Jobling, 2002). Besides, fish in captivity have little or no chance of escaping predation via habitat segregation (Baras and Jobling, 2002). As a result, intracohort cannibalism may cause high commercial losses (Parazo et al., 1991, Smith and Reay, 1991, Hecht and Pienaar, 1993, Ruzzante, 1994, Hseu et al., 2007).

Several fish species are highly cannibalistic at the early larval stage because larvae grow with large mouth dimensions that differ from the adult phenotypes, as recently reviewed by Baras (2013). At this stage, cannibalism does not require substantial prey-cannibal size difference, and normally the prey is captured tail-first and incompletely ingested and digested by the cannibal (Baras and Jobling, 2002, Baras, 2013). With the increase in size variation through the exercise of incomplete cannibalism among other factors such as food competition, incomplete cannibalism is replaced by complete cannibalism, *i.e*. larger individuals ingest the conspecific prey whole with the head being sucked in first (Baras and Jobling, 2002). The switch between types of cannibalism usually coincides with the metamorphosis stage from larvae to juvenile, when individuals are weaned to formulated diets, enhancing food competition, size heterogeneity and consequently the incidence of complete cannibalism (Kestemont et al., 2003, Baras and Jobling, 2002). Once complete cannibalism starts, it becomes permanent during the juvenile stage, as size heterogeneity is both the cause and the consequence of cannibalism (Kestemont et al., 2003, Hecht and Pienaar, 1993, Baras and Jobling, 2002). Therefore, a comprehensive understanding of the dynamics of the event in a captive population is crucial when designing the method for cannibalism control. Several studies have used models and/or empirical data to estimate the maximum prey size that cannibals can ingest (Baras and Jobling, 2002). However, as observed in many piscivorous fish (Juanes, 1994), cannibals may prefer to eat prey that are much smaller than the largest prey size they can ingest.

The optimal foraging theory (OFT) has been used to predict diet selection of predators (Stephens and Krebs, 1986) based on the concept of profitability maximization, *i.e.*, predators select prey that give the higher energy gain per unit effort (Stephens and Krebs, 1986, Gill, 2003, Sih and Moore, 1990, Mittelbach, 2002). While the energy value of a prey is a straightforward estimation, the assessment on the cost of predation may be subjective and dependent on factors which are ultimately related to both predator and prey behaviour (Sih and Christensen, 2001). The OFT makes feeding predictions based on active predator choice, *i.e.*, the predator’s behaviour. As a result, although the OFT is generally applicable for planktivores, it does not necessarily predict optimal foraging for piscivorous fish mainly due to the mobile nature of fish prey (Sih and Moore, 1990).

Consequently, the OFT normally overestimates the preferable prey size in piscivorous fish (Sih and Christensen, 2001) as the predation components associated with prey behaviour are not considered. Recent studies have been intensively focused on whether prey size selection in piscivorous fish is a result of active predator choice or simply a result of passive prey selection where predators ingest the most vulnerable prey leading to predator choice for weak individuals (Sih and Christensen, 2001). For instance, Juanes and Conover (1994) found strong selection for smaller Atlantic silversides *Menidia menidia* (L. 1766) by the bluefish *Pomatomus saltatrix* (L. 1766). Further, they claimed that predatory bluefish attack all prey size at similar rates but ingest the smaller prey more often, suggesting that the observed preference is simply a reflection of size-dependent capture success or passive prey selection for smaller prey. Conversely, Turesson et al. (2002) suggested that the selection for smaller and most profitable fish prey by the piscivorous pike-perch *Sander lucioperca* (L. 1758) is a result of active predator choice. Corroborating with most of piscivorous fish species, cannibalistic species usually show strong selection for small conspecific prey, as observed in *Pangasius djambal* Bleeker 1846 (Baras et al., 2010), Arctic char *Salvelinus alpinus* (L. 1758) (Amundsen et al., 1995) and Amazonian catfish *Pseudoplatystoma punctifer* (Castelnau 1855) (Baras et al., 2011). However, these studies only addressed prey size selection and did not further investigate the nature of the predator choice.

Barramundi *Lates calcarifer* (Bloch 1790) is a carnivorous fish and has important economic value for fisheries and aquaculture in tropical and subtropical regions. Cannibalism could be a partial way for *L. calcarifer* to obtain food in natural populations (Davis, 1985), but in aquaculture, like other piscivorous fishes, intracohort cannibalism is strong at the juvenile stage, making size grading crucial for cannibalism reduction (Schipp et al., 2007). A previous study shows that *L. calcarifer* of 25 – 131 mm total length (L_T) are able to ingest sibling conspecifics of 78 – 72% of their body size (Ribeiro and Qin, 2013). However, it is unclear whether the selection for maximal prey size is more energetically profitable than the selection for smaller prey. Therefore, the aims of this study were to (1) investigate size-dependent prey selection of cannibals; (2) estimate the cost of prey capture using behavioural analysis; (3) determine the profitability of fish exercising cannibalism; and (4) address the active or passive nature of prey size selection in exercising cannibalism. The outcomes of the present study will provide insight into our understanding on fish cannibalism and prey-predator relationships in piscivorous fish.

3.3. Material and Methods

3.3.1. Animals and Holding Conditions

A total of 2500 hatchery produced *L. calcarifer* of 40 days old from the same cohort were obtained from West Beach Hatchery, South Australia, and transported to the Animal House, Flinders University. Upon arrival, the largest 300 fish were visually graded and stocked into a 300 l holding tank. The remaining 2200 fish were maintained in two groups of similar number of fish and stocked into two other 300 l tanks. Each tank was filled with freshwater, equipped with an external biofilter and individual heaters and maintained at 28.1 ± 0.3 °C. Pelleted feed (NRD$^{®}$ range, 0.4-2.0 mm; 55% protein, 9% lipid, INVE Aquaculture Ltd., Thailand) was offered to all fish. In order to create size differences among groups, the largest fish were fed to apparent satiation whereas others were fed restricted rations prior to the experiments. After the experiments had begun, all fish were fed twice a day to apparent satiation in the three holding tanks. Tanks were cleaned twice a day to remove uneaten pellets, faeces and dead fish. Water quality was maintained at 5.9 ± 0.9 mg l^{-1} dissolved oxygen (mean ± SD), 7.5 ± 0.2 pH, and <0.5 mg l^{-1} ammonia or nitrite nitrogen. A photoperiod of 12L:12D was used at a light intensity of 350 lux during the hours of light and zero lux during the hours of dark.

3.3.2. Experimental Designs

Three experiments were conducted using cannibalistic *L. calcarifer* of 40 – 140 mm total length (L_T). In experiment 1, the prey size selection of five cannibal size classes was investigated over five consecutive days with eight replicates per cannibal size class (Table 3.1). In experiment 2, a behavioural-energy analysis was used to assess the prey and cannibal size-dependent profitability. The prey handling time and capture success were used to measure predation costs and the prey energy body content was used to measure predation gain. Five cannibal size classes and seven prey size classes with three replicates were used in this experiment (Fig. 3.1). Lastly, in experiment 3, a behavioural analysis was used to differentiate the active versus passive nature of cannibalism on prey size selection. The attack rate and capture success of five cannibal size classes were examined in nine replicates for each cannibal size class and the prey of three sizes (small, medium and large) were simultaneously offered to each cannibal during the experiment (Fig. 3.1).

3.3.2.1. Prey Size Selection

Experiment 1 started when substantial difference of fish size was established. All cannibals in the five size classes (41.4 ± 2.5, 63.9 ± 4.0, 87.3 ± 4.0, 105.5 ± 3.2, 125.4 ± 2.2 mm L_T; mean ± SD; Table 3.1) were obtained from the largest fish tank to test the size-dependent prey selection. In each cannibal size class, putative cannibals were measured for L_T (1.0 mm) and eight cannibals of similar size were selected and individually stocked into separate 20 l aquaria (30 × 30 × 25 cm), which were connected to a communal 200 l biofilter to stabilise water quality. Subsequently, potential prey individuals were obtained from the other two tanks holding the smaller fish. The prey fish were measured for L_T (mm) and divided into eight groups of heterogeneous body length. Then, each prey group was correspondingly offered to one of the eight cannibals. Each prey group consisted of 20 – 28 prey and all were within 28 – 68% of cannibal L_T (Table 3.1). No pelleted feed was provided to the fish once the predator-prey pairs started.

The following criteria proposed by Baras et al. (2010) on the number and size of prey used for fish size selection were adopted in this study, *i.e.*, (1) prey size distributions were broad enough to allow cannibals to select prey of different sizes and tight enough to avoid ingestion between prey; (2) prey fish size and number were selected to avoid cannibal ingestion outside of their preferred size range and to avoid low prey density affecting the cannibal feeding behaviour; and (3) the range of prey size was within the upper gape limit of each cannibal (Ribeiro and Qin, 2013). In this study, 20 – 28 prey individuals were offered to each cannibal to meet the above criteria and the lower prey density was still much over the daily ration of a cannibal.

Cannibals were studied over five consecutive days, while prey groups were daily renewed because the small fish might not be suitable as prey due to a day-long starvation or gained experience to escape from the cannibal’s attack. Both the cannibal and surviving prey were daily removed and the aquaria were cleaned. Each cannibal was measured for L_T (mm) and restocked into the same aquarium after cleaning. New batches of pre-measured prey (20 – 28 individuals per batch) were paired up with each cannibal. Surviving prey in each day were counted and measured for L_T (mm) before returning to the holding tanks. Before reintroducing the surviving prey into the holding tanks, they were kept in 10 l floating buckets in the holding tanks and fed with pelleted diet for one day for recovery. This procedure avoided reusing prey in the following day. The same protocol was used for all five size classes of cannibals, which were examined sequentially for prey selection (Table 3.1). After finishing with a cannibal size class, cannibals were euthanized and never tested again as a subsequent cannibal size class. Water quality and physical parameters were kept the same as those as in the holding tanks.

Each ingested individual prey was identified by comparisons between the final and initial prey L_T (mm) distribution where the missing fish in the final distribution were considered the prey succumbed to cannibalism. Normalised preference index was used to assess the prey size selection of cannibals (Baras et al., 2010, Baras, 1999). Cannibals were grouped into five size classes and the prey size was expressed as percentage of the cannibal L_T. Prey size was categorized into classes by 2.0% increment of cannibal L_T. For each cannibal size class, preference indices (I_P) for each prey size class were calculated as the number of prey consumed (N_C) in relation to the number of prey available (N_A) in that size class ($I_P = N_C N_A^{-1}$). The preference index was normalised with the highest value fixed at 1.0 to enable direct comparisons between cannibals in different size classes.

3.3.2.2. Cannibalistic Behaviour

In experiment 2, five cannibal size classes were tested, including class 1 (45.5 ± 1.4 mm L_T, mean ± SD), class 2 (65.1 ± 1.0 mm L_T), class 3 (88.2 ± 1.0 mm L_T), class 4 (110.9 ± 1.6 mm L_T) and class 5 (134.0 ± 2.4 mm L_T) (Fig. 3.1, Table 3.2). Each cannibal class was tested against seven prey size classes in triplicate, with one cannibal and nine prey individuals of similar size in each aquarium. These seven prey classes included (1) 30.0 – 35.0%, (2) 35.1 – 40.0%, (3) 40.1 – 45.0%, (4) 45.1 – 50.0%, (5) 50.1 – 55.0%, (6) 55.1 – 60.0%, and (7) 60.1 – 65.0 % of cannibal L_T (Fig. 3.1). In each cannibal size class, two days prior to the trial, 21 cannibals were selected from the tanks holding the largest fish, measured for L_T and individually stocked into twenty one 10 l aquaria (20 × 20 × 25 cm). These cannibals were acclimated for two days without feeding for habitat adaptation and hunger standardisation. Subsequently, all prey individuals were collected from the tanks holding the smaller fish and divided into seven prey size classes. In each prey size class, a single putative cannibal co-inhabited with nine prey individuals of similar size in a single aquarium in triplicate (Fig. 3.1). Soon after all prey had been released to the aquarium with one cannibal, a video camera recorded the cannibalistic behaviour for at least 30 min on each of the 21 aquaria containing one cannibal and nine prey. After video recording, the cannibal and the surviving prey were collected and returned to the respective holding tanks. All combinations of cannibal size classes (1-5) and prey size classes (1-7) were tested using the same design (Fig. 3.1). Cannibal size classes were sequentially tested, but alternated with experiment 1. Due to the relative short-time pairing period and the 5-day intervals (at least) between testing each cannibal size class (*i.e*. learning skills from both cannibal and prey were considered negligible), both cannibals and surviving prey were considered for retesting in the subsequent size class trials. Water quality and physical parameters were kept the same as those as in the holding tanks.

In experiment 3, five cannibal size classes, similar to those in experiment 2, were tested (Fig. 3.1, Table 3.3). Each cannibal size class was tested against a mix of three prey sizes including small (34.0 – 44.0% cannibal L_T), medium (44.1 – 53.0%

cannibal L_T) and large prey (60.0 – 65% cannibal L_T, Fig. 3.1, Table 3.3). In each cannibal size class, one cannibal co-inhabited with nine prey individuals with three prey from each size (small, medium and large), in each aquarium with nine replicates (Fig. 3.1). The nine cannibals were selected from the tank holding the largest fish, individually stocked into nine 10 l aquaria and acclimated for two days without feeding. Subsequently, prey individuals were collected from the tanks holding the smaller fish and divided into similar groups of mixed prey sizes for co-habitation with the putative cannibal. Soon after the mixed sized groups of nine prey were introduced to each aquarium with a single cannibal, a video camera recorded the cannibalistic behaviour for 30 min. After video recording, the cannibal and the surviving prey were collected and returned to the respective holding tanks. Cannibals from all five classes were examined using the same protocol. Cannibal size classes were sequentially tested, in parallel with experiment 2. The reason why the number of replicates increased to nine in this experiment was due to the low propensity of cannibalistic events in this test regime.

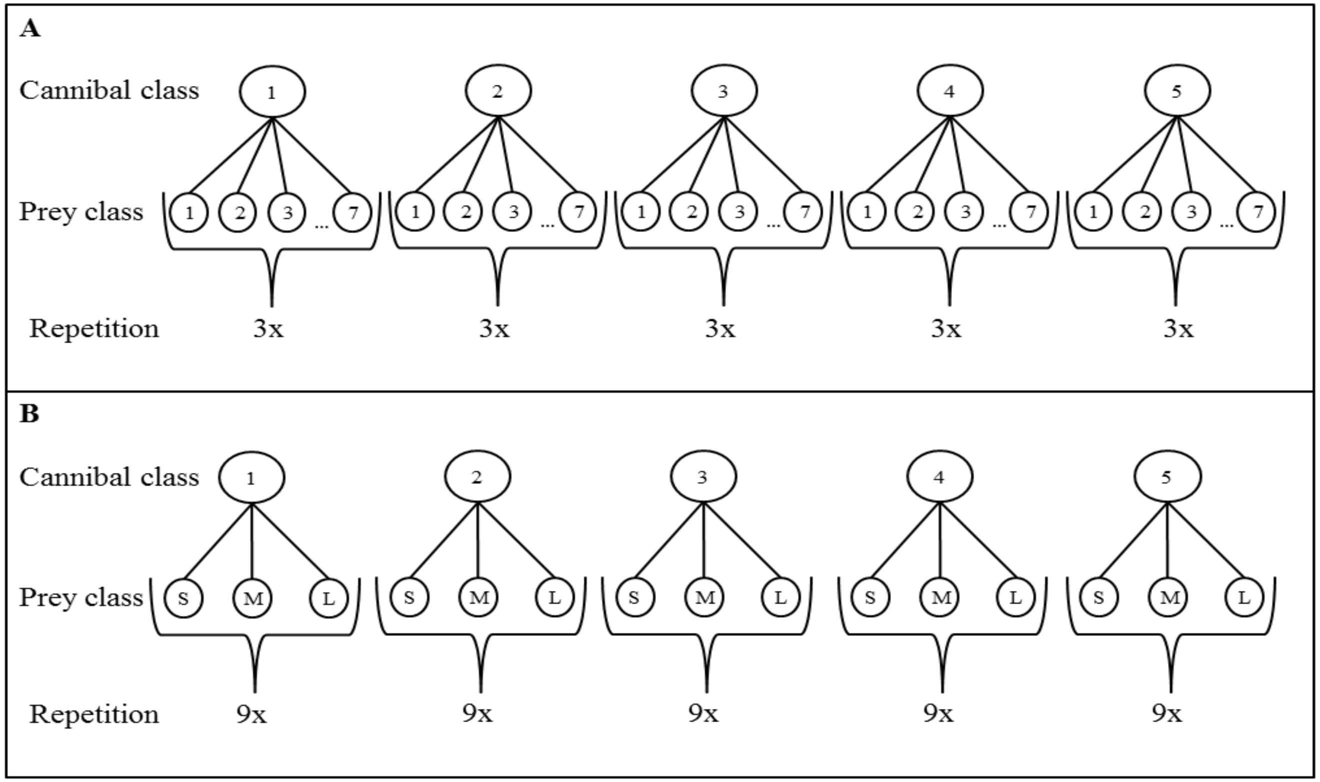

Fig. 3.1. Experimental design. Panel A shows the design of experiment 2. Five cannibal size classes include (1) 45.5 ± 1.4; (2) 65.1 ± 1.0; (3) 88.2 ± 1.0; (4) 110.9 ± 1.6; (5) 134.0 ± 2.4 mm total length (L_T; mean ± SD). Seven prey size classes include: (1) 30.0 – 35.0; (2) 35.1 – 40.0; (3) 40.1 – 45.0; (4) 45.1 – 50.0; (5) 50.1 – 55.0; (6) 55.1 – 60.0; (7) 60.1 – 65.0% cannibal L_T. Panel B shows the design of experiment 3. Five cannibal size classes include (1) 45.7 ± 0.9; (2) 64.8 ± 1.8; (3) 88.9 ± 0.8; (4) 111.4 ± 1.6; (5) 133.6 ± 2.7 mm L_T. Three prey size classes are a mix of small (34.0 – 44.0% cannibal L_T), medium (44.1 – 53.0% cannibal L_T) and large (60.0 – 65% cannibal L_T) prey.

The following behavioural parameters were analysed from the video recordings: (1) Attack rate (R_A, n) as the frequency of attacks observed for each cannibal tested; (2) capture success (S_C, proportion of attacks) as the proportion of cannibalistic attacks that result in successful ingestion; and (3) handling time (T_H, min) as the time between the capture and successful ingestion. Only the situations ending in successful ingestion were included in data analysis. Once a successful ingestion was detected, the observation was ended and no other cannibalistic behaviour analysis was included for that replicate. While the three behavioural parameters were analysed from the footages in experiment 2, only the first two parameters were analysed in experiment 3 where the prey size (i.e. small, medium and large) was also recorded. Handling time was not assessed in experiment 3 because this behavioural parameter was not a component in the analysis of active or passive nature of prey size selection. In experiment 2, profitability 1 was estimated from the relative prey gross energy content as energy intake and the handling time (min) as predation cost, *i.e.* Profitability 1 (% cannibal's energy content per min) = $(E_P E_C^{-1}) T_H^{-1}$. Profitability 2 was estimated by incorporating the unsuccessful capture attempts as an additional predation cost, *i.e.*, Profitability 2 (% cannibal's energy content per min) = $[(E_P E_C^{-1}) T_H^{-1}] S_C$. E_P and E_C are the energy contents (J) of the prey and cannibal, respectively in both profitability equations. The fish energy content (E_F, J) was inferred from an independent data set ($E_F = 0.0429 L_T^{3.1053}$, $R^2 = 0.9985$, $n = 23$, L_T range = 20 – 140 mm) obtained from fish sampled from a population kept in the same condition as those fish in the holding tanks. Fish energy content was measured with the combustion calorimeter system C2000 basic (IKA, Germany).

3.3.3. Statistical Analyses

In experiment 1, chi-square (x^2) test for goodness of fit was used to test the null hypothesis of random prey size selection for each cannibal size class. The average values of daily prey ingestion (DPI, n cannibal^{-1} day^{-1}) was compared between cannibal size classes by the Welch robust test of equality of means. Tukey’s test was performed for *post hoc* multiple range comparisons. In experiment 2, handling time and capture success were regressed against prey size using a power equation for each cannibal size class. ANCOVA was used to test the effect of cannibal size classes on the slopes and intercepts of the regressions of handling time and capture success against prey size. Handling time and capture success were linearised by log (x + 1) and log (x^{-1}), respectively. In experiment 3, the prey-size-dependent attack rate was analysed using chi-square (x^2) test to detect differences from random attack for each cannibal size class. Within each cannibal size class, attack success was analysed using chi-square (x^2) test to assess whether the frequencies of successful and unsuccessful attacks were independent of prey size. Replicates in each cannibal size class were pooled for both analyses in experiment 3. A significant level of $P < 0.05$ was used for all statistical analysis. All statistics were performed using SPSS (IBM®) statistics software, version 20.

3.3.4. Ethical Note

This study was carried out in strict accordance with the recommendations in the Animal Welfare Act 1985 and the Australian Code of Practice for the Care and Use of Animals for Scientific Purpose 7th Edition. The protocol, species, and number of animals used in this study were approved by the Flinders University Animal Welfare Committee (Project No. E347). Euthanasia procedures were by overdosing animals at 43 mg l^{-1} of Aqui-S® (New Zealand Ltd., Lower Hutt, New Zealand). All fish handlings were followed by light anesthesia (15 mg l^{-1}) with Aqui-S®, and all efforts were made to alleviate fish suffering.

3.4. Results

3.4.2. Prey Size Selection

In experiment 1, a total of 4529 prey (28.6 – 67.2% cannibal L_T) were offered to the cannibals (38 – 133 mm L_T) and a total of 574 prey (12.67%) were ingested (Table 3.1). In general, the daily prey ingestion (DPI, n cannibal^{-1} day^{-1}) decreased as the body size of cannibals increased (Welch, $F_{4,\,14.67} = 44.04$, $P < 0.0001$; Table 3.1). In all cannibal size classes, the null hypothesis of random prey size selection was rejected. Cannibals showed size selection for the smallest prey (x^2, $P < 0.01$; Table 3.1). The normalised preference index (I_P) for cannibal size classes 1 – 4 showed a clear trend of decrease with the increase of prey size, but this declining trend fluctuated over prey size in cannibal size class 5 (Fig. 3.2).

Table 3.1. Details of the experiment addressing prey size selection in cannibalistic *L. calcarifer*. Chi-squared (x^2) test for goodness of fit was used to test the null hypothesis of random prey size selection for each cannibal size class. Prey size is given as percentage of the cannibal total length (L_T). Different letters in the row of DPI (daily prey ingestion) means significant differences between cannibal size classes ($P < 0.05$). Eight replicates were used for each cannibal size class and each replicate was observed for five consecutive days.

Cannibal size class	**Statistics**	**1**	**2**	**3**	**4**	**5**
Cannibal L_T (mm, mean ± SD)		41.4 ± 2.5	63.9 ± 4.0	87.3 ± 4.0	105.5 ± 3.2	125.4 ± 2.2
Prey offered (*n*)		874	923	900	918	914
Prey Ingested (*n*)		172	155	129	88	30
DPI (*n* cannibal^{-1} day^{-1}, mean ± SD)		4.3 ± 0.2a	3.9 ± 0.3ab	3.2 ± 0.2b	2.2 ± 0.1c	0.8 ± 0.3d
Prey L_T range (mm)		13.0 – 28.0	18.0 – 46.0	25.0 – 61.0	35.0 – 72.0	47.0 – 83.0
Prey size range (% cannibal L_T)		31.0 – 65.8	28.6 – 67.2	29.1 – 66.3	33.6 – 65.5	38.2 – 66.4
Selectivity	x^2	140.3	80.5	49.7	35.8	22.0
	df	17	14	12	13	9
	P	<0.0001	<0.0001	<0.0001	<0.0001	<0.01
Selected prey size (% cannibal L_T)		31.7 ± 0.3	30.4 ± 0.8	30.3 ± 0.8	30.9 ± 0.5	39.4 ± 0.6

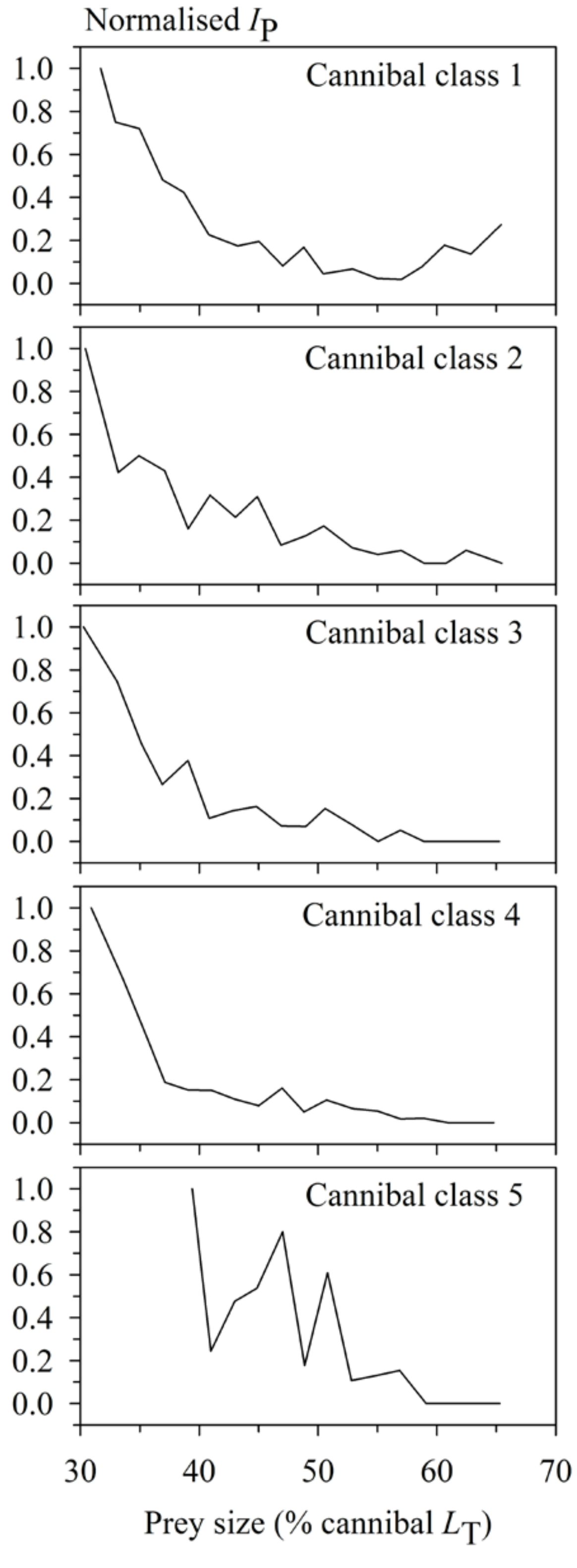

Fig 3.2. Prey size selection in cannibalistic *L. calcarifer* in experiment 1. Prey size is given as percentage of cannibal total length (L_T). The preference index (I_P) is normalised to allow comparisons between cannibal size classes. Cannibals were grouped into five cannibal size classes according to their L_T (mean ± SD): (1) 41.4 ± 2.5 mm L_T; (2) 63.9 ± 4.0 mm L_T; (3) 87.3 ± 4.0 mm L_T; (4) 105.5 ± 3.2 mm L_T; (5) 125.0 ± 2.2 mm L_T.

3.4.2. Cannibalistic Behaviour

Among 150 replicates tested in the behaviour experiments (105 in experiment 2 and 45 in experiment 3), 72 replicates ended up with successful cannibalism acts (49 in experiment 2 and 23 in experiment 3) and were then used for analyses. The cannibalistic behaviours were similar among successful cannibals in both experiments. Once prey were introduced into an aquarium, the cannibal usually remained motionless on the bottom of the aquarium using ambush predatory behaviour to attack the prey. Attacks were normally orientated head first, and if the first attack was not successful, the cannibal would remain motionless until the next attack started. All successful cannibalistic events were oriented with ingesting the head of prey first. During the observations on all replicates, some experimental fish did not show any aggressive or predatory behaviour at all, and remained motionless on the bottom of the aquaria. In other cases, experimental fish did show aggressive or predatory behaviour such as chasing and attacking attempts, but mostly resulted in unsuccessful capture. In cases where the experimental fish successfully captured the prey after chasing, the predation was orientated tail-first and the prey managed to escape. No successful ingestion was observed when the tail of a prey was ingested first.

Table 3.2. Statistical details of experiment 2. Handling time (T_H, min) represents the time elapsed between the capture and the complete ingestion. Capture success (C_S) is the proportion of attacks that result in successful ingestion. The variable "x" in the models represents prey size (% cannibal L_T). Cannibals were grouped into five cannibal size classes according to their total length (L_T, mm).

Cannibal size class	**1**	**2**	**3**	**4**	**5**
Cannibal L_T (mean ± SD)	45.5 ± 1.4	65.1 ± 1.0	88.2 ± 1.0	110.9 ± 1.6	134.0 ± 2.4
Handling time (T_H)	$y=5.62E\text{-}9x^{5.37}$	$y=1.38E\text{-}9x^{5.80}$	$y=1.40E\text{-}8x^{5.24}$	$y=1.55E\text{-}12x^{7.58}$	$y=1.19E\text{-}10x^{6.47}$
r^2	0.9	0.86	0.92	0.99	0.98
F	107.90	61.15	92.15	499.19	234.06
Df	1,12	1,10	1,8	1,4	1,5
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Capture success (C_S)	$y=518355x^{-3.96}$	$y=33453180x^{-5.15}$	$y=11295398x^{-4.95}$	$y=71046x^{-3.56}$	$y=4088x^{-2.85}$
r^2	0.66	0.82	0.89	0.81	0.89
F	23.46	46.21	61.49	17.41	40.84
Df	1,12	1,10	1,8	1,4	1,5
P	<0.0001	<0.0001	<0.0001	<0.05	0.001

In experiment 2, 49 successful cannibalistic acts were included in the analyses of handling time and capture success. In all cannibal size classes, handling time (T_H) significantly increased as the prey size increased ($P < 0.0001$; Table 3.2, Fig. 3.3). There were no significant differences between slopes (ANCOVA, $F_{1,48}$ = 1.21, $P > 0.05$) and intercepts (ANCOVA: $F_{1.48}$ = 1.09, $P > 0.05$) in the regression analysis, indicating that T_H was similar among the cannibal size classes and increased at a similar rate with the increase of prey size. Conversely, capture success (S_C) significantly decreased in all cannibal size classes as the prey size increased ($P < 0.05$; Table 3.2, Fig. 3.3). The regression slopes were similar (ANCOVA, $F_{1, 48}$ = 1.41, $P > 0.05$) between cannibal sizes indicating that S_C decreased at a similar rate with the increase in the prey size in all cannibal size classes. However, the intercepts of the regression analysis were significantly different (ANCOVA, $F_{1,48}$ = 12.48, P = 0.001) between cannibal classes, indicating that as cannibal size increases, S_C decreases (Fig. 3.3). Capture success was highly variable on small prey. In cannibal size class 1, S_C varied from 10% to 100% in the prey size of 35 – 40% of cannibal L_T (Fig. 3.3). The variability among the smallest prey size was a result of the mathematical nature of the S_C index, where any missing attack dropped the index exponentially.

Cannibalistic profitability 1 (y, % cannibal’s energy content per min) was negatively correlated with the increase of the prey size (x, % cannibal L_T) for all cannibal size classes (Fig. 3.4). However, in cannibalistic profitability 2, where capture success was incorporated in calculation, the profitability (y, % cannibal’s energy content per min) was more negatively correlated with prey size (x, % cannibal L_T) and decreased sharply to near zero in all cannibal size classes when the prey size was over 50% of cannibal L_T (Fig. 3.4).

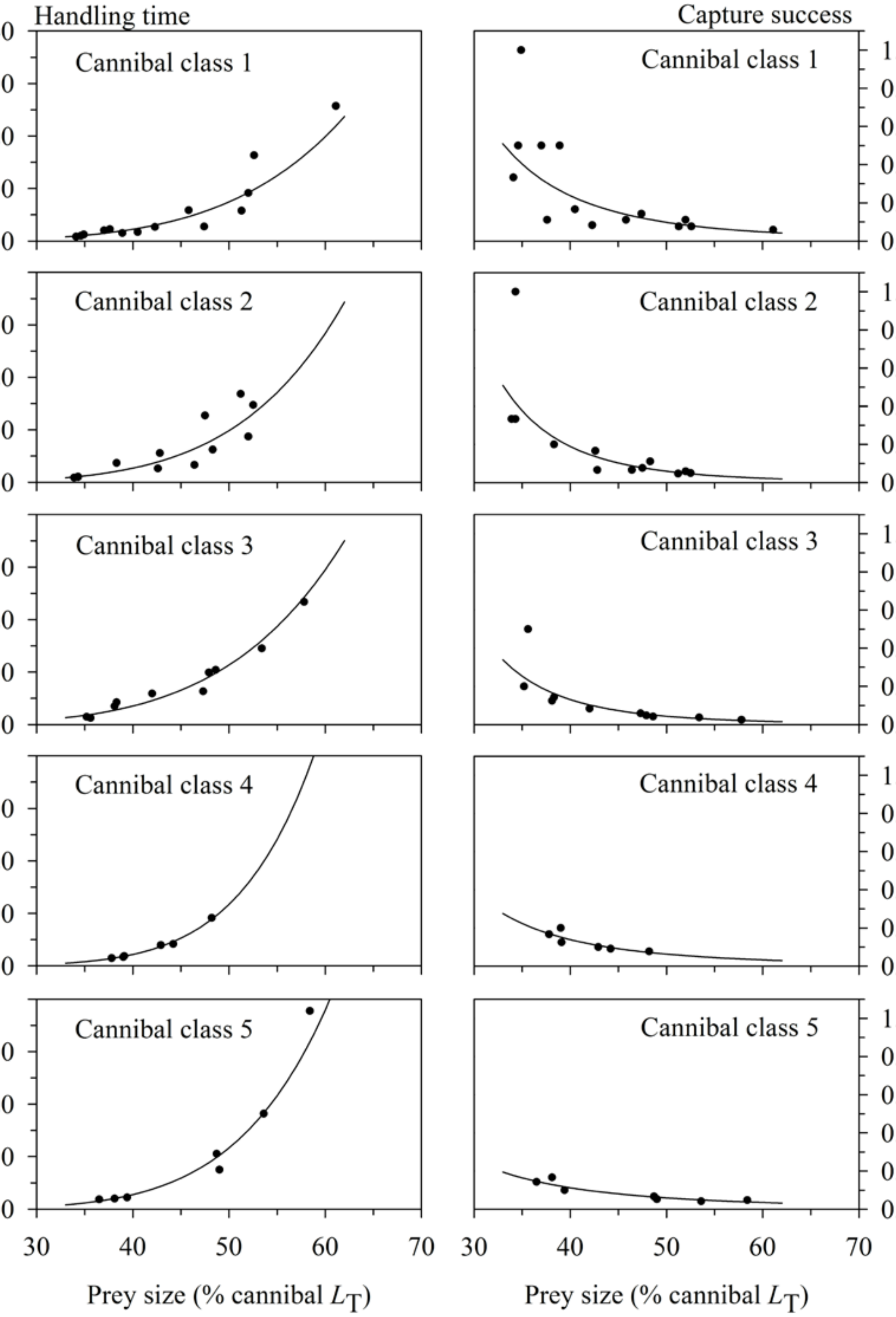

Fig 3.3. Handling time (T_H, min) and capture success (S_C, proportion of attacks resulting in sucessfully ingestion) of cannibalistic *L. calcarifer* preying on conspecific prey in experiment 2. Only the replicates resulted in successful prey ingestion were used in the behavioural analysis. Prey size is expressed as percentage of cannibal total length (L_T). Cannibals were grouped into five cannibal size classes according to their L_T (mean ± SD): (1) 45.5 ± 1.4 mm L_T; (2) 65.1 ± 1.0 mm L_T; (3) 88.2 ± 1.0 mm L_T; (4) 110.9 ± 1.6 mm L_T; (5) 134.0 ± 2.4 mm L_T. Data points represent individual value collected from individual replicate. Lines represent the average value of the dependent variable estimated by regression models using the prey size as the independent variable.

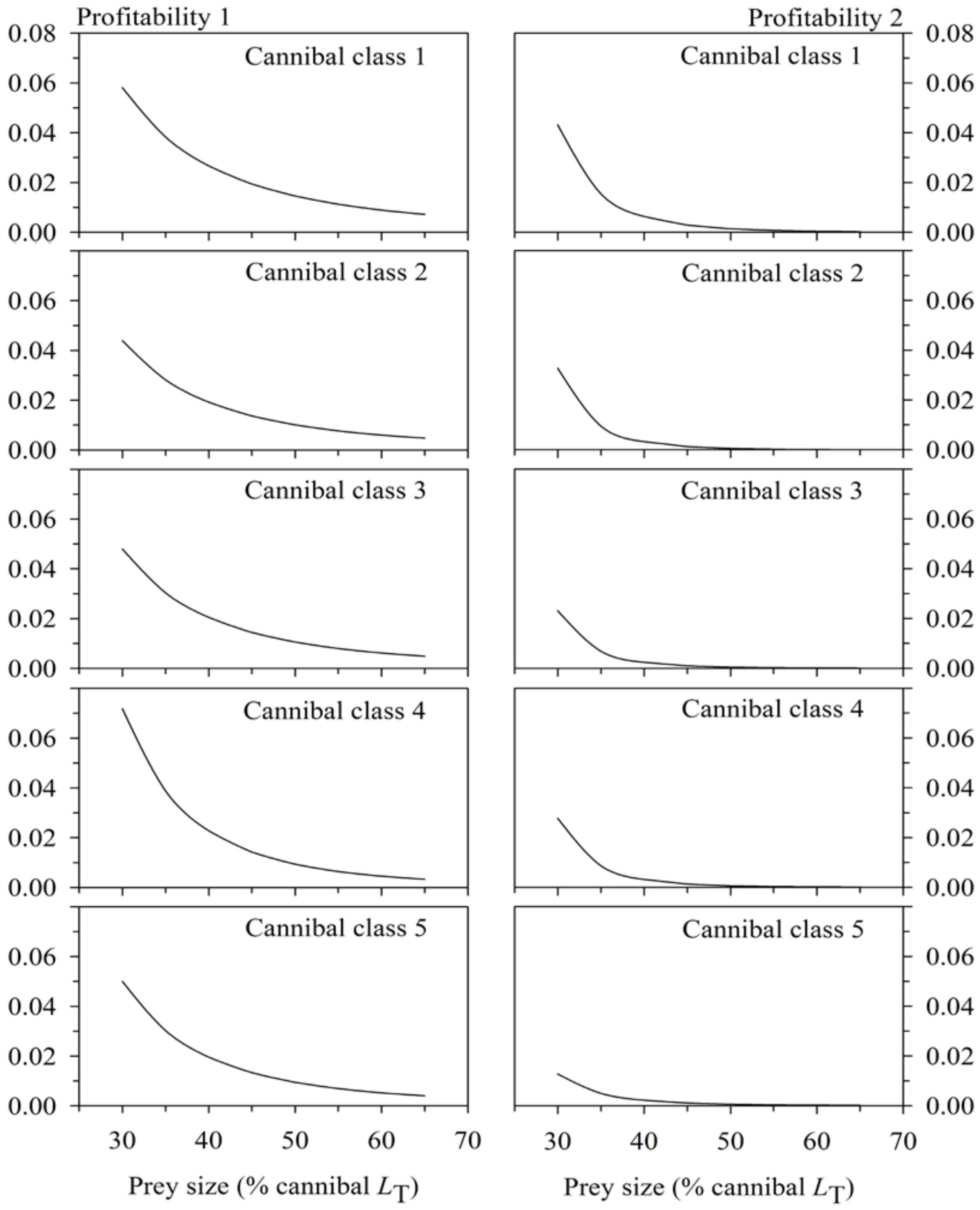

Fig 3.4. Prey size profitability calculated for cannibalistic *L. calcarifer*. Prey size is expressed as percentage of cannibal total length (L_T). Cannibals were grouped into five size classes according to their L_T (mean ± SD): (1) 45.5 ± 1.4 mm L_T; (2) 65.1 ± 1.0 mm L_T; (3) 88.2 ± 1.0 mm L_T; (4) 110.9 ± 1.6 mm L_T; (5) 134.0 ± 2.4 mm L_T. Profitability 1 (% cannibal's energy content per min) curves were estimated using the relative prey gross energy content as measurement of energy intake and handling time ($T_{H,}$ min) as a measurement of predation cost (Profitability 1: Cannibal class 1: $y = 574x^{-2.70}$; Cannibal class 2: $y = 765x^{-2.87}$; Cannibal class 3: $y = 1148x^{-2.96}$; Cannibal class 4: $y = 57380x^{-4.00}$; Cannibal class 5: $y = 3279x^{-3.26}$). In profitability 2 (% cannibal's energy content per min), capture success (S_C, proportion of attacks resulting in sucessfully ingestion) was incorporated into the equation for calculating profitability 2 (Profitability 2: Cannibal class 1: $y = 3^{8}x^{-6.66}$; Cannibal class 2: $y = 2^{10}x^{-8.02}$; Cannibal class 3: $y = 1^{10}x^{-7.92}$; Cannibal class 4: $y = 4^{9}x^{-7.56}$; Cannibal class 5: $y = 1^{7}x^{-6.11}$).

In experiment 3, 23 out of 45 observations resulted in successful cannibalistic acts and were included in the analysis for active or passive prey selection. None of the replicates from the cannibal size class 5 resulted in successful cannibalism and this size class was not included in the analysis. When cannibal size classes 1 – 4 were offered with prey of three sizes at the same time, no differences were detected in the attack rate between small, medium and large prey classes ($P > 0.05$; Table 3.3), suggesting that cannibals attack different prey sizes at similar rates. However, cannibals in classes 1, 2 and 3 were more successful in capturing smaller prey ($P < 0.05$; Table 3.3). By contrast, in cannibal size class 4, cannibals were equally successful on capturing all prey size classes (chi-square test: $x^2 = 3.45$ $P > 0.05$; Table 3.3).

Table 3.3. Details of experiment 3. Only replicates ending in successful capture were included into the analysis. The number of attacks and failed captures represents the total number of events observed prior successful captures for all replicates for each cannibal size class. Cannibals were grouped into size classes according to their total body length (L_T, mm). Prey were grouped into small (S), medium (M) and large (L) size classes, and prey size is given as percentage of the cannibal total length (% cannibal L_T). Values in parenthesis are mean ± SD. Cannibal size class 5 (133.6 ± 2.7 mm L_T) was not included into the analysis because no successful capture were observed for this cannibal size class.

Cannibal size class	**Replicates**	**Prey size class**	**Attack rate**				**Capture success**				
			n	x^2	***df***	***P***	**Success (*n*)**	**Fail (*n*)**	x^2	***df***	***P***
1 (45.7 ± 0.9)	7	S (35.0 ± 0.5)	6	3.31	2	>0.05	5	1	14.50	2	0.001
		M (48.0 ± 1.5)	7				2	5			
		L (62.4 ± 0.5)	13				0	13			
2 (64.8 ± 1.8)	6	S (36.6 ± 2.5)	14	2.22	2	>0.05	5	9	12.21	2	<0.01
		M (48.2 ± 1.1)	18				1	17			
		L (62.3 ± 0.8)	23				0	23			
3 (88.9 ± 0.8)	7	S (39.0 ± 1.5)	19	4.43	2	>0.05	5	14	6.89	2	<0.05
		M (47.9 ± 0.6)	20				2	18			
		L (62.2 ± 0.2)	10				0	10			
4 (111.4 ± 1.6)	3	S (40.1 ± 1.6)	12	4.79	2	>0.05	2	10	3.45	2	>0.05
		M (49.9 ± 0.7)	10				1	9			
		L (60.0 ± 1.0)	21				0	21			

3.5. Discussion

A juvenile *L. calcarifer* is morphologically capable to ingest conspecific individuals up to 78% of its body length (Ribeiro and Qin, 2013). However, when given a choice for prey, the present study shows that *L. calcarifer* cannibals prefer ingesting much smaller prey. Other cannibalistic fish species show a general trend for preying on much smaller conspecific prey than they are morphologically capable as observed in giant grouper *Epinephelus lanceolatus* (Bloch 1790) (Hseu et al., 2007), orange-spotted grouper *E. coioides* (Hamilton 1822) (Hseu and Huang, 2014), Atlantic cod *Gadus morhua* L. 1758 (Blom and Folkvord, 1997), yellow perch *Perca flavescens* (Mitchill 1814) (Post and Evans, 1989), *S. alpinus* (Amundsen et al., 1995), *P. djambal* (Baras et al., 2010), *B. moorei* (Baras et al., 2000) and *P. punctifer* (Baras et al., 2011). This general selection for smaller prey corroborates with most cases of interspecific predation of piscivorous fish (Juanes, 1994).

Prey size selection is defined as any difference in prey size composition in the predator diet compared to the composition of available prey sizes in the environment (Chesson, 1978, Baras, 1999, Baras et al., 2010, Ivlev, 1961). According to the optimal foraging theory (OFT), predators actively select prey individuals that bring maximum energy return per unit of time (Stephens and Krebs, 1986). In order to test if prey size selection in *L. calcarifer* cannibals supports the OFT, profitability indices were inferred in the present study using relative gross energy content of the prey (% cannibal’s energy content) as a measure of energy intake, and prey size dependent handling time (min) and capture success (a proportion of successful attacks) as measures of predation costs for each cannibal size class tested. While the energy intake is obviously directly and positively dependent on prey size, the costs of predation may vary according to predator and prey behaviours (Mittelbach, 2002, Sih and Christensen, 2001, Sih and Moore, 1990, Juanes, 1994). For piscivorous fish, predation can involve pre-capture behaviours (*i.e.* encounter rate, pointing, approach, follow and pursuit times), capture success, and post-capture behaviour (*i.e.* handling time) (Johansson et al., 2004, Mittelbach, 2002). In the present study, the experimental condition minimized the dependence of prey-cannibal size on encounter rate, as all prey sizes were within the visual spectrum of the cannibal and prey were morphologically within the ingestible range by a cannibal. In fact, the estimation of encounter rate in the study of piscivorous fish and their prey is elusively problematic (Juanes et al., 2008). However, this issue has been minimized in other studies by designing experimental conditions similar to the present study (Baras et al., 2011, Baras et al., 2010, Amundsen et al., 1995, Juanes, 1994, Juanes and Conover, 1994). Furthermore, as *L. calcarifer* uses an ambush foraging mode (Juanes et al., 2002, Moore, 1982, Dowling et al., 2000) similar to the pike *Esox lucius* L. 1758 (Nilsson and Brönmark, 2000), pre-capture behaviours such as follow and pursuit were not included in profitability analysis. In the present study, capture success and post-capture behaviour were used to estimate prey size profitability and prey size selection in cannibalistic *L. calcarifer*.

In the present study, handling time increased exponentially with the increase of prey size in all cannibal size classes. The prey-size dependent handing time has been found in other carnivorous fish such as *P. saltatrix* preying on *M. menidia* (Scharf et al., 1998, Juanes and Conover, 1994) and striped bass *Morone saxatilis* (Walbaum 1792) (Scharf et al., 1998), *G. morhua* preying on common dab *Limanda limanda* (L. 1758) and European plaice *Pleuronectes platessa* L. 1758 (Ellis and Gibson, 1997) and *S. lucioperca* preying on roach *Rutilus rutilus* (Linnaeus 1758) and rudd *Scardinius erythrophthalmus* (L. 1758) (Turesson et al., 2002). Handling time includes the time elapsed between a successful strike and completion of prey ingestion (Nilsson and Brönmark, 2000). Obviously, longer handling time incurs high energy costs due to prey manipulation and it may be associated with other negative events such as losing the prey, exposing to predator or risks of kleptoparasitism (Nilsson and Brönmark, 2000). The trade-off of lowing handling time and energy cost while trying to maximise the energy intake is a central issue when selecting optimal diet items in piscivorous fish (Gill, 2003). In this study, handling time is assumed as a measure of predation cost, and the positive dependency on prey size, regardless of cannibal size, indicates that the selection for smaller prey yields high profitability and energy gain in cannibals of all size classes.

Handling time has been a parameter subject to criticism as it frequently used for primary energy cost of feeding in theoretical foraging models (Juanes et al., 2002, Mittelbach, 2002). However, some models incorporating differential capture probabilities based on prey size have proven to be more successful in predicting piscivorous fish diets (Mittelbach, 2002, Rice et al., 1993, Juanes, 1994). In the present study, capture success was negatively dependent on prey size in all cannibal size classes, indicating that smaller prey are more easily captured. Similar results were also found in *P. saltatrix* preying on *M. menidia* (Scharf et al., 1998, Juanes and Conover, 1994) and *M. saxatilis* (Scharf et al., 1998). In contrast to planktivores, capture success rates of piscivorous fish are negatively prey-size dependent (Juanes, 1994). Prey behaviour, such as escape skill, is usually enhanced as body size increases, resulting in reduction of predator capture success (Juanes, 1994). In this study, when capture success was included, the profitability 2 curves were more pronounced than those from profitability 1, suggesting that capture success is an important component when predicting optimal prey size for a *L. calcarifer* cannibal.

Any prey larger than 50% of cannibal L_T has little energy gain on the cannibal. Irrespective of capture success inclusion, both profitability 1 and 2 indices showed that the smaller prey provide the most energy return to the cannibals in all cannibal classes. As *L. calcarifer* cannibals selected smaller prey, the results of the present study corroborate with the OFT that predators select the most profitable prey regardless the size of cannibals.

Optimal foraging theory predicts diet selection based on active predator choice (Sih and Christensen, 2001). In other words, given a similar encounter, predators attack a particular prey more often than others (Sih and Christensen, 2001). Active predator choice is one of the components in the overall predation process affecting prey selection (Juanes, 1994, Sih and Moore, 1990). It can be influenced by unequal attack and capture probabilities and other behavioural traits of both predator and prey (Sih and Moore, 1990). Capture success is an important component determining prey size selection as it leads to selection for a particular prey size in absence of active predator choice (Sih and Christensen, 2001, Sih and Moore, 1990, Juanes, 1994). The higher capture success for smaller and usually more vulnerable prey with lower escape ability can cause passive selection for small prey, even if all prey size classes are attacked at similar rates (Juanes, 1994). In the present study, similar attack rates were observed when *L. calcarifer* cannibals (<100 mm L_T) were simultaneously offered with small, medium and large prey. However, cannibals significantly ingested small prey more often than in the larger prey size groups. Similar results were observed on *P. saltatrix* preying on different sizes of *M. menidia* (Juanes and Conover, 1994), suggesting that the selection for the smaller prey is a result of negatively prey-size dependent capture success. In the present study, all prey sizes were within the morphological limit of the ingestible capacity for cannibals, but the most vulnerable individuals were more likely ingested, resulting in apparent size selection for smaller prey. Consequently, prey size selection in *L. calcarifer* became passively orientated and determined by size-dependent capture success, rather than active cannibal choice for the most profitable prey. Similar conclusions were drawn by Juanes and Conover (1994) and Sih and Moore (1990) that prey behaviour and prey size dependent vulnerability may be as important as active predator choice in determining predators diets. In addition, the passive selection for the most vulnerable prey size further supports the opportunistic nature of the foraging behaviour of *L. calcarifer* (Davis, 1985).

According to Ribeiro and Qin (2013), intracohort cannibalism in *L. calcarifer* is more likely to occur in the early juvenile stage than in latter development. In the present study, daily prey ingestion was negatively correlated to cannibal size, reflecting the reduction of capture success as cannibals grow larger. The difficulty of the larger cannibals preying siblings was evident in all experiments in the present study. In the prey size selection experiment, the largest cannibal size class showed fluctuations in selecting prey sizes and might have ingested larger prey not as profitable as the smaller ones. In addition, very few cannibalistic events were observed in the larger cannibal (>100 mm L_T) size classes in the behaviour experiments. Such a reduction in capture success may reflect the better ability of prey to avoid predation as a prey grows larger (Fessehaye et al., 2006, Hseu and Huang, 2014). In addition, the negative allometric growth of the *L. calcarifer* mouth in relation to their size may have contributed to the reduction in capture success, as the morphological capacity to ingest conspecific prey is reduced as *L. calcarifer* grow larger (Ribeiro and Qin, 2013). In addition, the decline in capture success with the increase of cannibal size could be an experimental artefact due to the relative small environmental dimensions, and result in confinement stress or greater difficulties by a large fish to manoeuvre in a small aquarium. However, considering that *L. calcarifer* is an ambush predator (Moore, 1982, Dowling et al., 2000), the confined environment in the present study was unlikely to significantly affect the predatory acts, since ambush predators present a relative simple pre-capture repertoire. Nevertheless, low capture success would contribute to reduction of cannibalism profitability as *L. calcarifer* grow larger.

The dynamics of intracohort cannibalism in piscivorous fish is dependent on multiple factors that are ultimately influenced by fish size heterogeneity. The present study showed that *L. calcarifer* cannibals selected smaller prey available in the environment. In an ungraded *L. calcarifer* population under captivity, once smaller prey have been succumbed to cannibalism, cannibals are forced to move up to consume larger prey. However, such a size shift represents a substantial increase in handling time and reduction in capture success, leading to low profitability for cannibals. In a farming scenario, the capture of larger prey is expected to be even more complicated due to a relatively larger environment compared with the small aquaria in the present study. Therefore the progressive reduction of size heterogeneity would lead to a significant reduction of cannibalism as *L. calcarifer* grow larger. However, if cannibalistic individuals do enjoy the growth advantage over siblings feeding on alternative diets, cannibalism will hardly become to an end as fast growing cannibals would always find smaller, profitable and slow-growing conspecifics to prey on. On the other hand, if alternative food is supplied accordingly, non-cannibalistic individuals may achieve more competitive growth rates (Baras et al., 2011) and grow beyond the prey spectrum of the cannibals (Baras and Jobling, 2002). Further studies should assess the energetics of intracohort cannibalism in order to compare growth rates between cannibalistic individuals and conspecifics feeding on inert diets.

In summary, this study showed that *L. calcarifer* cannibals select smaller conspecific prey when given a choice on a range of prey sizes. Although cannibalistic individuals select for smaller prey to gain higher energy intake per unit of time, prey size selectivity is a result of passive selection rather than active predator choice. Further studies should assess the bioenergetics of cannibalism, particularly addressing the conversion efficiency of cannibals preying on different sizes of prey to further validate the higher profitability found in the smallest prey in the present study. Capture success is an important component of the general predation cycle among cannibalistic *L. calcarifer*, as it depends on prey and cannibal sizes. Capture success passively contribute to the selection of the smaller and most vulnerable conspecific prey for the size range of cannibals. The decrease on capture success as cannibals grow larger, mediated by the increase of prey escaping ability and morphological constraints of the predator, play an important role on the reduction of intracohort cannibalism as fish grow larger. The smaller prey will be the most selected conspecific prey by cannibalistic *L. calcarifer* in a captive population as long as they are available. In practice, to reduce intracohort cannibalism *L. calcarifer* in captivity, size grading should focus on the early juvenile phase of development as the profitability for preying on conspecifics reduces when cannibals grow larger, thus the penalty for not exercising size grading is expectedly more severe on small than on large fish.

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Chapter 4

Bioenergetics of Cannibalism in Juvenile Barramundi: Exploring Growth Advantage of Fish Fed Live Prey and Formulated Diet

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4.1. Abstract

The present study investigated the growth performance of juvenile barramundi *Lates calcarifer* (Bloch) fed conspecific prey (CP), formulated diet (FD) and a mix of both (MIX) using a bioenergetics approach through comparison of the energy budget equation components. Fish in the MIX treatment ingested significantly more conspecific prey than formulated diet by dry mass. However, prey mass ingestion and cannibalism rate in the MIX treatment were significantly lower than in the CP treatment. This indicates that the provision of alternative food does not completely mitigate cannibalism, but it can significantly reduce cannibalism in barramundi. Fish in the FD treatment showed a significantly higher daily food intake than fish in the CP and MIX treatments. However, fish fed conspecifics showed significantly better feed conversion efficiencies, apparent digestibility rates and growth performances. Exclusive cannibalistic barramundi assimilated significantly more energy consumed, with 1.5% of energy lost in faeces comparing with 7.3% of siblings feeding exclusively on formulated diet. Consequently, exclusive cannibals channelised more energy consumption into growth (57.1%) than those fed solely on formulated diet (43.9%). Therefore, high energy allocation into growth promoted better feed conversion efficiency and growth performance of cannibalistic barramundi than siblings fed solely on formulated diets. This study implies that fast-growing cannibals may continuously prey on slow-growing conspecifics due to growth advantage through cannibalism.

Keywords: Cannibalism, Conspecific predation, Nursery, Energy budget, Growth advantage

4.2. Introduction

Intracohort cannibalism is a major concern in the culture of piscivorous fish and severe cannibalism can result in production losses during the period of larval and juvenile rearing (Hecht and Pienaar, 1993, Baras and Jobling, 2002). Intracohort cannibalism can start within a day after hatch as observed in dorada *Brycon moorei* (Steindachner) (Baras et al., 2000). At the early stage, intraspecific predation is not much limited by size heterogeneity as cannibals ingest conspecific prey tail-first and discard the uningested part, which is termed as incomplete cannibalism (Baras and Jobling, 2002). Nevertheless, this type of cannibalism can lead to growth heterogeneity as fish usually obtain better nutrition from conspecific prey than from live food prey such as rotifers and *Artemia* commonly used in larviculture (Kubitza and Lovshin, 1999). Other factors also promote size heterogeneity during larval rearing, such as genetic factors, resource competition, variation on developmental ontogeny and morphological deformities (Baras and Jobling, 2002, Kestemont et al., 2003, Baras, 2013). The weaning process coinciding with the metamorphosis from larvae to juvenile can further expedite size heterogeneity since individuals accepting formulated diets with high nutrition usually exhibit faster growth than their siblings solely fed on live zooplankton (Kestemont et al., 2003). As a result, if size heterogeneity is high at the start of the juvenile stage, complete cannibalism is likely to occur in a non-size graded population and cause significant mortality in nursery.

Unlike incomplete cannibalism, complete cannibalism depends on fish size heterogeneity as cannibals ingest the whole prey with head first (Hecht and Pienaar, 1993, Baras and Jobling, 2002, Baras, 2013). Complete cannibalism is morphologically restricted by the gape size of the cannibal and prey body dimensions (Qin and Fast, 1996, Baras and Jobling, 2002, Hseu et al., 2007). Particularly, the positive allometric growth of body dimensions, and the negative allometric growth of the mouth size result in a declining trend of ingestible prey size as fish grow larger, as observed in snakehead *Channa striatus* (Bloch) (Qin and Fast, 1996) and Atlantic cod *Gadus morhua* L. (Otterå and Folkvord, 1993). However, as in most interspecific predation events in fish, cannibals are unlikely to use the full morphological potential for prey intake, but prefer to select a smaller prey if given a choice of prey size (Baras and Jobling, 2002). Consequently, cannibalism can significantly reduce fish size heterogeneity as cannibals remove the smaller fish from the population (Baras and Jobling, 2002, Baras, 2013). In this scenario, cannibalism could be considered a “self-sabotage” strategy as its practice will proportionally decrease the number of ingestible prey and size heterogeneity in a captive fish population. But an important component has been overlooked in cannibalistic dynamics, *i.e.*, the growth rate differential between cannibals and non-cannibals. If a cannibal does not gain growth advantages over non-cannibalistic siblings, as observed in the larvae of Asian catfish *Pangasius djambal* (Bleeker) (Baras et al. 2010), cannibalism should disappear at some stage during the nursery period since cannibals would remove all potential prey from the population (Baras, 2013). However, if cannibals do possess growth advantage over their siblings ingesting formulated diet, cannibals will grow faster than the non-cannibalistic siblings, leading to even greater size heterogeneity in the population (Baras, 2013). In such a scenario, larger cannibals would always seek for victims of suitable sizes to prey on, resulting in long-lasting cannibalism.

In order to test the hypothesis that cannibals have a growth advantage over their siblings fed a formulated diet, this study used a bioenergetics approach through quantifications of food consumption and the energy budget aiming to compare the consumed energy allocation into growth and then assess the growth differences between cannibalistic and non-cannibalistic barramundi *Lates calcarifer* (Bloch). Barramundi was selected as the model species to study because it is an important fish for aquaculture and also shows severe cannibalism during the nursery stage. Our previous study has demonstrated that cannibalism in barramundi becomes progressively restricted as fish grow larger due to allometric growth of body parts (Ribeiro and Qin, 2013). Furthermore, the general preference for smaller conspecific prey as evidenced in various cannibalistic fish species (Baras, 2013) and the reduction of prey vulnerability as fish grow (Baras and Jobling, 2002) suggest that cannibalism in barramundi aquaculture would gradually phase out as fish grow larger. However, the growth advantage of cannibalistic barramundi over non-cannibalistic siblings is still not clear. Should growth advantage exists in cannibalism, the fast growing cannibals would overcome the cannibalistic size-restrictions as fish grow larger and socially dominate over other conspecifics, leading to persistence of cannibalism in the population. Therefore, the result of this study will improve our understanding on the dynamics of cannibalism from nutrition and energetic perspectives, which would contribute to developing management strategies for cannibalism control in fish culture.

4.3. Material and Methods

4.3.1. Ethical Note

This study was carried out in strict accordance with the recommendations in the Animal Welfare Act 1985 and the Australian Code of Practice for the Care and Use of Animals for Scientific Purpose 7th Edition. The protocol, species, and number of animals used in this study were approved by the Flinders University Animal Welfare Committee (Project No. E347). Euthanasia procedures were performed under overdose (43 mg L^{-1}) of AQUI-$S^{®}$ (New Zealand Ltd). All fish handling were followed by light anesthesia (15 mg L^{-1}) with AQUI-$S^{®}$, and all efforts were made to alleviate fish suffering.

4.3.2. Fish and Holding Conditions

A total of 3300 hatchery raised barramundi *L. calcarifer* of 30 days old (15 – 20 mm total length, TL) from the same cohort were obtained from the West Beach Hatchery, West Beach, SA, and transported to the Animal House, Flinders University. Upon arrival, the largest 300 fish were visually selected and stocked into a 300 L holding tank. The remaining 3000 fish were divided into two groups of similar numbers and stocked into two other 300 L holding tanks. Each holding tank was filled with freshwater, equipped with an external biofilter, air diffusers and individual heaters. Water temperature was kept at 28.2 ± 0.2°C (mean ± SD), dissolved oxygen at 7.6 ± 0.9 mg L^{-1}, 7.4 ± 0.1 pH, and <0.5 mg L^{-1} ammonia and nitrite nitrogen. A photoperiod of 12L:12D was followed at a light intensity of 350 lux during the light hours with abrupt transition between dark and light periods. While the larger fish were fed twice a day to apparent satiation, fish from the other two groups were food restricted and fed once a day to promote size differentiation before the experiment. After the experiment had begun, all small fish were fed twice a day to apparent satiation. Commercial formulated diets were offered to all fish ($NRD^{®}$ range, 400 to 2000 µm; 55% protein, 9% lipid, INVE Ltd, Thailand). Tanks were cleaned twice a day to remove uneaten feed, faeces and dead fish.

4.3.3. Experimental Design and Management

This study involved three feeding treatments: conspecific prey (CP), formulated diet (FD), and both conspecific prey and formulated diet (MIX). The experiment was implemented in fifteen 36 L tanks (40 × 30 × 30 cm) with five replicates per treatment. All tanks were connected to a recirculation system with a communal 200 L biofilter. Water quality and physical parameters were kept the same as those in the holding tanks.

After the size difference had been established, a batch of fish was collected from the larger fish group, and measured for total length (TL, mm) and wet body mass (WBM, g). A total of 50 larger fish of similar size (66.00 ± 1.55 mm TL and 3.57 ± 0.31 g WBM; mean ± SD) were selected and 45 of these larger fish were randomly released into the 15 experimental tanks with three fish per tank. The remaining five fish were stocked into a separate tank to be used as the initial sample of the experiment. Fish were acclimated for one week prior to the experiment and all fish received a formulated diet (3 mm long; 54% crude protein; 10% lipid; Ridley Agriproducts, Australia). Prior to the experiment, all fish were deprived of food overnight. Each fish was measured for TL (mm), weighed for WBM (g) and returned to the respective experimental tanks. Those five fish in the separate tank were measured, weighed and stored at -20°C for further analysis. The experiment lasted 20 days and at the end of the experiment, all experimental fish were deprived of food overnight, measured, weighed and stored at -20°C for further analysis.

In the treatments of CP and MIX, 15 conspecific prey, ranging from 40 to 50% of the average cannibal TL (22130 J g dry mass^{-1}), were added into each tank and replaced every 24 h to avoid substantial body mass loss due to food deprivation. Every morning, a batch of prey fish was collected from the smaller fish holding tanks and measured for TL. Then, 165 of these smaller fish with similar size were selected and kept in a floating bucket in the holding tank. In the afternoon, 150 of these smaller fish were selected from the floating bucket and randomly divided into 10 groups of 15 fish each. After the bulk wet weight was measured to estimate the offered prey biomass (OPB, g), these 10 groups of 15 smaller fish were released into the experimental tanks as live prey for the cannibals. The number of offered prey (OPN) fish for each tank was recorded. The remaining 15 smaller fish in the floating bucket were stocked into an empty tank as the control to estimate the proportion of prey biomass loss (PBL) during the 24 h period of food deprivation, calculated as $PBL = (PB_i – PB_f) \times PBi^{-1}$, where PBi and PB_f are the initial and final prey biomass (g), respectively. After 24 h, unconsumed prey were collected, counted to estimate the number of surviving prey (SPN) and weighed to estimate the surviving prey biomass (SPB, g). Meanwhile, a new batch of 15 prey with similar size (40-50% cannibal TL) and pre-weighed were offered to the cannibals in each tank. In 5-day intervals, experimental fish were measured for TL to adjust the prey size (% cannibal TL) to be offered for the following 5-day period. In the same time intervals, five prey samples (n = 30 each) were collected from the small fish population, measured for TL, individually weighed (g) and stored at -20°C for further analysis. As the exact time of prey ingestion was not known, the time of prey ingestion was assumed at the mid of the 24 h period. Then, in each replicate, the daily ingested prey biomass (DIPB, g) was estimated as $DIPB = OPB – \{SPB + [(0.5 \times PBL) \times OPB]\}$ (Baras and Jobling, 2002). The daily number of ingested prey was estimated as $DIPN = OPN – SPN$. The procedures of offering prey and estimating DIPB and DIPN were repeated daily. At the end of the experiment, the total ingested prey mass (IPM, g) and the total number of ingested prey (IPN) in each replicate during the 20-day period were estimated as $IPM = \Sigma DIPB$ and $IPN = \Sigma DIPN$, respectively.

In the treatments of FD and MIX, 10 containers were filled with the same formulated diet during acclimation (20997 J g^{-1} dry mass) and weighed (g) at the start of the experiment. A sample of the formulated diet was stored at -20°C for further analysis. The formulated diet was offered to the fish twice a day to apparent satiation by hand-feeding. Pellet size was large enough to avoid ingestion by the prey in the MIX treatment. Thirty minutes after feeding, the number of uneaten pellets was counted, multiplied by the average pellet weight, and deducted from the total weight of pellets offered. The remaining pellets in the containers were re-weighed at the end of the experiment to determine the total ingested formulated diet mass (IFDM, g) in each replicate.

Faeces were collected three times a day by siphoning the bottom of the tanks and transferred to containers. Faeces collection was performed before feeding fish with formulated diets and before lights turned off. The containers were placed into an oven at 60°C for a day to remove the excess amount of water by evaporation. The dried faecal material was then stored at -20°C for further analysis.

4.3.4. Chemical Analysis

Dry mass (g) of the experimental fish, prey, formulated diet and faeces were determined after samples had been oven-dried at 60°C to constant mass. Nitrogen content (% dry mass) was determined by the Dumas total combustion method using an elementar rapid N III nitrogen analyzer (Elementar Analysensysteme, Germany). Gross energy content (J g dry mass^{-1}) was measured by the combustion calorimeter system C2000 basic (IKA, Germany). Each sample had at least duplicate measurements.

4.3.5. Energy Budget

The energy budget was calculated according to Brafield (1985) by the following equation:

$C = G + R + U + F$

where, C is energy content of the food consumed; G is energy deposited for growth; R is energy lost as respiration; U is energy lost in ammonia excretion; and F is the energy lost in the faeces.

The estimation of U was based on the nitrogen budget equation (Fang et al., 2014):

$U = (C_N - G_N - F_N) \times 24830$

where C_N is the nitrogen content of the food consumed; G_N is the nitrogen deposited in the fish body; F_N is the nitrogen lost in the faeces; and 24830 is the energy equivalent of one gram of excreted ammonia ($\mathrm{J\ g^{-1}}$) (Elliott, 1976);

The energy lost as respiration (R) was calculated as the following energy budget equation:

$R = C - G - U - F$

4.3.6. Measurements

All wet mass (g) was converted to dry mass (g) prior to calculation. Specific growth rate [SGR, % dry body mass (DBM) $\mathrm{day^{-1}}$], daily food intake (DFI, % DBM $\mathrm{day^{-1}}$), feed conversion efficiency (FCE, %), apparent digestibility rate (ADR, %), and cannibalism rate (CR, $\mathrm{prey_{ingested}\ cannibal^{-1}\ day^{-1}}$) were calculated for the whole 20-day trial period as follow:

Specific growth rate: $\mathrm{SGR} = 100 \times [(\ln \mathrm{DBM_f} - \ln \mathrm{DBM_i}) / t]$;

Daily food intake: DFI = $100 \times \{\{FMI / [(DBM_i + DBM_f) / 2]\} / t\}$;

Feed conversion efficiency: FCE = $(DBM_f - DBM_i) / FMI$;

Apparent digestibility rate: ADR = $100 \times [(FMI - FM) / FMI]$;

Cannibalism rate: CR = $(IPN / N_{cannibal}) / t$.

where, DBM_f and DBM_i are final and initial dry body mass (g) of the experimental fish, respectively; t is time (days); FMI is food dry mass intake (in CP, FMI = IPM; in FD, FMI = IFDM; and in MIX, FMI = IPM + IFDM, g); FM is faeces dry mass production (g), and $N_{cannibal}$ is the number of cannibals;

4.3.7. Statistical Analysis

Each tank was considered an experimental unit and the average values of the five replicates in each treatment were used for data analysis. At the start of the experiment, differences in initial fish TL and WBM between treatments were assessed by one-way ANOVA. All performance parameters including final total length (TL_f, mm), final wet body mass (WBM_f, g), final dry body mass (DBM_f, g), specific growth rate (SGR, % DBM day^{-1}), daily food intake (% DBM day^{-1}), feed conversion efficiency (FCE, %), apparent digestibility rate (ADR, %) and the energy consumed (C), energy for growth (G) and respiration (R), energy loss as ammonia excretion (U) and in faeces (F), both in terms of J g DBM^{-1} day^{-1} and %C, were compared by analysis of variance to assess differences between treatments. One-way ANOVA after Levene's test for homogeneity of variance was used for all comparisons. When significance was detected, Tukey's test was performed for *post hoc* multiple range comparisons. T-test was used to compare the ingested prey mass (IPM, % DBM day^{-1}) and cannibalism rate (CR, prey$_{ingested}$ cannibal^{-1} day^{-1}) between treatments CP and MIX; ingested formulated diet mass (IFDM, % DBM day^{-1}) between FD and MIX; and the ingested mass (% DBM day^{-1}) between formulated diet and conspecific prey (IFDM vs. IPM, % DBM day^{-1}) in the MIX treatment. A significant level of $P < 0.05$ was used for all statistical analysis. All statistics were performed using SPSS (IBM®) statistics software, version 22.

4.4. Results

Survival of the experimental fish was 100% in all treatments. There were no significant differences in initial fish total length (77.04 ± 0.53 mm, mean ± SD; ANOVA: $F_{2,12} = 0.306$, $P = 0.742$) and initial wet body mass (5.91 ± 0.26 g; ANOVA: $F_{2, 12} = 0.649$, $P = 0.540$) between treatments. The one-way ANOVA showed no significant difference on the growth performance (TL_f, WBM_f, DBM_f and SGR) between conspecific prey (CP) and MIX treatments ($P > 0.05$, Table 4.1) at the end of the experiment. However, fish in both CP and MIX treatment grew faster than fish in the formulated diet (FD) treatment ($P < 0.05$, Table 4.1). Fish in the MIX treatment ingested significantly more conspecific prey than formulated diet (10.29 ± 0.58% *vs* 3.02 ± 0.49% DBM day^{-1}, mean ± SD; T-test, $t = 21.30$, $df = 8$, $P < 0.0001$). Fish in the FD treatment ingested significantly more formulated diet than those in the MIX treatment (15.62 ± 0.79% *vs* 3.02 ± 0.49% DBM day^{-1}; T-test, $t = 30.21$, $df = 8$, $P < 0.0001$). However, fish fed exclusively on conspecific prey (CP treatment) ingested significantly more prey mass (IPM) than those in the MIX treatment (12.58 ± 0.52% *vs* 10.29 ± 0.21% DBM day^{-1}; T-test, $t = 6.59$, $df = 8$, $P < 0.0001$). Similarly, cannibalism rate was significantly higher in the CP treatment comparing with MIX treatment (2.77 ± 0.45 *vs* 2.24 ± 0.08 prey$_{\text{ingested}}$ cannibal^{-1} day^{-1}; T-test, $t = 2.61$, $df = 8$, $P = 0.031$). Therefore, the provision of formulated diet reduced significantly cannibalism in the present study. When comparing the total of food ingested between the three treatments, fish fed exclusively on formulated diet in the FD treatment showed a significantly higher DFI than fish in the CP and MIX treatments ($P < 0.05$, Table 4.1). At the same time, both CP and MIX treatments presented significantly better FCE and ADR than in the FD treatment ($P < 0.05$, Table 4.1), indicating that cannibalistic fish ingest less food but convert food into body mass more efficiently than non-cannibalistic fish.

The energy consumption (C, J g DBM^{-1} day^{-1}) in the FD feeding treatment was significantly higher than in both CP and MIX treatments ($P < 0.05$, Table 4.2). However, there was no significant difference on the energy deposition for growth (G, J g DBM^{-1} day^{-1}) between treatments ($P > 0.05$, Table 4.2). Fish in the FD treatment spent significantly more energy on respiration (R, J g DBM^{-1} day^{-1}) than fish in the CP and MIX treatments ($P < 0.05$, Table 4.2). The fish energy loss as both ammonia excretion and faeces (U and F, J g DBM^{-1} day^{-1}) was significantly higher in the FD treatment than in the CP and MIX treatments ($P < 0.05$, Table 4.2).

Table 4.1. Performance parameters of juvenile barramundi *Lates calcarifer* (Bloch) fed on formulated diet (FD), conspecific prey (CP) and both diets (MIX). Each treatment had five replicates (mean ± SD). Different letters in the same rows mean significant differences between treatments ($P < 0.05$).

Variables	**Treatments**			**One-way ANOVA**	
	FD	**CP**	**MIX**	***F***	***P***
TL_f (mm)	121.27 ± 4.77^b	131.40 ± 5.02^a	134.33 ± 2.44^a	13.07	0.001
WBM_f (g)	22.69 ± 1.95^b	29.96 ± 4.63^a	32.17 ± 2.11^a	12.41	0.001
DBM_f (g)	6.03 ± 0.55^b	7.75 ± 1.37^a	8.19 ± 0.76^a	7.14	0.009
SGR (% DBM day^{-1})	7.51 ± 0.75^b	8.80 ± 0.90^a	9.13 ± 0.48^a	8.46	0.005
DFI (% DBM day^{-1})	16.05 ± 1.72^a	12.58 ± 0.52^b	13.31 ± 0.57^b	14.07	0.001
GCE (%)	40.45 ± 3.71^b	57.05 ± 3.71^a	55.41 ± 3.18^a	33.31	<0.0001
ADR (%)	87.75 ± 0.39^b	93.66 ± 0.94^a	93.06 ± 0.37^a	136.08	<0.0001

TL_f is final total length; WBM_f is final wet body mass; DBM_f is final dry body mass; SGR is specific growth rate; DFI is daily food intake; GCE is gross conversion efficiency; and ADR is apparent digestibility rate.

Table 4.2. Energy parameters of juvenile barramundi *Lates calcarifer* (Bloch) fed on formulated diet (FD), conspecific prey (CP) and both diets (MIX). Each treatment had five replicates (mean ± SD). Different letters in the same rows mean significant differences between treatments ($P < 0.05$).

Energy parameters ($\mathbf{J\ g\ DBM^{-1}\ day^{-1}}$)	**Treatments**			**One-way ANOVA**	
	FD	**CP**	**MIX**	***F***	***P***
C	3370.11 ± 362.18^{a}	2784.54 ± 114.44^{b}	2911.83 ± 123.34^{b}	8.92	0.004
G	1469.30 ± 70.78	1588.91 ± 185.00	1610.51 ± 74.69	1.94	0.19
R	1460.13 ± 280.39^{a}	1020.72 ± 177.65^{b}	1091.02 ± 119.31^{b}	6.72	0.01
U	195.59 ± 36.53^{a}	133.87 ± 14.98^{b}	140.10 ± 17.38^{b}	9.59	0.003
F	245.09 ± 40.47^{a}	41.04 ± 4.04^{c}	70.20 ± 7.37^{b}	106.94	<0.0001

C is energy consumed in food, *G* is energy deposited for growth, *R* is energy for respiration, *U* is energy excreted as ammonia, and *F* is the energy lost in the faeces.

Approximately 50% of the total energy consumption was deposited for fish growth in all treatments (Table 4.3). However, fish in treatments CP and MIX assimilated significantly more energy into growth (G, % C) than the fish in the FD treatment ($P < 0.05$, Table 4.3). The percentage of energy allocated to respiration (R, % C) was not different between the three treatments ($P > 0.05$, Table 4.3). The percentage of energy lost as ammonia excretion (U, % C) and in the faeces (F, % C) from the total of energy consumption was minimal in all treatments (Table 4.3). However fish in the FD treatment lost more energy as ammonia excretion and faeces than fish in the CP and MIX treatments ($P < 0.05$, Table 4.3).

Table 4.3. Energy budget of juvenile barramundi *Lates calcarifer* (Bloch) fed on formulated diet (FD), conspecific prey (CP) and both diets (MIX). Each treatment had five replicates (mean ± SD). Different letters in the same rows mean significant differences between treatments ($P < 0.05$).

Energy Budget (% *C*)	**Treatments**			**One-way ANOVA**	
	FD	**CP**	**MIX**	***F***	***P***
C	100	100	100		
G	43.91 ± 4.26^{b}	57.07 ± 6.18^{a}	55.38 ± 3.19^{a}	11.53	0.02
R	43.07 ± 3.81	36.65 ± 6.01	37.42 ± 2.93	3.11	0.082
U	5.77 ± 0.47^{a}	4.80 ± 0.30^{b}	4.80 ± 0.39^{b}	10.25	0.003
F	7.25 ± 0.55^{a}	1.48 ± 0.21^{c}	2.41 ± 0.20^{b}	375.75	<0.0001

C is energy consumed in food, *G* is energy deposited for growth, *R* is energy for respiration, *U* is energy excreted as ammonia, and *F* is the energy lost in the faeces.

4.5. Discussion

Once cannibalism has emerged in a fish population in aquaculture, its continuation and impact depend on the relative growth rate of cannibals to their potential victims or on the growth advantage of fish fed conspecific prey over formulated diet (Kubitza and Lovshin, 1999, Baras, 2013). The dynamics of size distribution of a fish population shows that cannibalistic individuals present growth advantage over non-cannibalistic siblings, as suggested by McIntyre et al. (1987) in walleye *Stizostedion vitreum* (Mitchill), Hecht and Appelbaum (1988) in catfish *Clarias gariepinus* (Burchell), van Damme et al. (1989) in koi carp *Cyprinus carpio* L., Folkvord and Otterå (1993) in Atlantic cod *G. morhua*, and Katavić et al. (1989) in European sea bass *Dicentrarchus labrax* L. However, none of the above studies have deeply explored the mechanism regulating the growth pattern between cannibals and non-cannibals. In a recent study, cannibalistic larvae of Asian catfish *P. djambal* did not show consistently better conversion efficiency over siblings feeding on *Artemia* (Baras et al., 2010). Conversely, cannibalistic larvae of other species such as the catfishes *Pseudoplatystoma punctifer* (Castelnau) and *Hemibagrus nemurus* (Valenciennes) and dorada *B. moorei* consistently showed better conversion efficiency over siblings ingesting *Artemia* (Baras et al., 2011, Baras et al., 2013, Baras et al., 2000). However, these studies explored the cannibalistic performance of fish larvae comparing with siblings ingesting brine shrimp which normally have low nutritional value (Kubitza and Lovshin, 1999). To our best knowledge, the present study was the first to compare the growth performance and bioenergetics of individuals feeding smaller conspecific prey and their siblings feeding on formulated diet during the juvenile stage. The present study showed that barramundi cannibals indeed presented growth advantage over siblings feeding exclusively on high energetic formulated diets. This result suggests that cannibalism in barramundi will be never ending if prey less than 50% of cannibal size is present in the captive population and may explain why cannibalism is so intense during the nursery period of barramundi culture if size grading is not performed.

In aquaculture, once a fish becomes a cannibal, it is unlikely to reverse the food type to formulated feed (Paller and Lewis, 1987). This was not the case in barramundi as fish could ingest both when conspecific prey and formulated diet were provided simultaneously, but the intake of live prey was significantly higher than on formulated diet. Despite the preference for live conspecific prey, the cannibalistic fish still ingested the artificial pellets, but did not significantly improve growth due to pelleted feed intake. Furthermore, exclusive cannibals in the treatment CP ingested significantly more prey mass and numbers comparing with cannibals in the MIX treatment where formulated diet was also provided. Clearly, the provision of alternative food items significantly reduced cannibalism in barramundi, which agrees with the reports on other cannibalistic fish species such as European sea bass *D. labrax* (Katavić et al., 1989), snakehead *C. striatus* (Qin and Fast, 1996) and Japanese flounder *Paralichthys olivaceus* (Temminck & Schlegel) (Dou et al., 2000). Despite cannibalism in barramundi being reduced by the provision of alternative food, it cannot be complete mitigated as conspecific prey is still the main food item in the cannibal diet as suggested for European sea bass *D. labrax* (Katavić et al., 1989, Hatziathanasiou et al., 2002). Future investigation should be performed with the consideration on the frequency and quantity associated with formulated food supply to further assess the effects of food availability on cannibalism reduction in barramundi aquaculture.

In the present study, barramundi feeding exclusively on formulated diet ingested significantly more food, both in terms of dry mass and energy, comparing with those feeding on conspecific prey. However, cannibalistic fish exhibited faster growth rate, higher feed conversion efficiency and higher apparent digestibility rate, indicating that feeding on conspecifics is more efficient in converting food into body mass. Michael et al. (2010) found that the faster growth rate and higher conversion efficiency in barramundi fed on live mosquito fish *Gambusia holbrooki* (Girard) than their siblings fed commercial formulated diet were due to a higher protein, lower carbohydrate and higher protein to energy ratio in mosquito fish. The formulated diet in this study was a commercial diet specifically developed for barramundi. Although it closely matches the protein and energy requirements of barramundi (Glencross, 2006), the ingredient composition may be poor in digestibility. Nevertheless, the apparent digestibility rate of live prey (93.66%) in the present study was similar to the 94% found by Bermudes et al. (2010) in barramundi under similar experimental conditions and fed a practical diet containing 70% fish meal. However, despite the similar digestibility rate, the energy lost to the faeces in cannibalistic fish was lower (1.5%) than those fed on commercial formulated diet in the present study (7.3%) and also lower than the barramundi fed a practical formulated diet (10.0%) (Bermudes et al., 2010). As a consequence, the cannibalistic barramundi channelised more energy consumption (57.1%) into growth than barramundi fed commercial formulated diet (43.9%) in this study and those fed a practical formulated diet (49.4%) (Bermudes et al., 2010). Therefore, the higher energy channelised into growth by feeding on conspecifics promotes higher conversion efficiency and growth of cannibals than the counterparts feeding on a formulated diet.

In the aquaculture of piscivorous fish species, cannibalism usually tends to reduce as fish get larger due to morphological constraints as a result of allometric growth of body parts, and reduction of prey vulnerability through improved prey escape skills (Baras and Jobling, 2002). In barramundi, the maximum prey size that cannibals are able to ingest reduces from 78% to 72% of their size when the cannibal size increased from 25 to 131 mm in total length during the nursery stage (Ribeiro and Qin, 2013). Cannibalistic fish under captivity usually select the smaller prey size when given a size choice (Baras, 2013). According to the optimal foraging theory, studies on interspecific piscivorous predation in fish suggest that prey size selection is positively correlated to prey profitability (Turesson et al., 2002, Juanes, 1994, Juanes and Conover, 1994, Sih and Moore, 1990, Mittelbach, 2002). Therefore, in an non-size graded fish population in aquaculture, once smaller prey have succumbed to cannibalism, cannibals are forced to move up to consume larger prey not as profitable as the smaller ones. However, the present study shows that the cannibal barramundi grow faster than siblings feeding on formulated diet. Therefore, once enough size heterogeneity is attained in the population, cannibals will emerge as long as the smaller fish are <50% of the larger barramundi (Ribeiro and Qin, 2013). Consequently, fast-growing cannibals would continuously find slow-growing and high profitable victims to prey on since the higher cannibal growth may compensate cannibalistic restrictions promoted by morphological constraints and prey vulnerability as fish get larger. Cannibalism would possibly come to an end only when all edible prey sizes have vanished, and only those large cannibalistic fish remain in the population.

In summary, the present study shows that barramundi cannibals do have growth advantages over siblings feeding formulated diet through a higher consumed energy allocation into growth. This indicates that once cannibalism emerges in barramundi aquaculture, it would progressively continue as cannibals would continually find victims to prey on. Therefore, an initial size grading should be performed during the early stage to remove all potential cannibals in the population and further size grading must be conducted to keep fish size differences under 50% in order to avoid the emergence of new cannibals. In order to reduce the necessity of frequent size grading during nursery of barramundi, the effects of environmental manipulation on size heterogeneity and cannibalism rate in barramundi aquaculture must be further investigate.

4.6. Acknowledgements

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Chapter 5

Dynamics of Intracohort Cannibalism and Size Heterogeneity in Juvenile Barramundi at Different Stocking Densities and Feeding Frequencies

This chapter has been accepted by Aquaculture after major revision as:

Ribeiro FF, Forsythe S, Qin JG. Dynamics of intracohort cannibalism and size heterogeneity in juvenile barramundi (*Lates calcarifer*) at different stocking densities and feeding frequencies. Aquaculture (accepted after major revision on 6/11/2014).

5.1. Abstract

Intracohort cannibalism is a major concern for the aquaculture of piscivorous fish species. This study investigates the effects of stocking density and feeding frequency on dynamics of cannibalism, growth and size distribution in juvenile barramundi (*Lates calcarifer*) over time. Cannibalism was significantly reduced or delayed by the use of high feeding frequency and low fish density. In the fish fed once a day, food restriction stimulated cannibalism through heterogeneous growth and size dispersal due to hunger and food competition. The number of potential cannibals gradually emerged from the conspecifics and provoked cannibalism over time. The increase of feeding frequency from one to three times a day enabled most fish to gain access to the formulated diet and achieve homogeneous growth rates through the reduction of food competition. Consequently, the emergence of potential cannibals and the dynamics of size dispersal, skewness and cannibalism at a high feeding frequency were postponed in comparison with the feeding once a day. The increase in fish density intensified cannibalism regardless of feeding frequency. The present study indicates that the increase of feeding frequency and decrease of fish density can significantly reduce size heterogeneity and consequently alleviate or postpone the cannibalism in barramundi.

Keywords: Cannibalism, Nursery, Feeding frequency, Stocking density, Size heterogeneity

5.2. Introduction

Intracohort cannibalism is a major concern for the aquaculture of piscivorous fish species (Hecht and Pienaar, 1993, Baras and Jobling, 2002, Baras, 2013). For instance, cannibalistic predation in fish can start within a day after hatch and between individuals of similar size, as observed in dorada (*Brycon moorei*) (Baras et al., 2000), when the victim is incompletely ingested and gape size is not limited. In contrast, in some marine fish species, cannibalism occurs after completion of body metamorphosis (Baras and Jobling, 2002). At this stage, complete prey ingestion, termed as type II cannibalism, is limited by the mouth size of cannibals, and the size heterogeneity of population is the core regulatory factor for cannibalism (Hecht and Pienaar, 1993, Baras, 2013). After metamorphosis, size heterogeneity may become conspicuous due to resource competition during the period of fish larval feeding and weaning (Baras and Jobling, 2002, Kestemont et al., 2003, Baras, 2013, Kubitza and Lovshin, 1999). Therefore, size grading practices have been the most frequently used method to control cannibalism during the nursery period of many piscivorous fish species in aquaculture (Hecht and Pienaar, 1993, Baras and Jobling, 2002). However, size grading is costly, labour intensive and in many instances, it can be inefficient (Hecht and Pienaar, 1993, Baras and Jobling, 2002) causing stress and injury to the fish (Hseu, 2002). Therefore, it is necessary to explore alternative methods to reduce fish size heterogeneity and cannibalism in fish aquaculture. Among various factors causing size heterogeneity and cannibalism (Hecht and Pienaar, 1993, Baras, 2013), food abundance, feeding frequency and animal density are the most significant and common measures to manage cannibalism in fish hatchery (Mélard et al., 1996, Baras, 2013, Baras et al., 2011).

Food abundance is one of the major factors influencing cannibalism in many reared fish species during the early life stages (Polis, 1981, Hecht and Appelbaum, 1988, Smith and Reay, 1991, Hecht and Pienaar, 1993, Dou et al., 2000, Baras and Jobling, 2002, Baras, 2013). Restriction on food supply usually leads to cannibalism simply by means of hunger motivation (Katavić et al., 1989, Baras and Jobling, 2002, Baras, 2013). Indirectly, food restriction can also lead to food competition and consequently provoke size heterogeneity and emergence of new cannibals in the population (Baras and Jobling, 2002, Baras, 2013). Consequently, fish size heterogeneity can be further intensified and lead to long-lasting cannibalism (Baras, 1999, Baras, 2013). Since fish require a relative high feed supply during nursery, the knowledge on appropriate feeding frequency is crucial to work out a feeding protocol to reduce cannibalism (Baras, 2013).

Fish stocking density also can significantly affect fish cannibalism (Hecht and Pienaar, 1993, Baras, 2013, Baras and Jobling, 2002, Baras et al., 2003). Strong cannibalism is generally associated with high fish densities as reported in European sea bass (*Dicentrarchus labrax*) (Hatziathanasiou et al., 2002), European perch (*Perca fluviatilis*) (Kestemont et al., 2003) and fat snook (*Centropomus parallelus*) (Corrêa and Cerqueira, 2007). The enhanced cannibalism in high stocking density can be simply a result of a high prey encounter frequency (Sogard and Olla, 1994, Baras, 2013). Conversely, high stocking densities can also reduce cannibalism in visually orientated fish due to perceptual confusion for prey capture (Mélard et al., 1996, Baras and Jobling, 2002, Baras et al., 2003, Baras, 2013). However, high fish density coupled with insufficient food supply can stimulate food competition, social hierarchy, depensatory growth, size heterogeneity and cannibalism (Baras and Jobling, 2002) as seen in European sea bass and European perch (Katavić et al., 1989, Baras et al., 2003). Therefore, the impact of stocking density on cannibalism in fish under captivity may depend on food provision.

Barramundi (*Lates calcarifer*) is a carnivorous fish with important economic value for aquaculture in tropical and subtropical regions. Like other piscivorous fishes, intracohort cannibalism in the early juvenile stage is a major concern in aquaculture. Our previous study indicates that the increase in size heterogeneity leads to the emergence of cannibalism (Ribeiro and Qin, 2013). In the present study, we hypothesised that size heterogeneity and cannibalism can be controlled through manipulation of environmental and biotic factors. Specifically, we aimed to test the dependent effects of feeding frequency and stocking density on the temporal dynamics of fish growth, size heterogeneity and cannibalism in juvenile barramundi. The outcomes of this study will contribute to our knowledge on the nature of fish cannibalism and improvement of hatchery technology on cannibalism control of barramundi juveniles and other similar carnivorous fish in the nursery period.

5.3. Materials and Methods

5.3.1. Animals and Experimental Conditions

A total of 10500 hatchery raised juvenile barramundi (15 – 20 mm total length, TL; 35 days old) from the same cohort were obtained from the West Beach Hatchery and transported to South Australia Research and Development Institute, Adelaide. Upon arrival, fish were size-graded by removing the top and bottom 5 percentile individuals from the population. The graded fish were stocked into six 170-L tanks and acclimated for three days. Each tank was supplied with flow-through seawater at an increasing exchange rate of 34 – 68 times the tank volume every day, as fish grew. Two airstones were used in each tank to keep dissolved oxygen levels near saturation. Water quality parameters were daily monitored and maintained as follows: water temperature 28.0 ± 0.2 °C (mean ± SD), dissolved oxygen 5.5 ± 0.4 mg L^{-1}, salinity 37.0 ± 1.0 mg L^{-1}, pH 7.9 ± 0.3 and ammonia and nitrite nitrogen <0.5 mg L^{-1}. A photoperiod of 12L:12D was followed at light intensity of 500 lux at the water surface with abrupt changes between light and dark. Formulated feed (NRD® range, 800 to 2000 µm; 55% protein, 9% lipid, INVE Ltd, Thailand; and Start 3 mm; 54% protein, 10% lipid, Ridley Agriproducts, Australia) were offered to apparent satiation three times a day at 0900, 1200 and 1600 h. The transition between the two types of feed was done progressively over 10 days once fish reached 50 mm TL. Tanks were cleaned twice a day to remove uneaten feed, faeces and dead fish.

A 3 × 2 factorial design including three stocking density (1, 5 and 10 fish L^{-1}) and two feeding frequencies (once and three times day^{-1}) in triplicate was used in eighteen 100-L tanks. After acclimation, fish were randomly assigned to each tank until the target stocking densities were attained. Fish were fed either three times a day at 0900, 1200 and 1600 h or once a day at 1200 h. The experiment lasted 40 days. Regardless of the feeding frequency treatments, food was offered to apparent satiation. Formulated feed, water quality parameters and tank management were similar to the period of acclimation.

Prior to the start of the experiment, fish were randomly collected from the acclimation tanks, anesthetized (AQUI-S®, 15 mg L^{-1}) and placed onto a tray containing 10 mm deep water. Each batch of 100 fish was photographed with a graduated mark on the bottom as the reference to estimate fish length. The anesthetized fish were recovered in buckets and then randomly stocked into the 18 experimental tanks until the target fish density for each tank was reached. In a 10-day interval, all fish from the experimental tanks were collected, counted, anesthetized and the photo procedure was repeated as before. Photos containing fish images were further analyzed to measure fish standard length (SL, mm) using ImageJ software, version 1.45s. In order to validate digital fish measurements, an additional group of 400 fish was kept in a 100-L tank following the same experimental conditions and fed to apparent satiation twice a day. Every 10 days, a random batch of 100 fish was photographed following the aforementioned protocol for further digital fish SL_{photo} measurements. Fish were then allowed to recover and after 30 minutes they were re-anesthetized (15 mg L^{-1} AQUI-$S^{®}$) and measured for standard length using a graduated scale (SL_{eye}, mm). SL_{eye} measurements were plotted against SL_{photo} measurements and a linear regression ($SL_{photo} = 1.031 \times SL_{eye} – 0.367$, $r^2 = 0.99$, n = 275) with strong Pearson's correlation ($r = 0.99$) indicated a strong reliability of the digital measurement protocol.

5.3.2. Population Parameters and Mortalities

Throughout the experiment, all fish in the tanks were counted and digitally measured for SL (mm) at a 10-day interval. Individual SL was further converted into total length (TL, mm) using the linear regression equation from an independent dataset: $TL = 1.163 \times SL + 1.244$ ($r^2 = 0.99$, $n = 368$, TL range = 20 – 140 mm). The dataset was obtained from a fish population in a similar condition as those in the present study. The SL was converted to TL to facilitate comparisons with other studies. Therefore, the observed fish numbers (N), mean fish total length ($Mean_{TL}$, mm), coefficient of variation [$CV_{TL} = (SD_{TL} / Mean_{TL}) \times 100$], and skewness of the fish total length distribution ($Skew_{TL}$) were estimated. The percentage of potential cannibals (PC) was estimated as: PC (% N_{start}) = (N_{PC} / N_{start}) × 100], where N_{PC} is the numbers of potential cannibals assuming that a fish must be 50% larger than the smallest fish in the population to show cannibalistic tendencies ($N_{\mathrm{PC}} = N_{\mathrm{fish}} \geq 0.5 \times \mathrm{Small}_{\mathrm{TL}}$) (Ribeiro and Qin, 2013, 2014, 2015) and N_{start} is the number of fish at the start of the experiment.

Daily mortalities (% N_{start} day^{-1}) owing to cannibalism (DCM = $([(N_{\mathrm{i}} - N_{\mathrm{f}} - N_{\mathrm{dead}}) / N_{\mathrm{start}}] / \Delta t) \times 100$) and other causes (DOM = $[(N_{\mathrm{dead}} / N_{\mathrm{start}}) / \Delta t] \times 100$), daily total mortality (DTM = $([(N_{\mathrm{i}} - N_{\mathrm{f}}) / N_{\mathrm{start}}] / \Delta t) \times 100$) and specific growth rate (SGR, % $\mathrm{Mean}_{\mathrm{TL}}$ day^{-1} = $[(\ln \mathrm{Mean}_{\mathrm{TLf}} - \ln \mathrm{Mean}_{\mathrm{TLi}}) / \Delta t] \times 100$), were calculated for each 10-day period (days 0 – 10; 11 – 20; 21 – 30; 31 – 40), where N_{i} and N_{f} are the initial and final numbers of fish in the time interval, N_{dead} is the number of dead fish during the time interval, N_{start} is the number of fish at the start of the experiment, Δt is the time interval (day) and $\mathrm{Mean}_{\mathrm{TLf}}$ and $\mathrm{Mean}_{\mathrm{TLi}}$ are the final and initial mean total length (mm) in the time interval.

Mortality (% N_{start}) owing to cannibalism (CM = $[(N_{\mathrm{start}} - N_{\mathrm{end}} - N_{\mathrm{dead}}) / N_{\mathrm{start}}] \times 100$), to other causes (OM = $[N_{\mathrm{dead}} / N_{\mathrm{start}}] \times 100$) and total mortality (TM = $[(N_{\mathrm{start}} - N_{\mathrm{end}}) / N_{\mathrm{start}}] \times 100$) were estimated at the end of the experiment, where N_{end} is the observed number of fish at the end of the experiment.

5.3.3. Statistical Analyses

All data were subjected to log-transformation prior to statistical analysis to homogenize the variance. The average values of triplicates were used for statistical comparisons between treatments. A two-way ANOVA was used to assess the effects of feeding frequency (two levels) and stocking density (three levels) on population parameters ($\mathrm{Mean}_{\mathrm{TL}}$, $\mathrm{CV}_{\mathrm{TL}}$, $\mathrm{Skew}_{\mathrm{TL}}$, PC), specific growth rate (SGR), daily mortalities (DCM, DOM and DTM) and final mortalities (CM, OM and TM). When two-factor interaction or main effect was significant, Tukey's test was used for post hoc multiple range comparisons. Differences were considered significant at $P < 0.05$.

5.4. Results

5.4.1. Mortalities

Mortality owing to cannibalism was significantly higher in barramundi fed once a day than in those fed three times daily ($P < 0.05$, Table 1), though the increase of stocking density further intensified cannibalism, regardless of the feeding regime ($P < 0.05$). The low fish density and high feeding frequency suppressed cannibalism by 1.33%, which was significantly lower than in any other treatment combinations ($P < 0.05$). Mortality owing to other causes, such as wounds and suffocation, was enhanced by the increase of both feeding frequency and stocking density ($P < 0.05$), though the effect of the stocking density was stronger in fish fed three times a day than in fish fed once a day ($P < 0.05$). In general, the total mortality was reduced by increasing feeding frequency, but amplified at high stocking density, irrespective of the feeding regime ($P < 0.05$). Cannibalism contributed to >90% of the total mortality in the fish fed once a day, while mortality due to other causes was attributed to the most total mortality (>50%) in the fish fed three times a day.

Table 5.1. Mortalities in juvenile barramundi stocked at three densities (1, 5 and 10 fish L^{-1}) and fed at two feeding frequencies (once or three times a day) during a 40-day experimental period. CM is cannibalism mortality, OM is mortality owing to other causes and TM is total mortality (% N_{start} day^{-1}). Values are the average percentage (mean ± SD) of fish mortality at the end of the experiment, in three replicate groups. Different capital letters within the same column represent significant effect of feeding frequency, and different low-case letters within the same feeding frequency represent significant differences between stocking density ($P < 0.05$).

Feeding frequency (N day^{-1})	**Stocking density (N L^{-1})**	**CM (%)**	**OM (%)**	**TM (%)**
1	1	57.14 ± 11.28Ac	4.98 ± 0.02Bb	62.12 ± 11.28Ac
1	5	78.51 ± 6.82Ab	5.28 ± 1.61Bab	83.78 ± 2.29Ab
1	10	88.05 ± 0.44Aa	7.31 ± 1.37Ba	95.36 ± 5.1Aa
3	1	1.33 ± 0.88Bc	9.69 ± 2.17Ac	11.02 ±1.79Bc
3	5	12.59 ± 0.44Bb	16.98 ± 2.53Ab	29.57 ± 1.48Bb
3	10	19.78 ± 3.09Ba	25.38 ± 1.51Aa	45.15 ± 2.28Ba
Two-way ANOVA (P value)				
Feeding frequency (FF)		<0.0001	<0.0001	<0.0001
Stocking density (SD)		0.003	0.001	<0.0001
Interaction (FF×SD)		0.517	0.008	0.927

5.4.2. Dynamics of Cannibalism, Growth and Size Distribution

There were no significant differences in initial Mean_{TL}, CV_{TL} and Skew_{TL} between treatments ($P > 0.05$) and no potential cannibals were present in all treatments at the start. Nevertheless, daily mortality owing to cannibalism (DCM) started in the first 10 days and progressively increased with time (Fig. 1A). Throughout the experiment, DCM increased with density and decreased with feeding frequency ($P < 0.05$). Daily mortality owing to other causes (DOM) occurred in the first 10 days and increased with density at day 20 disregarding feed frequency ($P < 0.05$, Fig. 1B). While DOM was progressively replaced by DCM from day 20 onwards in the fish fed once a day, DOM increased by day 30 in the fish fed three times daily and remained higher until the end of the experiment ($P < 0.05$). In general, daily total mortality consistently increased with density and decreased with feeding frequency throughout the experiment ($P < 0.05$, Fig. 1C).

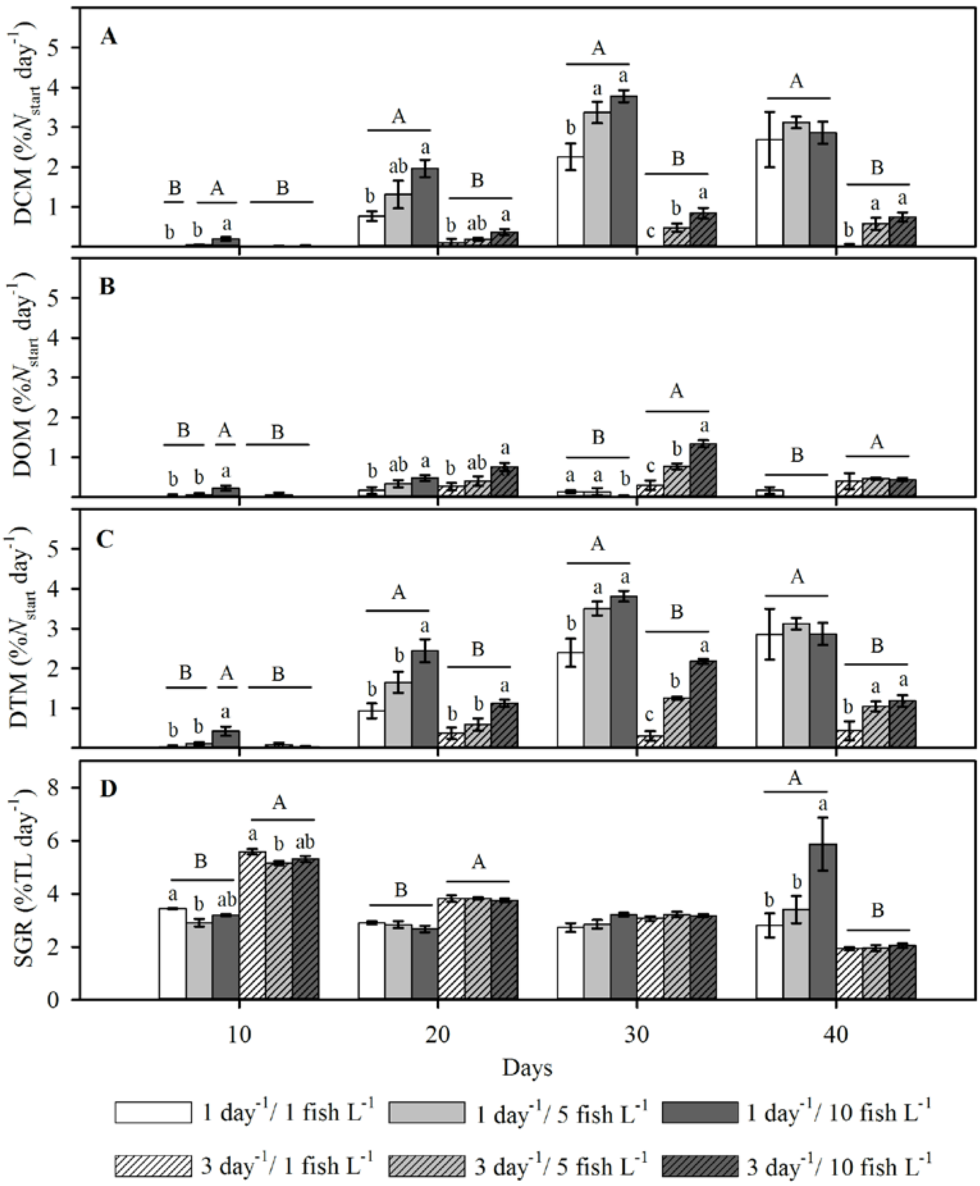

Fig. 5.1. Daily mortalities (% N_{start} day^{-1}) and specific growth rate (%TL day^{-1}) in juvenile barramundi stocked at three densities (1, 5 and 10 fish L^{-1}) and fed at two feeding frequencies (once or three times a day) during a 40-day experimental period. Panel A: daily mortality owing to cannibalism (DCM); Panel B: daily mortality owing to other causes (DOM); Panel C: daily total mortality (DTM); and Panel D: specific growth rate (SGR). Bars and whiskers are the means and standard deviations of three replicates. Different capital letters on the same day represent significant differences between feeding frequency, and different low-case letters within the same feeding frequency represent significant differences between stocking density ($P <$ 0.05).

Barramundi fed three times daily grew faster than those fed once a day in the first 20 days ($P > 0.05$, Fig. 1D). However, the specific growth rate (SGR) of those fish fed three times daily decreased continuously over time, showing an inverse relationship between growth and fish size. By contrast, SGR did not decrease over time in the fish fed once daily and even tended to increase in the high density groups. This unusual pattern was a reflection of intense cannibalism as smallest fish in the population were continuously consumed by fast growing cannibals. Apparently, SGR was extremely high in the last 10 days in the fish fed once daily and at the density of 10 fish L^{-1} leading to a comparable $Mean_{TL}$ with the fish fed three times daily at the end of the experiment (Fig. 2A).

Fish size heterogeneity (CV_{TL}) was consistently higher in barramundi fed once a day than in those fed three times daily throughout the experiment ($P < 0.05$, Fig. 2B). In the latter groups, CV_{TL} remained almost unchanged with the course of the experiment whereas it continuously increased in those fish fed once a day. The increase of CV_{TL} was faster in the fish at intermediate or high density than at low density, where the impact of cannibalism was higher during the experiment.

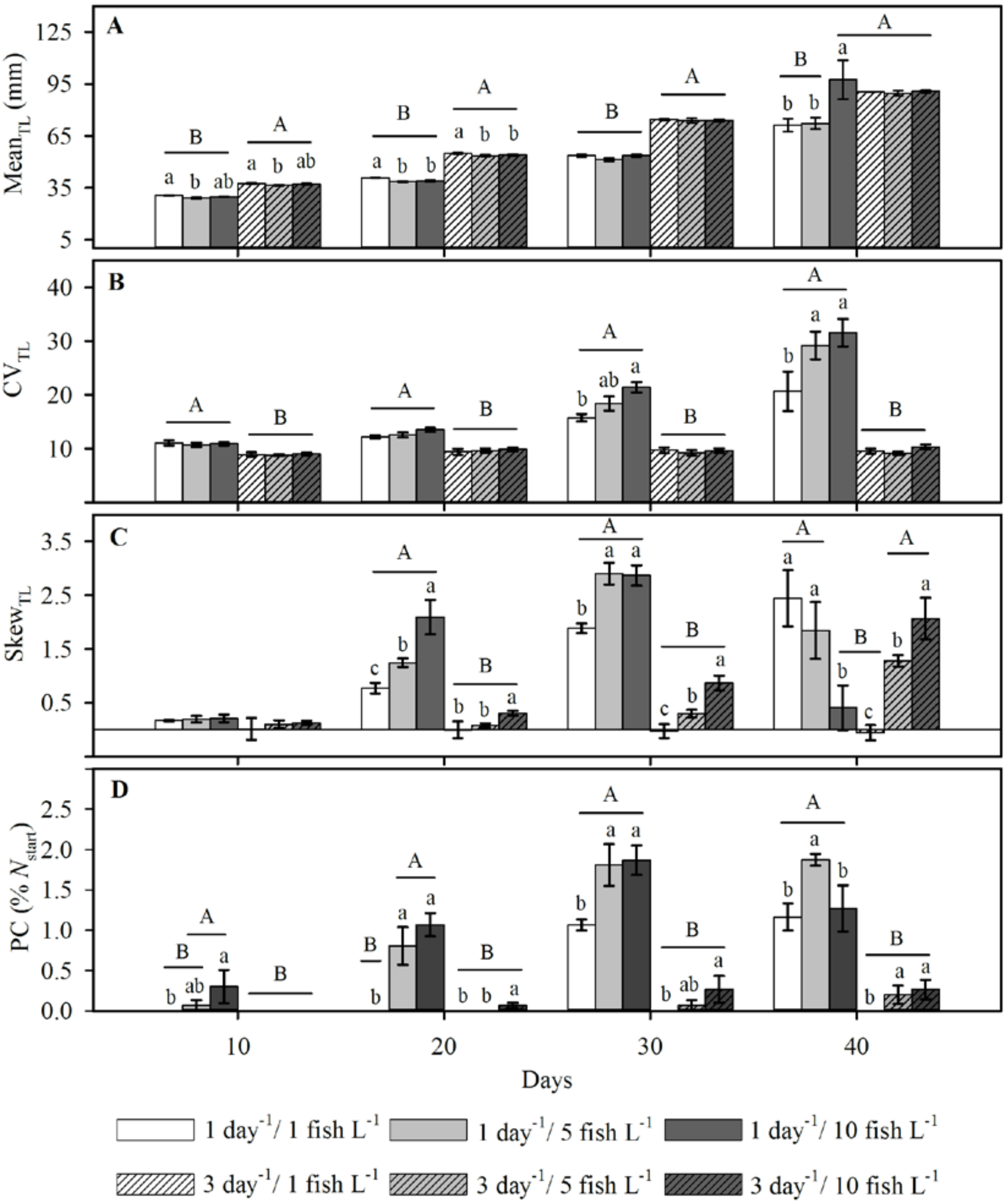

Fig. 5.2. Population parameters of juvenile barramundi stocked at three densities (1, 5 and 10 fish L^{-1}) and fed at two feeding frequencies (once or three times a day) during a 40-day experimental period. Panel A: mean total length (Mean$_{TL}$, mm); Panel B: coefficient of variation of total length (CV$_{TL}$); Panel C: the population skewness of total length (Skew$_{TL}$); and Panel D: percentage of potential cannibals (PC, % N_{start}), assuming that a fish must be 50% larger than the smaller fish to exercise complete cannibalism. Bars and whiskers are the means and standard deviations of three replicates. Different capital letters on the same day represent significant differences between feeding frequencies, and different low-case letters within the same feeding frequency represent significant differences between stocking density (P < 0.05).

Barramundi size populations in all treatment combinations showed narrow shaped bell-curve distributions in the first 10 days (Fig. 3) with low but positive skewness (Fig. 2C). Nevertheless, a low percentage of potential cannibals (PC) was detected in the fish fed once a day and stocked at intermediate and high densities by day 10 (Fig. 2D). From day 10 onwards, Skew_{TL} and PC gradually increased in the fish fed once a day, suggesting that restricted feeding did not suffice to support the growth of small fish and induced the emergence of cannibalistic fish, leading to the wide spread of fish size distributions (Fig. 3A, B and C). The Skew_{TL} and PC continuously increased until day 40 in fish fed once a day at low and intermediate densities, but both variables decreased in fish at high density (Fig. 2C and D) where most small fish were consumed by cannibals. However, as long as some small fish survived, CV_{TL} remained high (Fig. 2B). The dynamic patterns of increasing Skew_{TL}, PC and DCM over time in the fish fed three times daily were delayed compared with those in the fish fed once a day. By day 20, the Skew_{TL} of barramundi fed more frequently increased slowly and the fish size distributions were more evenly spread comparing with the fish fed once daily (Fig. 3D, E and F). As a result, the percentage of potential cannibals in the fish fed three times a day was low by day 20 in comparison with the fish fed less often (Fig. 2D). In contrast, from day 30 onwards, fish size distributions became more positively skewed at intermediate and high densities, leading to the increase in the percentage of potential cannibals. By day 40, the situation of a few cannibals and many small individuals in the fish fed three times daily resembled that on day 20 when fish were fed once daily, a similar pattern delayed by 20 days due to the increase of feeding frequency (Fig. 2C, 3F and E). Nevertheless, at low density both Skew_{TL} and PC were not detected throughout the experiment (Fig. 2C, D) and the size distribution was evenly spread (Fig. 3D).

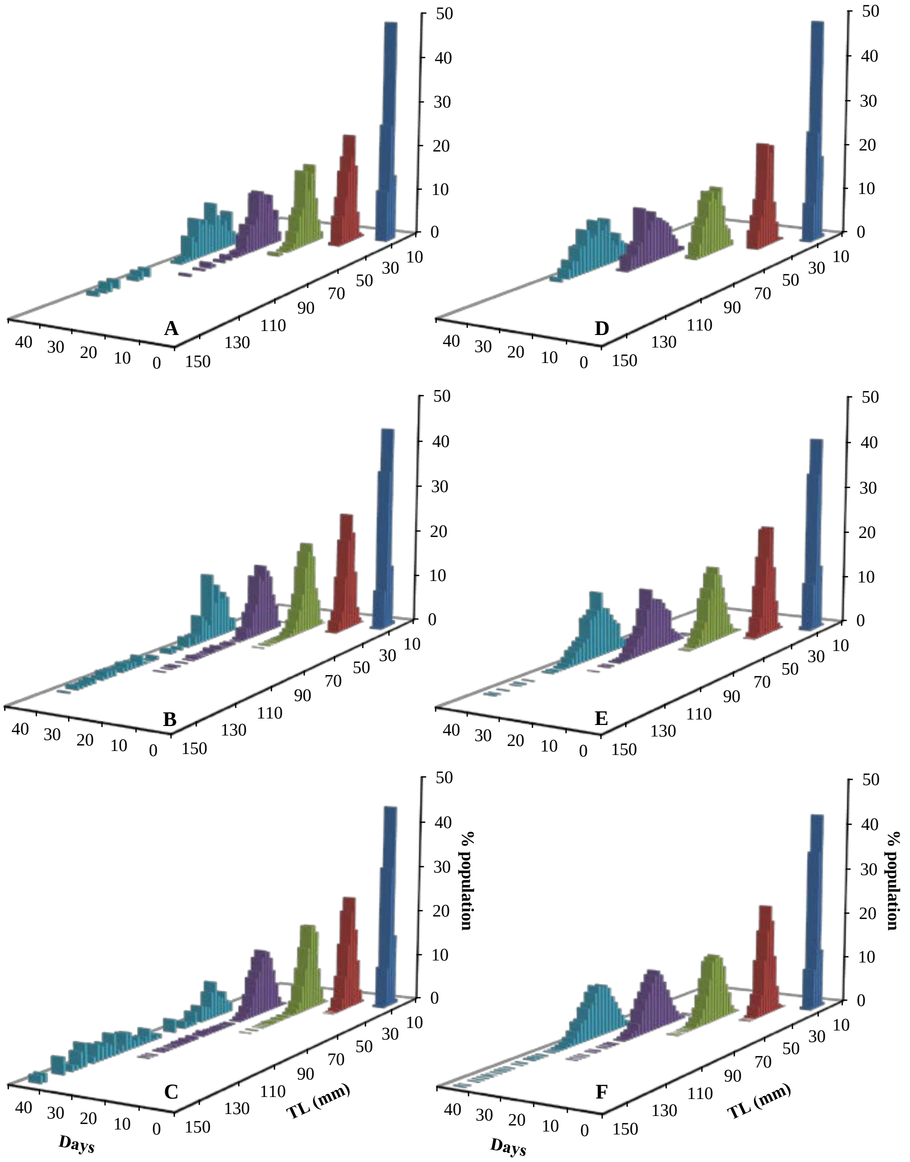

Fig. 5.3. Barramundi length distribution at three stocking densities (1, 5 and 10 fish L^{-1}) and fed at two feeding frequencies (once or three times a day) over time. Histograms represent fish mean length distribution on each sampling day (0, 10, 20, 30 and 40 days). Left panels are fish fed once a day and stocked at 1 (Panel A), 5 (Panel B) and 10 (Panel C) fish L^{-1}; Right panels are fish fed three times a day and stocked at 1 (Panel D), 5 (Panel E) and 10 (Panel F) fish L^{-1}. X-axis is fish total length (TL, mm); Y-axis is days; Z-axis is fish size frequency (% population).

5.5. Discussion

In the present study, cannibalism in barramundi (*L. calcarifer*) was substantially reduced or delayed by the increase of food availability, which is consistent with the findings on other cannibalistic fish species such as European sea bass (*D. labrax*) (Katavić et al., 1989), snakehead (*Channa striatus*) (Qin and Fast, 1996) and Japanese flounder (*Paralichthys olivaceus*) (Dou et al., 2000). Furthermore, cannibalism in barramundi was positively density-dependent disregarding the feeding regime, as also documented in other fish species such as in European sea bass (Hatziathanasiou et al., 2002), European perch (*P. fluviatilis*) (Kestemont et al., 2003) and fat snook (*C. parallelus*) (Corrêa and Cerqueira, 2007).

Cannibalism in barramundi was directly affected by both feeding frequency and stocking density but also indirectly regulated by fish size heterogeneity in the present study. Cannibalism is size restricted in the early life stages due to gape size limitation. Cannibalistic barramundi are morphologically capable of ingesting a maximum conspecific prey up to 78% of their body length (Ribeiro and Qin, 2013), but the predation efficiency is negatively correlated to prey size as prey smaller than 50% of the cannibal size can result in high energetic return to the cannibal (Ribeiro and Qin, 2014). Therefore, once the 50% threshold of prey-cannibal size difference was attained, potential cannibals emerged and their relative abundance dictated the severity of the upcoming cannibalism mortality. This is especially true in the condition of low feeding frequency as observed in barramundi fed once a day, where fast growing cannibals continuously preyed on slow growing conspecifics.

The restricted feeding regime of one meal a day induced the emergence of predatory behaviour in a few days as observed by the frequent attacking behavior and dead fish with body injury, reflecting the incapability of cannibalistic barramundi to consume the whole prey due to low size heterogeneity and gape size restrictions. Feeding restriction not only promotes forage activities by hunger motivation, encounter rates of conspecifics and prey vulnerability (Polis, 1981, Elgar and Crespi, 1992, Hecht and Pienaar, 1993, Baras, 2013, Cuff, 1977). but can also lead to food competition, variability in food intake and establishment of a dominant hierarchy, resulting in growth depensation and size heterogeneity (Hecht and Pienaar, 1993, Baras and Jobling, 2002, Kestemont et al., 2003, Baras, 2013). Therefore, the intense food competition in fish fed once daily together with initial cannibalism led to the increase of size heterogeneity, the emergence of individuals large enough to exercise complete cannibalism, and the progressive shift from non-cannibalism by cannibalism mortality. Since cannibalistic barramundi gain growth advantage over non-cannibalistic siblings (Ribeiro and Qin, 2015), cannibalism became intense under food restriction as the cannibal would persistently find conspecifics to prey on, further increasing size dispersal (Baras, 2013). Therefore, cannibalism prevailed throughout the experimental period due to a combination of hunger, size heterogeneity and improved cannibalistic skills under restricted food supply.

Barramundi fed three times a day grew more rapidly than those fed once a day, though the growth of less frequently fed fish was apparently accelerated toward the end as a result of intense cannibalism. Furthermore, the size heterogeneity of fish fed three times daily was also low, which collaborates the notion that high feeding frequency enables most fish to gain access to food and achieve homogeneous growth (Baras and Jobling, 2002, Katavić et al., 1989, Kestemont et al., 2003). Such conditions almost suppressed the emergence of cannibalism and consequently reduced fish mortalities when they were fed three times a day. The dynamics of cannibalism, size dispersal and skewness indicate that the cannibalism in these groups was not only reduced, but also delayed when compared with fish fed once a day. The situation of a few cannibals and many small individuals in more frequently fed fish towards the end of the study resembled that on day 20 in fish fed once daily. It is possible that if the experiment had lasted longer, cannibalism rates would have increased in the fish fed three times a day at the intermediate to high stocking densities, while they would inevitably be reduced in fish fed once a day due to the substantial reducing in the number of fish small enough to be ingested by cannibals.

In the present study, mortality owing to cannibalism was increased at high fish density, but was further amplified under restricted feeding. In European perch, the density-dependent cannibalism is mediated by food availability as each fish cannot equally access food in spatially restricted tanks (Kestemont et al., 2003). In the present study, the high density combined with low feeding frequency potentially provoked intense food competition leading to growth depensation, size heterogeneity and cannibalism mortalities in barramundi. In fish fed once a day, high cannibalism occurred at moderate and high stocking densities in the first 30 days but the fish density-dependent cannibalistic mortality wan not detected after 30 days as a result of reduction of small fish in the early period. Consequently, by the end of the experiment, the fish population under restricted feeding consisted of a few large cannibalistic individuals with a uniform size and a very low number of small prey. Similar pattern was observed in less frequently fed population of European sea bass, where cannibalistic individuals eliminated most of the small fish and cannibalism ceased thereafter (Katavić et al., 1989). As an indirect effect of cannibalism, the barramundi fed once a day and at high density reached similar size to the fish fed three times a day by the end of the experiment. The removal of small fish by cannibalism and the faster growth rate of cannibals in the less often feeding group contributed to an apparent faster growth rate when compared with fish fed three times a day and with low incidence of cannibalism.

In summary, the increase of population size skewness can be an indicator for the contingent risk of cannibalism, whereas the increase of size heterogeneity together with abnormally rapid growth of the population is the reflection of ongoing cannibalism, which agrees with the view of Baras (2013) on cannibalism dynamics. Although cannibalism in barramundi cannot be completely eradicated by the manipulation of fish density and feeding frequency, it can be significantly reduced or delayed by the increase of feeding frequency coupled with low fish stocking density.

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Chapter 6

General Discussion

Intracohort cannibalism is a major problem in the aquaculture of piscivorous fish, potentially resulting in significant production losses during the larviculture and nursery period (Hecht and Pienaar, 1993, Baras and Jobling, 2002, Baras, 2013). Although cannibalism can start from the early ontogeny of fish larvae, in most fish species cannibalism becomes evident after the metamorphosis (Baras and Jobling, 2002). At this life stage, such an intraspecific predation becomes size-limited since cannibals ingest the whole prey with head first, termed complete cannibalism (Baras and Jobling, 2002). Consequently, the risk of cannibalism is mirrored by fish size heterogeneity in the population (Baras, 2013). Therefore, the understanding on the functional processes and the size-dependent mechanisms behind cannibalism are essential to develop protocols for fish cannibalism control in aquaculture. The present thesis contributes to new knowledge in the piscivorous prey-predator relationship and in the growth – size heterogeneity – cannibalism dynamics, through morphological (chapter 2), behavioural (chapter 3), bioenergetics (chapter 4) and biotical (chapter 5) approaches. The outcomes of this thesis have achieved the main research objective and provided insights into the understanding on the functional mechanisms underlying fish cannibalism, ultimately contributing to fundamental knowledge for cannibalism control in the hatchery phase of fingerling production. As a model fish species, the barramundi *Lates calcarifer* adequately illustrated the characteristics of cannibalism through all the approaches used in the present thesis.

6.1. Knowledge Advance and Research Significance

6.1.1. The impacts of polyphenism and allometric growth on fish cannibalism

As demonstrated by the prey-predator relationships across a variety of teleost species, morphological traits determine the maximum prey size that predators can ingest (Dabrowski and Bardega, 1984). Assuming that a cannibal can ingest a prey if the cannibal mouth size is equal or larger than the prey body depth, species-specific models have been developed to predict the maximum prey size that cannibals are morphologically capable of ingesting (Chapter 2). A previous model predicted that barramundi of 10 – 50 mm in total length (TL) have a morphological capacity to ingest prey with 67 – 61% of the cannibal TL, respectively (Parazo et al., 1991). However, the concept of morphological polyphenism has never been considered in the development of fish cannibalistic model. Polyphenism refers to phenotypic changes in a single genotype in response to environmental stimuli (West-Eberhard, 1989, Hoffman and Pfennig, 1999, Smith and Skulason, 1996, Closs et al., 2003). For instance, in a challenging environment, some fish individuals can evolve into a cannibalistic morph (Smith and Reay, 1991, Polis, 1981) by further extending their mouth opening with the provision of progressively larger prey.

In Chapter 2, the morphological model based on the open mouth width predicts that cannibals from 30 to 140 mm TL can ingest a maximum prey of 61% of the cannibal TL, which corroborates the predictive model proposed by Parazo et al. (1991). However, the empirical observations showed that barramundi cannibals could ingest conspecific prey larger than the model prediction. Cannibals of 25 – 131 mm TL have the capacity to ingest conspecific prey up to 78 – 72% of cannibal TL, respectively, with the challenge of increasingly large prey. This finding suggests that the models based on the average morphological traits of a population largely underestimate the maximum prey size that a cannibal can possibly ingest. However, when polyphenism was considered on measuring the width of mouth opening, the model prediction became closer to the realistic observations. Therefore, when predicting the upper limit of a prey size in complete cannibalism, the assumption of cannibalistic polyphenism must be considered to keep a safe margin and avoid significant losses due to cannibalistic mortality in fish farming.

The reduction of the predation capacity as cannibals grow larger is a result of the negative-allometric growth of the mouth size, suggesting that cannibalism is more likely to occur at the early stage. This notion is supported by the results from other cannibalistic fish species such as Atlantic cod *Gadus morhua* (Otterå and Folkvord, 1993) and snakehead *Channa striatus* (Qin and Fast, 1996). Furthermore, naïve barramundi could become cannibals when the first-offered prey size was <50% of the cannibal TL, but fish would never become a cannibal when the size of the first offered prey was >58% of cannibal TL. This indicates that the challenge with a prey smaller than 50% of the largest fish in the population can trigger cannibalism in barramundi.

6.1.2. Cannibals select for small prey despite the availability of large prey

Chapter 2 indicates that barramundi cannibals are morphologically capable of ingesting conspecific prey up to 78% of their sizes. However, as observed in many piscivorous fishes (Juanes, 1994), cannibals may prefer ingesting a prey that is much smaller than what they are capable of preying though it may be sometimes at odds with the optimal foraging theory (Baras, 2013). Chapter 3 of the present thesis documented that barramundi cannibals (40 – 140 mm TL) select for smaller prey, which corroborates with the findings on other cannibalistic fish species such as giant grouper *Epinephelus lanceolatus* (Hseu et al., 2007), orange-spotted grouper *E. coioides* (Hseu and Huang, 2014), Atlantic cod *G. morhua* (Blom and Folkvord, 1997), yellow perch *Perca flavescens* (Post and Evans, 1989), Arctic char *Salvelinus alpinus* (Amundsen et al., 1995), catfish *Pangasius djambal* (Baras et al., 2010), dorada *Brycon moorei* (Baras et al., 2000) and Amazonian catfish *Pseudoplatystoma punctifer* (Baras et al., 2011). According to the optimal foraging theory, Chapter 3 showed that barramundi cannibals select prey for high profitable return, since a smaller prey demands shorter handling time and is easier to capture. Therefore, any prey smaller than 50% of the cannibal length can result in higher energy return to the cannibal. The behavioural analysis also indicated that the prey size selection in barramundi cannibals is passively orientated towards the small and most vulnerable prey, supporting the opportunistic foraging behaviour of barramundi (Davis, 1985). Capture success significantly reduced as cannibal grew larger, reflecting the better ability of larger prey for predation avoidance (Fessehaye et al., 2006, Hseu and Huang, 2014) and low predatory capacity of larger cannibals (Chapter 2), ultimately contributing to the reduction of energy profitability for cannibals as barramundi grow larger. Furthermore, in an ungraded barramundi population under captivity, once smaller and more vulnerable prey have succumbed to cannibalism, cannibals are forced to move up to consume larger prey that are not as profitable as smaller prey. This scenario would lead to a progressive reduction of size heterogeneity and consequently reduce the propensity of cannibalism as barramundi grow larger.

6.1.3. Discovery of the growth advantage in cannibalistic barramundi over siblings fed formulated diet

The outcomes from Chapters 2 and 3 indicate that barramundi cannibalism in a captive population is progressively reduced as the cannibal size gets larger due to morphological constraint of cannibals and reduction of prey vulnerability. In addition, the preference for smaller prey found in Chapter 3 indicates that cannibalism can be a “self-sabotage” strategy as the cannibalistic events progressively decrease the number of ingestible small prey in the population, ultimately reducing fish size heterogeneity and cannibalism. In Chapter 4, this thesis addresses an important component in fish cannibalism that is usually overlooked in other studies, *i.e.*, the growth advantage of cannibals over their siblings feeding on formulated diets. For example, cannibalistic larvae of catfish *Pangasius djambal* did not show consistently better conversion efficiency over siblings feeding on *Artemia*, therefore cannibalism did not bring any growth advantage and rapidly vanished during the ontogeny (Baras et al., 2010). Conversely, cannibalistic larvae of *Hemibagrus nemurus* indeed showed better conversion efficiency and growth advantage over siblings feeding on *Artemia*, overcompensating size-dependent constraints upon growth and ingestion capacity, leading to even greater size heterogeneity and long-lasting cannibalism (Baras et al., 2013, Baras, 2013). Barramundi preying on conspecific <50% of cannibal size clearly demonstrated growth advantage by channelising more energy intake into growth than conspecifics fed exclusively on formulated diets (Chapter 4). The growth advantage was obtained by a higher conversion efficiency and higher apparent digestibility. Therefore, once a size difference below 50% is attained, cannibals will emerge in a barramundi captive population and fast-growing cannibals will continuously find slow-growing and high profitable victims to prey on. The higher cannibal growth may over-compensate cannibalistic restrictions by morphological dimension (Chapter 2) and prey vulnerability (Chapter 3) as fish grow larger. The provision of formulated diet to cannibalistic fish significantly reduced cannibalism rate compared with cannibals feeding exclusively on conspecific (Chapter 4), indicating that cannibalism in barramundi can be reduced by increasing the amount of alternative food.

6.1.4. Relationship between feeding frequency and stocking density on cannibalism reduction

The severity of fish cannibalism can be substantially regulated by biological variables in aquaculture (Hecht and Pienaar, 1993, Baras and Jobling, 2002, Baras, 2013). Any restriction on food supply can lead to cannibalism simply by hunger motivation (Katavić et al., 1989). Similarly, the increase in fish density can also enhance cannibalism due to a high predator-prey encounter frequency (Sogard and Olla, 1994). High fish density coupled with insufficient food supply can stimulate food competition, social hierarchy, growth discrepancy, size heterogeneity and cannibalism as observed in European sea bass *Dicentrarchus labrax*, European perch *Perca fluviatilis* and other fishes (Baras et al., 2003). Therefore, Chapter 5 explored the effects of feeding frequency and fish stocking density on the temporal dynamics of growth, size heterogeneity and cannibalism in barramundi. Chapters 2 and 3 demonstrate that cannibalism in barramundi will emerge when the prey are smaller than 50% of the cannibal size. Chapter 4 reveals that once cannibals start to prey on conspecifics smaller than 50% of the cannibal size, cannibalism would become permanent since the fast-growing cannibals would continuously find slow-growing and high profitable victims to prey on. Thus, Chapter 5 tested the hypothesis that once an initial size grading is performed, size heterogeneity and cannibalism in barramundi can be controlled by reducing fish density and increasing feeding frequency. Cannibalism in barramundi was positively density-dependent and negatively food-dependent (Chapter 5). The combination of low feeding frequency and high stocking density promoted food competition and variability in food intake, causing size heterogeneity within a few days in a population started with a uniform size distribution. Within 10 days of experiment, fish smaller than 50% of the largest fish size emerged in the treatment of low feeding frequency, leading to the emergence of cannibalism. Fast growing cannibals were subsequently found due to predation on slow-growing conspecifics under food restriction and cannibalism lasted until the end of the experiment. This study confirms the hypothesis that frequent feeding can lead to fast fish growth, uniform fish size and reduction or postponed cannibalism. Nevertheless, a few potential cannibals still emerged at high stocking density despite high feeding frequency, causing low but steady mortalities owing wounds or suffocation. Therefore, although complete cannibalism (*i.e*. swallowing the whole prey) was reduced through the increase of feeding frequency, cannibalistic attempts were still observed and contributed to fish injury and non-ingested mortalities, indicating an intrinsic aggressive – cannibalistic motivation in barramundi, which cannot be completely eradicated only through manipulation of environmental and biotic factors.

6.2. Conclusions and Recommendations

This thesis explored the mechanisms of morphological limitation, prey size selection and growth advantage in fish cannibalism and assessed the effects of biotic factors on the temporal dynamics of cannibalism. The outcomes of the thesis research not only contribute to better understanding of the prey-predator relationship in piscivorous fish, but also provide crucial information to guide size grading practices and protocols for cannibalism control in barramundi aquaculture. The major conclusions and recommendations are summarized below:

6.2.1. Cannibalism is morphologically determined by the mouth dimension of cannibals and the body depth of prey

Morphological models predicted that barramundi are capable of ingesting conspecific prey up to 61% of the cannibal size. However, in a challenging study by progressively offering large prey, cannibalistic polyphenism may escalate the upper limit to 78% of the cannibal size. Cannibalism is more likely to occur at early juvenile stages since the predation capacity decreases as the size of cannibal increases. Therefore, it is recommended an efficient size grading at early juvenile stages in barramundi aquaculture by removing all potential cannibals that are larger than 1.28 (*i.e.*, predator/prey ratio of 1:0.78) times of small fish. Once all cannibals are removed, a size difference of 50% should be set as the threshold to avoid the emergence of new cannibals.

6.2.2. Cannibals select for a smaller prey than they are morphologically able to ingest

Although barramundi are morphologically able to ingest a conspecific prey up to 78% of their size, cannibals prefer selecting smaller prey to gain higher energy intake per unit of catching effort. However, the selection for smaller and most profitable prey is passively orientated towards the most vulnerable prey size, rather than being an active choice by cannibals. Cannibalism profitability decreases as cannibals grow larger due to reduction in capture success. The increase of prey escape skills and the progressively morphological constraint of cannibals would lead to the reduction of cannibalism in large barramundi. Therefore, it is recommended that size grading practices be focused on the early phase of larvae/juvenile culture as the cannibalistic events tend to phase out as fish size is over 10 cm TL.

6.2.3. Exclusive cannibalistic individuals grow faster than siblings feeding on high energetic formulated diets

Cannibalistic barramundi exclusively preying on conspecifics grow faster than those fed with formulated diet, suggesting that cannibals can obtain energy from live prey more effectively than from the formulated diet. This explains the empirical observation that once cannibalism emerges in barramundi aquaculture, the fast-growing cannibals can easily find slow-growing conspecifics and cause great mortality due to cannibalism. This finding corroborates with the previous recommendation highlighting the importance of size grading in the early juvenile stages. However, this study reinforces the necessity to keep the size difference below 50% after the initial size grading. Otherwise, efficient cannibals would emerge from the population leading to ever-lasting cannibalism in barramundi aquaculture.

6.2.4. High stocking density provokes cannibalism while the increase in feeding frequency suppresses cannibalism

After an initial size grading, cannibalism in barramundi was significantly reduced or postponed by feeding the fish three times a day and stocking them at the low fish density of 1 fish L^{-1}. The reduction of feeding frequency to once a day and the increase in stocking density up to 10 fish L^{-1} induced or anticipated cannibalism not only by hunger motivation and high encounter rate, but also by the increase in food competition, food intake variability and size heterogeneity. However, regardless of the feeding frequency and stocking density, mortalities owing to other causes, such as wounds and suffocation still occurred, indicating that aggressive or predatory behaviour is inherent to barramundi. Therefore, the present thesis recommends a two-step management strategy: first by size grading at the early stage of larva/juvenile culture, and then by feeding fish at least three times a day. Although fish cannibalism can be reduced by lowing fish density, the hatchery manager should consider the trade-off between fish density and profitability to decide the realistic stocking density in hatchery operation. The management through manipulating of environmental and biotic factors would reduce or postpone size heterogeneity and consequently lead to low cannibalism during nursery, but cannibalism could not be totally eradicated as it is inherent to barramundi.

6.3. Future Research

Although this thesis provides new understanding in fish cannibalism, ultimately contributing to the development of size grading practices and general protocols for fish cannibalism control in hatcheries, some questions still remain outstanding. Therefore, future research should focus on tackling the following issues:

6.3.1. Cannibalistic polyphenism was only inferred and further study is needed to define its variation

Although this thesis has inferred the existence of cannibalistic polyphenism in barramundi, cannibalistic traits were not fully examined and evidenced. Its existence was based on the discrepancy between model predictions and empirical observations on fish cannibalism. Future studies should investigate the cannibalistic traits in barramundi. Particularly, detailed histological structure should be examined on the mouth apparatus of cannibals challenged with progressively increasing prey sizes. Further studies on cannibalistic polyphenism would benefit the cannibalism control practice, especially considering it as a part of selective breeding programs to cull out potential “super” cannibals.

6.3.2. The energetic profitability of cannibals preying on different prey sizes should be further validated through bioenergetic analysis

In Chapter 3, a behaviour-energy analysis was used to tackle the prey size selection and profitability for cannibals preying on conspecifics of different sizes, leading to the conclusion that the selection for smallest prey is a foraging strategy to acquire the most profitable food item. Although such a conclusion was based on a variety of prey sizes, the energetic analysis for growth and gross conversion efficiency was not done across different prey sizes. Such an energetic study would require a dedicated experiment with a substantial more number of fish and more complex facility, but both timing and logistics were restricted in this study. Therefore, the bioenergetics of cannibals preying on different fish sizes is worth further investigation.

6.3.3. The impact of abiotic factors on cannibalism in barramundi needs further exploration

The original research proposal of the present thesis included the examination of the effect of abiotic factors such as light properties, tank colour and water mixing regimes on cannibalism in cultured fish. However, preliminary trials and deeper literature review indicated that cannibalism in fish is mainly dependent on size heterogeneity. Since a detailed examination on the relationship of size variation and cannibalism in barramundi has never been conducted, the research was then focused on the exploration of the functional mechanisms underlying cannibalism and centred at the size differences between cannibals and conspecific prey. Therefore, the outcomes of the present thesis provide a solid base for further testing the effects of abiotic factors on cannibalism. Further knowledge on the impacts of light regimes, background colour and water mixing on cannibalism would provide alternative methods to control cannibalism in barramundi.

6.3.4. Cannibalism potential should be considered a trait in further selective breeding programs for barramundi

Cannibalism is a strong inherent trait in some fish at least during a certain period of life history. In Chapter 5, environmental biotic management reduced or postponed size heterogeneity and consequently resulted in low cannibalism in barramundi, but predatory behaviour still existed even in the presence of high food abundance, low fish density and low size heterogeneity. Such outcomes indicate that cannibalism is inherent to barramundi. Therefore, future research on the genetic contribution of cannibalism is recommended to develop a breeding program to cull out individuals with more cannibalistic traits such as large mouth dimension, aggressive attacking behaviour and low body depth.

6.4. References

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