Towards understanding the effect of size variation on the aggressive and feeding behaviours of juvenile dusky kob *Argyrosomus japonicus* (Pisces: Sciaenidae)

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Abstract

Many studies have been conducted on the effect of size-grading in other fish species. However, there is a paucity of scientific information on the effects of size variation on cannibalism of juvenile dusky kob. Thus, a study focusing on the effect of size variation on juvenile dusky kob aggressive and feeding (browsing) behaviours was conducted.

Three separate groups of hatchery-reared juvenile dusky kob of were obtained from Oceanwise (Pty) Ltd for use in a series of three experimental trials. In all trials, juvenile fish of the same age were size-graded and the COV-value was used to determine the size variation. The focal fish (largest fish) was exposed to groups of fish with different size variation for 30-min. behavioural observations before and after feeding in randomised trials. The first experimental trial (Chapter 2) quantified the effect of increasing size variation and observation time on the aggressive and browsing behaviours of juvenile dusky kob. On average, juvenile dusky kob weighed 3.60 ± 0.68 g $fish^{-1}$ and measured 5.8 ± 0.41 mm. Each focal fish was observed (a) before feeding in the morning, (b) 2 h after feeding, (c) 6 h after feeding and (d) 12 h after feeding. Fish increased browsing behaviours (averaging 6.60 ± 0.56) and decreased intimidating aggressive behaviours (18.60 ± 1.39) 12 h after feeding. Other aggressive behaviours occurred but did not differ between observation times. Aggressive and browsing behaviours positively correlated with size variation variables, predominantly, 12 h after feeding. An average frequency of 19 chases were observed positively correlated with size variation, followed by average frequencies of 17 body bites and browses, and 11 tail bites per 30 min. Some behaviours including average frequencies of 0.2 chases, 4 tail bites, 2.4 intimidating and 0.3 browsing behaviours negatively correlated with the size variation, generally closer to the last meal. These preliminary observations thus showed that fish have become hungry approximately 6-12 h after feeding and substituted certain behaviours for others as time after feeding passed and as size variation increased. The second experimental trial (Chapter 3) consisted of the observations further testing the relationship between aggressive

behaviours and size variation of juveniles of dusky kob averaging 0.43 ± 0.27 g fish^{-1}. The focal fish was exposed to groups of fish of four size variation (COV) treatments for observations before and 12 h after feeding. Aggressive behaviours positively correlated with size variation both before and 12 h after feeding. An average frequency of 437 body bites positively correlated more often with COV, followed by average frequencies of 365 intimidating behaviours and 199 tail bites per 30 min., respectively, before and 12 h after feeding. The least often exhibited aggressive behaviours averaged 26 chases while positively correlating with size variation on the times specified. An average frequency of 311 intimidating behaviours before and after feeding including average of 28 tail bites after feeding negatively correlated with size variation. This may relate to shift of behaviours depending on the needs and capacity of the fish. Apparently, aggressive fish can change its behaviour as a function of COV-values rather than the mean size of the other fish. The third trial (Chapter 4) investigated the effect of the aggressor's (focal fish) size in relation its aggressive and browsing behaviours to other fish (non-focal fish). Juvenile fish used for this trial, on average, weighed 30 ± 7.63 g fish^{-1}. Aggressive and browsing behaviours were observed in four treatments of a) high COV and mean weight below, b) low COV and mean weight less, c) high COV and mean weight equivalent to and d) low COV and mean weight higher than that of the focal fish. Increased frequencies of aggressive and browsing behaviours per 30 min. occurred in treatment A, sharing similar frequencies in treatment C, compared to the other treatments (B and D) which shared certain frequencies. The intimidating behaviours predominated, followed by browsing, body bites, chases and tail bites, respectively. The results of the overall study suggest that the time passed after feeding and increasing size variation and differences facilitated aggressive and browsing behaviours in juvenile dusky kob. Dusky kob showed increasing aggressive behaviours as early as in the first two weeks after hatching, averaging 0.43 ± 0.27 g fish^{-1} with the frequency correlating with size differences. Consistent size-grading technique in the same-age fish should be used to manage size variation associated with aggressive behaviours. The period of about 4-6 h after feeding may explain the noticeable increased aggressive and browsing behaviours. Thus, fish should be fed immediately before or after evacuation of their guts to maintain less-aggressive behaviours of juvenile dusky kob. Fish generally increased aggressive and browsing acts before and long time after feeding than closer to after feeding. The study has provided the fundamental scientific groundwork for fish farmers and future researchers can further explore size variation, time after feeding and gut evacuation rate as critical components of aggressive behaviours. The scientific knowledge of aggressive and cannibalistic behaviours has essential application in farming management to achieve improved survival and growth rates in juvenile fish.

Table of contents

Declaration

I, **Siviwe Elvis Babane** (G08b0002), declare that this thesis is my own, original work and that it has not been submitted for any degree or examination in any other university and that all the resources used or quoted have been indicated and acknowledged by complete references.

Signature .... Dale 14/11/2017

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Chapter 1

General introduction

In order to create protocols for increasing growth and survival rate of fish in aquaculture, a clear insight of physically and biologically limiting factors in young fish is important (Downing and Litvak, 1999; Monk *et al*., 2006). Behavioural interactions between siblings and the effect of size-grading on growth and survival require a special attention to better understand how fish grow and behave under culture conditions (Jobling and Reinsnes, 1987). Cannibalism is a commonly occurring phenomenon and it is probably more widespread than reported in the literature (Kvarnemo *et al*., 1998), particularly when food resources are limited (Amundsen, 1994) under culture conditions. Cannibalistic behaviour has been considered an alternative strategy of feeding when resources are limited, more likely, employed by larvae and early juveniles that are carnivorous (Hecht and Pienaar, 1993). Information about cannibalistic behaviour and factors moderating it along with environmental improvements are prerequisites for reducing aggression between fish under aquaculture conditions (Baras and Jobling, 2002). The common key factors of cannibalistic behaviours include food availability and feeding frequency (McCarthy *et al*., 1992; Hecht and Pienaar, 1993; Goldan *et al*., 1998; Baras *et al*., 1999; Liao *et al*., 2001; Almaza´n-Rueda *et al*., 2004) and size variation (Hecht and Pienaar, 1993; Baras and Jobling, 2002; Solomon and Udoji, 2011; Baras, 2013). Additional factors include water quality (Jobling and Baardvik, 1994), stocking density (Fenderson and Carpenter, 1971; Kaiser *et al*., 1995a; PIRSA, 2003; Collett, 2007), availability of refuge (Hecht and Appelbaum, 1988) and light intensity (Britz and Pienaar, 1992; Almaza´n-Rueda *et al*., 2004; Collett, 2007).

Size variation and aggressive behaviours

Growth variation among individuals is a central problem in the culture of larvae, particularly in predatory species (Kestemont *et al*., 2003) and in commercial fish culture (Jobling, 1985). Fish vary in size (DeAngelis *et al.*, 1979; Jobling, 1985; Hecht and Appelbaum, 1988; Baras, 1998) and their increasing initial size difference would lead to cannibalistic responses (Hecht and Pienaar, 1993; Solomon and Udoji, 2011). Size variation among fish is associated with genetic differences in their growth capacity and patterns (Wallat *et al.*, 2005). However, certain factors could be manipulated to manage the genetically associated behaviours of fish without restraining normal behaviours under husbandry conditions (Thorpe and Cho, 1995). Cannibalism due to initial size variation has been reported in fish such as largemouth bass *Micropterus salmoides* (DeAngelis *et al.*, 1980), walleye *Stizostedion vitreum* (Loadman *et al.*, 1986), African catfish *Clarius gariepinus* (Hecht and Appelbaum, 1988), European seabass *Dicentrarchus labrax* (Katavic *et al.*, 1989), Atlantic cod *Gadus morhua* (Folkvord and Ottera, 1993) and Dorado *Brycon moorei* (Baras *et al.*, 2000). Growth variation has also been reported for a number of commercially important fish species such as rainbow trout *Oncorhynchus mykiss* (Li and Brocksen, 1977; Metcalfe, 1986), coho salmon *Oncorhynchus kisutch* (Fagerlund *et al.*, 1981), Atlantic salmon *Salmo salar* (Metcalfe *et al.*, 1990) and Arctic char *Salvelinus alpinus* (Jobling *et al.*, 1993). Yellow perch have been size-graded to achieve optimal growth and production in recirculation systems (Wallat *et al.*, 2005). It is important to minimise factors that lead to high size variation in combination with size-grading techniques to reduce loss of juvenile fish (Fessehaye *et al.*, 2006). Size variation promotes aggressive and cannibalistic behaviours in most cultured fish (Baras *et al.*, 2000; Liao and Chang, 2002; Valdimarsson and Metcalfe, 2000; Chang and Liao, 2003; Paulet, 2003; Zakes *et al.*, 2004; Clement *et al.*, 2005; Fessehaye *et al.*, 2006; Solomon and Udoji, 2011). Managing the size variation among cultured fish thus contributes to increasing production efficiency, reducing food wastage, and to improving water quality (McCarthy *et al.*, 1992; Jobling and Baardvik, 1994). To minimise shortcomings of size variation in commercial culture environments, fish are being size-graded (Goldan *et al.*, 1998; Zakes *et al.*, 2004). Minimal size variation within a group of fish of the same age may reduce cannibalism (Liao and Chang, 2002). In addition, Chang and Liao (2003) suggested that maintaining the size difference at or below the limit levels through size-grading techniques and increased food supply could be a good strategy for reducing the rate of sibling cannibalism. For example, cannibalism in snakehead *Channa striatus* was not avoidable, but could be minimised by grading juvenile fish into groups of similar average sizes (Qin and Fast, 1996) and maintaining fish with similar sizes in the rearing tanks also improved the utilisation of food (Collett, 2007). Cannibalistic behaviours within the rearing environment influence survival and growth in various species (Smith and Reay, 1991; Baras and Jobling, 2002). It is important to understand the average age and length when the fish change from larval to juvenile stage with notable changes in behaviour, physiology and ecology associated with metamorphosis (Fukuhara, 1991). This information is essential as feeding and aggression change after metamorphosis. For example, aggressive behaviours of Japanese flounder *Paralichthys olivaceus* started after the completion of metamorphosis (Sakakura and Tsukamoto, 2002) and a similar case was recognised in larval and juvenile goldfish, with aggression increased significantly after metamorphosis at 10 days after hatching (Paulet, 2003). Frequent cannibalism after metamorphosis, at about 35 days after hatching, was observed in different *Epinephelus* (grouper) species (Lim, 1993; Watanabe *et al.*, 1996) under culture conditions.

Access to resources and aggressive behaviours among fish

Like many other animals, some fish fight to obtain food and maintain their territory (Grant, 1997). Fish have to balance the need to search for food and the need to avoid being cannibalised (Thorpe and Cho, 1995). This makes fish at times become vulnerable to various diseases and weakness due to severe wounds, associated with high degree of cannibalism or even death (Pienaar, 1990; Kaiser *et al.*, 1995a). Fish may also suffer chronic stress, leading to appetite suppression, reduced food intake and slow growth (Refstie and Kittelsen, 1976) due to the formation of social interactions and dominance hierarchy (McCarthy *et al.*, 1992; Jobling, 1994). Generally, suppressive effect occurs on the growth of smaller fish in the presence of larger conspecifics (Jobling and Reinsnes, 1987). During intensive production, fish experience stress due to social interactions among them regarding high competition for food and space, poor water quality or procedures connected to cleaning tanks and size-grading practice (Zakes *et al.*, 2004). Species, however, differ in their ability to cope with stress under culture conditions (Davis and Parker, 1990). Competition for food limits growth of small fish (Holm *et al.*, 1990) and limited food supply is a major cue for sibling cannibalism (Baras *et al.*, 1999; Liao *et al.*, 2001). In turn, competition for resources leads to intraspecific dominance hierarchies leading to unequal benefits between fish (Pusey and Packer, 1997). Furthermore, feeding frequency affects fish growth (Wallace and Kolbeinshavn, 1988) and size variability (Basquill and Grant, 1998). Both size variation among fish and food deprivation facilitate cannibalism under culture conditions (Folkvord, 1991). In the fish hatchery environment, frequent feeding and food abundance have become the most commonly used approaches to maintain less cannibalism (Jobling, 1985; Mélard *et al.*, 1996; Baras *et al.*, 2011; Baras, 2013). However, increased feeding frequency did not influence aggression for some species including Atlantic Cod *Gadus morhua* (Folkvord and Ottera, 1993) and yellowtail flounder *Limanda ferruginea* (Dwyer *et al.*, 2002). Fish with high hunger levels are often more active and aggressive than less hungry fish (Metcalfe *et al.*, 1995). The quality and presence of food influence the development of dominance hierarchies, growth variation and cannibalism (Paller and Lewis, 1987; Hecht and Appelbaum, 1988; Hecht and Pienaar, 1993). The chances for subordinate fish to feed may be increased by higher feeding frequencies since dominant fish may become satiated and less aggressive, resulting in the reduction of size variation within a tank (Wang *et al.*, 1998). The quality and presence of food influence the development of dominance hierarchies, growth variation and cannibalism (Paller and Lewis, 1987; Hecht and Appelbaum, 1988; Hecht and Pienaar, 1993). A continuous provision of food helped to control sibling cannibalism in juvenile Atlantic cod *Gadus morhua* (Folkvord, 1991) and Atlantic mackerel *Scomber scombrus* (Fortier and Villeneuve, 1996). Kestemont (1995) reported that cannibalism is common among the larvae of goldfish *Carassius auratus* when given insufficient quantity of food, although they are rarely cannibals.

Size grading and aggressive behaviours of fish

Some authors, however, question whether size-grading interrupts the formation of dominance hierarchy and improves growth performance (Jobling and Reinsnes, 1986). For example, size-grading did not improve fish growth for Arctic charr *S. alpinus* (Baardvik and Jobling, 1990), eel *Anguilla anguilla* (Kamstra, 1993), and turbot *Psetta maxima* (Sunde *et al.*, 1998). Similar observations were reported for juvenile pikeperch *Sander lucioperca* (Zakes *et al.*, 2004), silver perch *Bidyanus bidyanus* (Barki *et al.*, 2000), yellow perch *Perca flavescens* (Wallat *et al.*, 2005) and white sea bream *Diplodus sargus* (Dikel *et al.*, 2016). Conversely, some studies have reported findings different from the above-mentioned observations (Wallace and Kolbeinshavn, 1988; Dou *et al.*, 2004). For example, size-grading enhanced growth in certain species such as juvenile Atlantic salmon (Gunnes, 1976), and Nile tilapia *Oreochromis niloticus* (Brzeski and Doyle, 1995). Therefore, the removal of the largest fish could reduce the adverse effects imposed by large individuals and improve growth (Barki *et al.*, 2000). As a result of aggression and cannibalism, some of the negative effects observed include physical damage of the fish, even death normally due to body and fin biting behaviours (Koebele, 1985), poor growth and increased susceptibility to infections (Bolnik, 1990). The high rate of cannibalism, therefore, limits the phase of larval rearing and often leads to skin lesions and fin damage (Kaiser *et al.*, 1995a). In the context of dominance hierarchies or cannibalism, the consequent benefit of size may lead to more intense and frequent aggressive interactions, depending on the morphology and behaviour of the species (Hecht and Appelbaum, 1988; Hecht and Pienaar, 1993; Braband, 1995; Baras, 1998; Baras *et al.*, 2000). The aggressive and cannibalistic behaviours thus cannot be avoided if there is still high size variation among fish (Jobling, 1985; Wang *et al.*, 1998; Lazo *et al.*, 2000; Valdimarsson and Metcalfe, 2000; Manley *et al.*, 2014; Obirikorang *et al.*, 2014). Size-grading is assumed to reduce impact related to behaviour interactions between fish (Jobling, 1982). The effectiveness of size-grading practice during early stages of fish development has been questioned (Kestemont *et al.*, 2003). It is thus necessary to study aggressive behaviours within this species to minimise the negative influence associated with this behaviour on production (Koebele, 1985).

Dusky kob and ecological conditions

Juvenile dusky kob are carnivorous (Griffiths and Hecht, 1995) and inhabit shallow coastal and estuarine waters (Nelson, 1994). They are found in the northern hemisphere along the coast of China, South Korea and Japan (Griffiths, 1997) and in the southern hemisphere along the eastern seaboard of Australia (Starling, 1993), South Africa and Mozambique (Griffiths and Heemstra, 1995). They serve as key commercial and recreational line-fish species in South Africa and Australia (Griffiths, 1997). As shoaling fish, they visit the coastline in large groups to both feed and spawn (Van der Elst, 1988). Naturally, dusky kob move in large groups (PIRSA, 2003) and can undertake coastal migrations (Griffiths, 1996; Taylor *et al.*, 2014), preferring turbid estuaries and surf zones as nursery habitats (Griffiths, 1997). Dusky kob can tolerate poor water quality, low oxygen levels (FitzGibbon *et al.*, 2007) and relatively high stocking densities (Collett, 2007) and they are euryhaline (Whitfield and Blaber, 1978).

Dusky kob aquaculture attributes

Dusky kob are teleost fish of the family Sciaenidae (Silberschneider and Gray, 2008). The taxonomy, life history, growth and feeding of dusky kob has been well-studied (Griffiths and Heemstra, 1995; Griffiths, 1996; Griffiths, 1997). Dusky kob have become important to aquaculture on a worldwide basis (Fitzgibbon *et al.*, 2007), following the severe overfishing of dusky kob stock in South Africa (Griffiths and Fennessy, 2000) and Australia (O'Sullivan and Ryan, 2001). They are important indigenous candidate marine culture species (Thorpe, 1991; Hecht, 2000), with excellent biology for aquaculture (PIRSA, 2001). Currently, the global farming of sciaenid species is rapidly growing (Hong and Zhang, 2003) and therefore, special attention is required to make appropriate research and development of management plans (Lamberth and Joubert, 2014). Dusky kob have many special attributes beneficial for aquaculture and commands high prices (Griffiths, 1996). They are a good table fish (Silberschneider *et al.*, 2009) with excellent food conversion ratios and fast growth between 15 and 30 ^{o}C (PIRSA, 2001; O'Sullivan and Ryan, 2001). There is however a paucity of information on dusky kob behaviour under aquaculture conditions. Feeding juvenile dusky kob twice per day with about 9 h-intervals at 4% body mass per day may achieve optimal growth, feed efficiency and body composition (Daniel, 2004; Kaiser *et al.*, 2011).

Fish aggressive and cannibalistic behaviours

Juvenile fish often experience disturbances by humans and other factors that may increase aggression and cannibalism in culture settings (Huntingford, 2004). Apparently, aggressive and cannibalistic behaviours of juvenile dusky kob *Argyrosomus japonicus* can lead to high mortalities

(Silberschneider and Gray, 2008). High frequencies of cannibalism resulting in mortalities in juvenile dusky kob (PIRSA, 2001) are often observed under culture conditions (Griffiths, 1996). In contrast, less frequencies of cannibalism are observed in the natural environment (Griffiths, 1996). In dusky kob, cannibalistic behaviours appear occur 18 days after hatching and decrease after fish reached 80 mm total length (TL) (O'Sullivan and Ryan, 2001). Increased cannibalism in culture settings could be associated with the fact that fish have limited space to escape predation (Baras and Jobling, 2002), among other factors. The present study is aimed at quantifying the effect of size variation and grading techniques on the aggressive and feeding (browsing) behaviours of juvenile dusky kob. It was hypothesised that size variation influences aggressive and browsing behaviours of juvenile dusky kob, leading to increased aggressive behaviours. The findings may assist in improving the aggressive behaviours, growth and production of juvenile dusky kob under culture conditions.

Thesis structure

This thesis is organised into five chapters.

Chapter 1 presents a comprehensive literature review that covers general aspects and historical information of aggressive and cannibalistic behaviours of fish, a range of factors influencing aggression and feeding, and the effect of size variation and size-grading methods on aggressive behaviours.

Chapter 2 comprises experiment one, focusing on the effect of increasing size variation and observation time on the aggressive and browsing behaviours of juvenile dusky kob. This section provides background information on aggressive and cannibalistic behaviours of fish, driving factors of aggression. It also gives an account and details of the methods and materials used.

Chapter 3 comprises of experiment two with observations on the relationship between increasing size variation and aggressive behaviours of juvenile dusky kob. The experiment conducted for this section was designed based on the findings and discussion of results of the previous experiment. This section also presents an account of the methods and the experimental equipment used.

Chapter 4 consists of experiment three focusing on the effect of aggressor's size on aggressive and browsing behaviours in of juvenile dusky kob. This section gives a brief introduction and the background to the study, following the significant findings and discussions of the previous experiment. It provides an explanation and details of the methods and experimental methods and materials used.

Chapter 5 concludes the thesis by recapping the findings of the experimental trials conducted and highlights the originality of the research. Limitations of the study have been indicated with recommendations provided for ongoing farming practice and future research.

Chapter 2

Size variation effect on aggression and browsing behaviours of juvenile dusky kob, *Argyrosomus japonicus*

1. Introduction

In South Africa, the commercial culture of dusky kob is new and growing (Thorpe, 1991) with culture technology requiring further improvement (O'Sullivan and Ryan, 2001). The potential to culture dusky kob on a commercial scale has been confirmed in many nutritional, biological and ecological studies (Marais, 1984; Daniel, 2004; Collett, 2007). Dusky kob have a high survival rate in both experimental culture and within the aquaculture setting. They can tolerate low oxygen levels and relatively high stocking densities of up to 50 kg m^{-3} (PIRSA, 2003; Collett, 2007). Numerous studies documented the effect of size distribution on growth of different species (Jobling and Wandsvik, 1983; Jobling and Reinsnes, 1986; Wallace and Kolbeinshavn, 1988; Baardvik and Jobling, 1990). Despite these research efforts, more attention is necessary to study fish behaviours and size variation under culture conditions (Grant, 1997; Almaza´n-Rueda *et al.*, 2004). This is particularly essential for commercial production of dusky kob as well as to assist in restocking and reducing the effects of fishing pressure in the natural population (O'Sullivan and Ryan, 2001).

In many cultured species, intraspecific aggression is a frequently occurring activity (Kaiser *et al.*, 1995b). Cannibalism commonly occurs in cultured species and among them, are species such as sharptooth catfish *Clarias gariepinus* (Baras and d'Almeida, 2001), red drum (Liao and Chang, 2002), Arctic charr *S. alpinus* (Svenning and Borgstrøm, 2004) and Nile tilapia *O. niloticus* (Fessehaye *et al.*, 2006). Dusky kob have shown aggressive and cannibalistic behaviours (PIRSA, 2001; Collett, 2007; Bernatzeder, 2008; Timmer and Magellan, 2011) during larval and early juvenile stages (O'Sullivan and Ryan, 2001). In comparison with other fish such as African catfish *C. gariepinus* (Hecht and Appelbaum, 1988), koi carp *Cyprinus carpio* (Van Damme *et al.*, 1989) and goldfish *C. auratus* (Paulet, 2003), the aggressive and cannibalistic behaviours of juvenile dusky kob decrease as fish grow (O'Sullivan and Ryan, 2001). Resultant cannibalism, however, becomes gradually less intense at a certain stage of young fish (Chang and Liao, 2003). In red drum *Sciaenops ocellatus*, this behaviour occurs at a particular phase of growth due to genetic inheritance and environmental conditions (Liao and Chang, 2002).

Size variation facilitates cannibalism (Fessehaye *et al.*, 2006) and juvenile fish usually have large mouth gape in relation to body size (Baras, 1998). Apparently, high size variation becomes more prevalent during juvenile stages than during adult stages (Baras, 1998). In turn, cannibalism influences size variation among fish (Jobling, 1982; Baras, 1998; Øverli *et al.*, 1999; Clement *et al.*, 2005). Larger fish usually attack or cannibalise on smaller ones (Baras, 1998). Interestingly, many studies have quantified aggressive and cannibalistic behaviours of other species (Hecht and Appelbaum, 1988; Britz and Pienaar, 1992; Hecht and Pienaar, 1993; Kaiser *et al.*, 1995b; Hossain *et al.*, 1998).

Grading fish based on size is a common rearing practice on farms with aim of increasing survival rate and improving weight gain. This technique is used to improve growth of small fish, reduce cannibalism, decrease size variability and improve feed uptake in the farming of many commercial fish species. Size variation and feeding regimens are among the most common factors resulting in aggression and cannibalism in many important farmed species. Juvenile dusky kob *Argyrosomus japonicus* are aggressive and this behaviour likely contributes to high mortality in aquaculture. Dusky kob are highly susceptible to stress under culture conditions and this stress is associated with increased aggressive behaviours, resulting in cannibalism. Further research is however required to explore various techniques to reduce fish aggressive behaviours (Hecht and Pienaar, 1993; Valdimarsson and Metcalfe, 2000). In ensuring the successful spawning and rearing of dusky kob in culture setting (Landman, 2006) in South Africa, the present study determines the effects of size variation and grading on juvenile dusky kob aggressive behaviours. This study was therefore designed to quantify the effect of size variation and observation time on the aggressive and feeding (browsing) behaviours of juvenile dusky kob, using small glass tanks to maintain less-aggressive behaviours. The results would be essential to ascertain the functions of size-grading towards reducing aggressive and cannibalistic behaviours of juvenile dusky associated with size variation to improve growth and survival rate of kob under culture conditions. The study contributes to better understanding of fish behaviour and may benefit both research and farming practices.

2. Materials and methods

Experimental system, fish and acclimation

To conduct the behavioural observation study, 155 hatchery-reared juvenile dusky kob of an average weight (± standard deviation) and length (± standard deviation) of 3.60 ± 0.68 g and 5.8 ± 0.41 mm $fish^{-1}$, respectively, were obtained from the hatchery stock of Espadon Marine (Pty) Ltd, now known as Oceanwise (Pty) Ltd located in East London Industrial Development Zone (ELIDZ), South Africa. In the hatchery, these fish had been spawned from one batch of six-year-old dusky kob broodstock. All experiments were performed at the Rhodes University Marine Research Laboratory in Port Alfred, South Africa. This research facility uses seawater pumped from the mouth of the Kowie River estuary into the recirculation water system. The indoor aquarium system was set up before the arrival of the fish. The system consisted of a glass tank (22 cm x 11.8 cm x 28.8 cm) which was used as the observation tank (Figure 1). The two separate 600-L green plastic tanks making up part of the recirculation water system outside were used to keep the fish before and after the observation. Following the establishment of the culture tank system, total ammonia, nitrite and nitrate concentrations were determined and recorded using a spectrophotomer between 08h00 and 09h00 ($NH_3 + NH_4^+$ (total ammonia), NO_2^-, NO_3^- Kits, Red Sea Fish Pharm, Israel) once a week. Total ammonia to nitrogen and nitrite concentrations ranged between 0.00 to 0.25 mg L^{-1} N throughout the study. Before the start of the experiment, the fish were allowed to acclimatise for about two weeks in the holding tanks. A 2 mm-pelleted food was provided to the fish in the holding tanks at 3.4% of their body weight day^{-1}. The pelleted food was a commercial trout pellet (45% protein; 14% lipid; Aquanutro, Nutroscience Pty Ltd, South Africa). The feeding intensity in percentage body weight day^{-1} was calculated based on weight gain = ((final weight – initial weight) / initial weight) x 100. The minimum feeding intensity of 3.4% body weight and maximum feeding intensity of 3.85% body weight day^{-1} had achieved good growth and a low food conversion ratio (FCR) of juvenile dusky kob under culture conditions (Collett, 2007). Throughout the duration of the experiment, the fish were fed three times day^{-1} (08h00, 13h00 and 17h00) in the holding tank. The water temperature was maintained between 24 and 26 °C and this temperature falls within the range recommended as the best temperature for growth of dusky kob. A temperature of 25.3 °C is best for growth when culturing juvenile dusky kob (Collett, 2007; Collett *et al.*, 2008a). In addition, Bernatzeder and Britz (2007) suggested the preferred temperature range of 25-26.4 °C for juvenile dusky kob cultured in South African hatcheries. Submerged thermostatically controlled 300-W aquarium heaters were used to heat water to the temperature required for the study and water was added from the holding tanks when necessary. Using a portable refractometer (Atago S/Mill-E, Tokyo, Japan), salinity was measured daily and maintained at approximately 35 parts per thousand (ppt) with fresh and saltwater added to the system when necessary. Dusky kob are euryhaline (Whitfield *et al.*, 1981) and this salinity level falls within the range reported for this species. Larvae and early juveniles grew well in salinities ranging from 5 to 35 ppt (Fielder *et al.*, 1999). Water pH was maintained between 7.4 and 8.5 with a 200-L drum, connected to the system, containing oyster shells to buffer pH. Aggression could be managed by manipulating light intensity (Collett, 2007; Collett *et al.*, 2008b) and in this case, low light intensity for the observation tank was maintained by keeping the light dim throughout the experiment. Furthermore, under partial shade conditions dusky kob showed less aggression (Timmer and Magellan, 2011). Water temperature and pH were measured every day using a portable electronic probe (Hanna Instruments HI 98128, Rhode Island, USA). Dissolved oxygen (DO) was kept between 80 and 100% of saturation and was measured once a week in tanks using DO meter (YSI 85 DO/SCT Meter, Ohio, USA). A constant gentle water flow through the observation tank was set and maintained throughout the experiment with seawater changed and when necessary, added to maintain water quality and keep the water level constant. Aeration with air stones in all tanks was maintained during day and night. Water quality and physical parameters were kept the same as those as in the holding tanks.

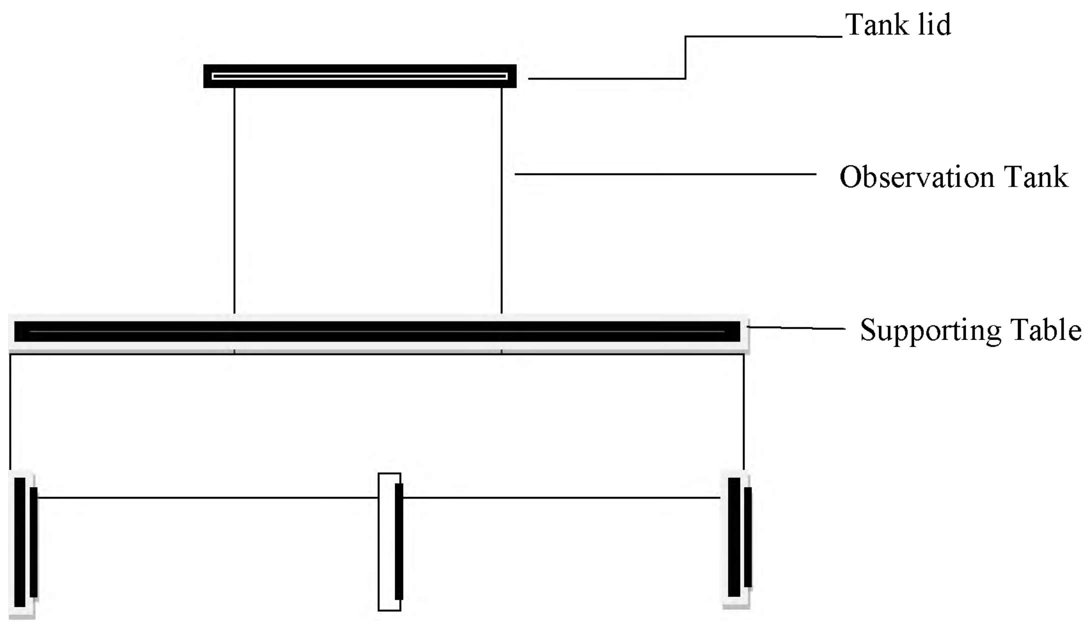


Figure 1: A schematic diagram showing an observation glass tank on a table stand

The observation tank was well covered to ensure that fish did not jump out and a black plastic sheet was used to cover the sides of the tank. This was done to minimise disturbance to the fish before and during observation. Only a small cut was made into the black plastic sheet on one side of the tank to enable the observer to observe the fish. Uneaten food and waste material was removed by siphoning from the bottom of the tank every morning and evening approximately 2 h after feeding to ensure that no food remained or faeces were left in the tank before and after observing the fish.

Experimental design and protocols

The juvenile dusky kob were size-graded visually to obtain a group of eight fish with different sizes (coefficients of variation) for behaviour observation. The coefficient of variation (COV) was used to determine and compare size variation for length and weight of fish prior observation. Each focal fish was observed among the non-focal fish at different observation times four times per day for observation before and after feeding. Each observation was set to start in the morning and the focal fish each observed (a) before feeding in the morning, (b) 2 h after feeding, (c) 6 h after feeding and (d) 12 h after feeding in the evening. The pelleted food was dropped by hand into the centre equidistant from each side of the observation tank for fish to feed to apparent satiation. In the evening 2 h after the last feeding, eight fish were selected from the holding tanks for the next observation. Fish were individually measured to obtain standard length (to the nearest 1.0 mm) and weighed (g) prior to observations and the COV of the group was calculated. From the raw data collected, derivatives of size variation (COV) were further explored to obtain minimum weight and length variation; maximum weight and length variation, size difference to mean length and weight; size difference to maximum weight and length as well as size difference to minimum weight and length of non-focal fish and the focal fish. Standard length was used as the caudal fin was sometimes damaged due to cannibalism and the wet weight of each fish was measured with an accuracy of 1.0 g using a digital scale. Once all fish measured, the largest-sized fish was chosen and observed as focal fish. In total, the experiment consisted of 20 behaviour observations.

Identification and quantification of fish behaviour

The behaviours of fish were recorded and each observation was made on the focal fish in the observation tank for 30 min. Before conducting the experiment, two observations on juvenile dusky kob behaviours before and after feeding were performed on two separate days. These observations were run to pilot and identify the aggressive behaviours of fish in the tanks. The largest fish was observed as the focal fish to identify and define the aggressive and browsing behaviours. Behaviours included fish browsing at the bottom of the tank, fish attacks shown by chasing, body or tail biting (nipping), with some of the smaller fish losing parts of their tails or fins and others being intimidated. For the present study, aggressive and browsing behaviours of the focal fish were identified and defined (Table 1). Only the focal fish aggressive and browsing behaviours towards non-focal fish were recorded. The behaviours were recorded, and tally numbers (1 to 5) were used and after observation, counted for representation of each of the five behaviour patterns. That is, 1 was denoted as chasing, 2 as body biting, 3 as tail biting, 4 as browsing and 5 as intimidating behaviours. A 30-min. period was allowed before each observation commenced to allow the fish to experience the presence of the observer. A countdown timer (stopwatch) with an alarm was used to keep the time the same for all observations and it was turned on when the observation started. The number of each of the aggressive and browsing behaviours were expressed as mean frequencies of behaviour $fish^{-1}$.

Table 1: Types and definitions of observed behaviors of juvenile dusky kob

Behaviour	**Definition**
Chasing	The focal fish pursues or chases another fish for one or more seconds.
Body biting	The focal fish swims towards or behind and bites another fish on its body; the bite is short or fast causing the other fish to dart away.
Tail biting	The focal fish intently moves more than one body length towards another fish from behind and bites it on its tail; the fish bites and / or holds to capture another fish on its tail, but is unable to consume it, i.e., Type 1 cannibalism.
Browsing	The focal fish moving along the bottom of the observation tank and appearing to search for food.
Intimidating	The focal fish faces and / or moves closer to another fish in an attacking way; the intimidated fish quickly darts away and continues swimming normally.

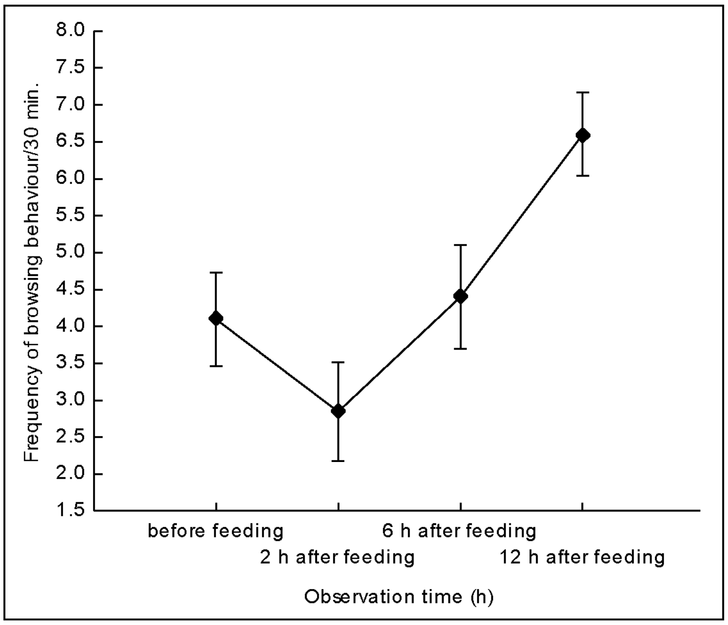
Data analysis

A repeated-measures analysis of variance (ANOVA) was applied to determine the effect of observation time on fish behaviours. The significance of differences in the frequencies of aggressive and browsing behaviours fish^{-1} was estimated using a Post hoc Tukey's Multiple Range Test. The error bars have been presented as standard deviation of the mean frequency and standard deviation was used throughout the rest of the analyses. The COV for standard weight and length was defined as standard deviation as percentage of the mean value of fish weight or length (COV = standard deviation / mean *100). Correlation between behaviours and size variation of weight or length was assessed using a least-squares linear regression model, with a test for significance of correlation based on the null hypothesis of no correlation between the dependent variable (behaviour) and the independent variable. The alpha-error value was 5% ($p < 0.05$). The weight and length means of fish were calculated to determine size differences between focal and non-focal fish so as to obtain an additional independent variable. Least-squares linear regression was used to determine correlation between fish behaviours and size variation including its derivatives such as minimum weight and length variation; maximum weight and length variation, size difference to mean length and weight; size difference to maximum weight and length as well as size difference to minimum weight and length of non-focal fish and the focal fish. All statistical analyses were done using Statistica version 9 (Statsoft, Tulsa, OK, USA).

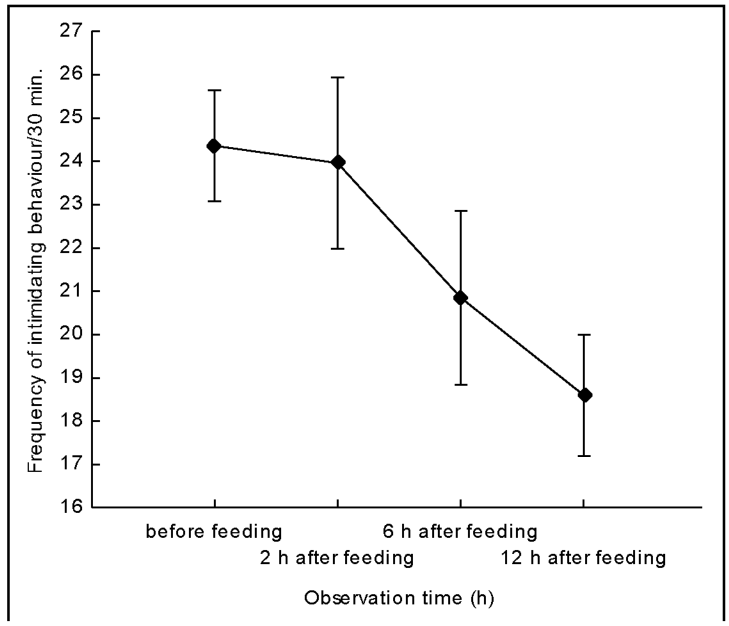
3. Results

Observation time and behaviours

Repeated measures ANOVA results showed that statistically significant differences occurred in the frequencies of browsing behaviour $fish^{-1}$ per 30 min. between observation times ($p = 0.001$). There were also significant differences in the frequencies of intimidating behaviour $fish^{-1}$ per 30 min. between observation times ($p = 0.010$). However, aggressive behaviours such as chasing ($p = 0.341$), body biting ($p = 0.510$) and tail biting ($p = 0.403$) behaviours did not differ between the observation times.



a)



b)

Figure 2: Frequency (± standard deviation) of a) browsing and b) intimidating behaviours fish^{-1} per 30 min. at different observation times (before feeding, 2 h, 6 h and 12 h after feeding). The vertical bars denote standard deviations of the frequencies.

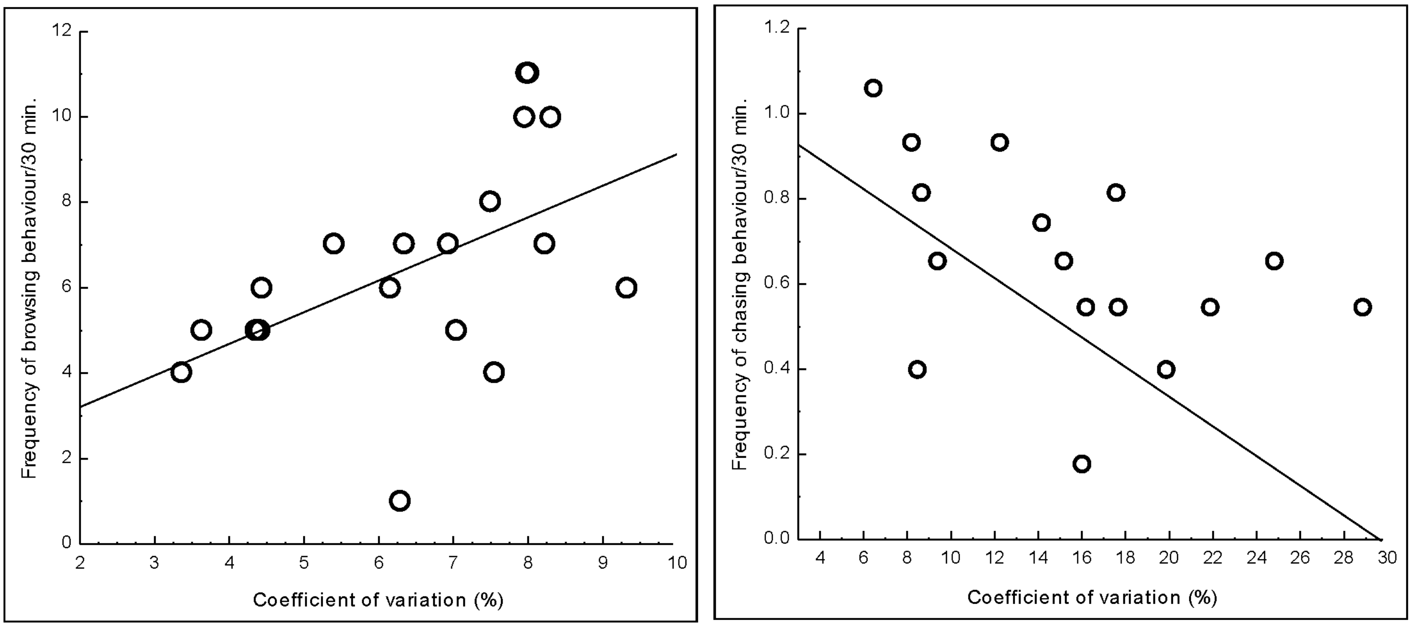
The frequency (2.85 ± 0.67) of browsing behaviour fish^{-1} 2 h after feeding and frequency (4.10 ± 0.63) before feeding were significantly lower than the values recorded 12 h after feeding (6.60 ± 0.56). Frequency (4.40 ± 0.70) 6 h after feeding did not significantly differ from values before and after feeding. However, behaviour frequency data 12 h after feeding did not significantly differ from average values 6 h after feeding. Therefore, the browsing behaviours fish^{-1} significantly increased 12 h after feeding as the time of no access to food increased (Post-hoc test, Table 1; Figure 2). The frequency (18.60 ± 1.39) of intimidating behaviour fish^{-1} 12 h after feeding was significantly lower than the frequency (24.35 ± 1.27) before feeding and the frequency (23.95 ± 1.98) 2 h after feeding. The frequency 12 h after feeding was not significantly different to the frequency (20.85 ± 2.00) observed 6 h after feeding. No significant differences were found in the frequencies of intimidating behaviour fish^{-1} before feeding, 2 h and 6 h after feeding. The increased frequencies of intimidating behaviour were more apparent before and 2 h after feeding than 12 h after feeding, without differing 6 h after feeding (Post-hoc test, Table 2; Figure 2).

Table 2: Post-hoc test showing the significant differences in the frequencies of browsing and intimidating behaviours fish^{-1} at different observation times. Different superscript letters within the same column indicate significant differences ($p < 0.05$).

Observation time (h)	**Frequency of browsing behaviour**	**Frequency of intimidating behaviour**
before feeding	4.10 ± 0.63^{a}	24.35 ± 1.27^{a}
2 h after feeding	2.85 ± 0.67^{a}	23.95 ± 1.98^{a}
6 h after feeding	4.40 ± 0.70^{ab}	20.85 ± 2.00^{ab}
12 h after feeding	6.60 ± 0.56^{b}	18.60 ± 1.39^{b}

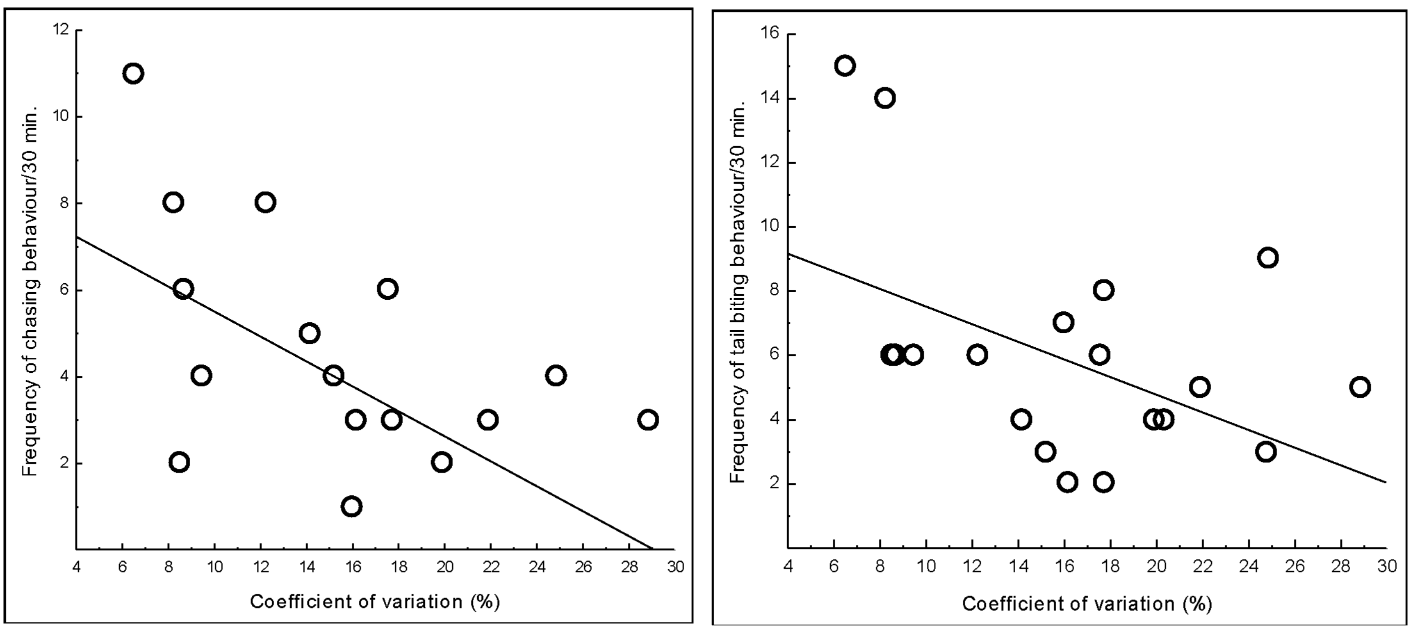
Size variation, its derivatives and behaviours

Frequency of browsing behaviour fish^{-1} per 30 min. was significantly positively correlated with the COV-value of the group of fish for length 12 h after feeding (least-squares linear regression; $p = 0.02$; $r^2 = 27\%$); Frequency of chasing behaviour fish^{-1} negatively correlated with COV for length 12 h after feeding (least-squares linear regression; $p = 0.03$; $r^2 = 24\%$) (Figure 3).



a) b)

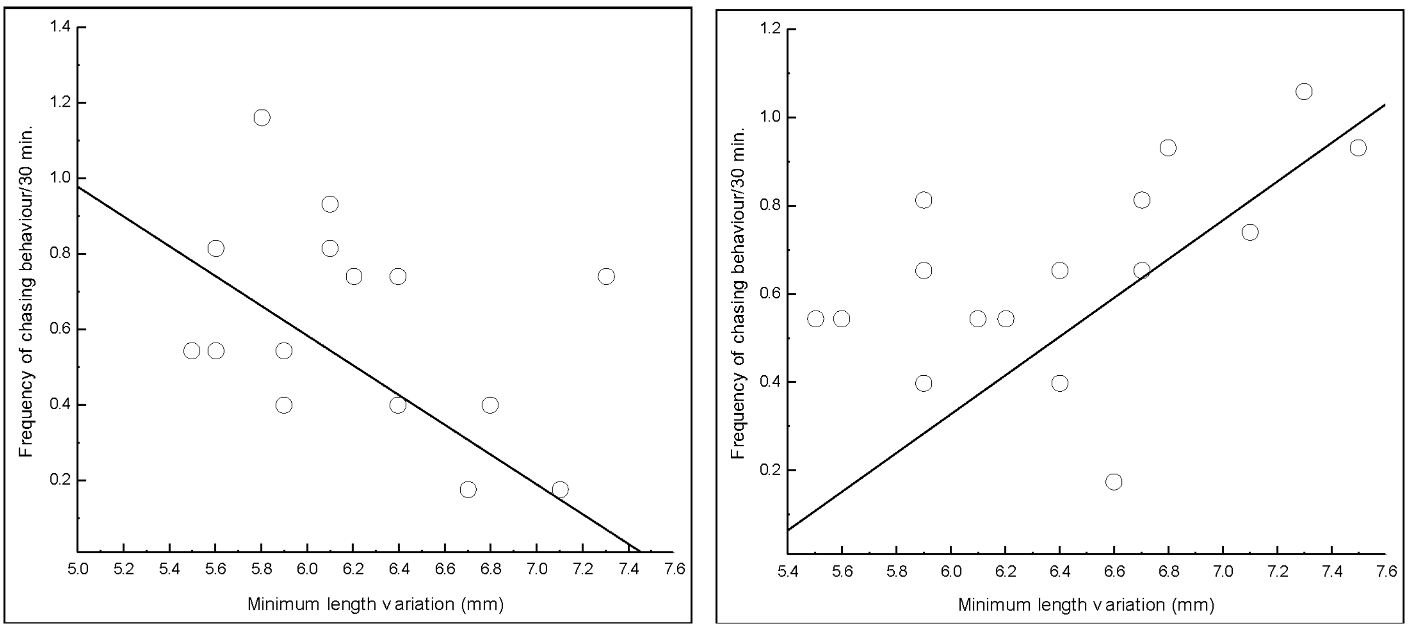
Figure 3: Frequency of a) browsing (y = 1.726 + 0.741x; p = 0.02; r^2 = 27%) and b) chasing (y = 1.033 - 0.035x; p = 0.03; r^2 = 24%) behaviours fish^{-1} 12 h after feeding as a function of the COV (%) for length.



a) b)

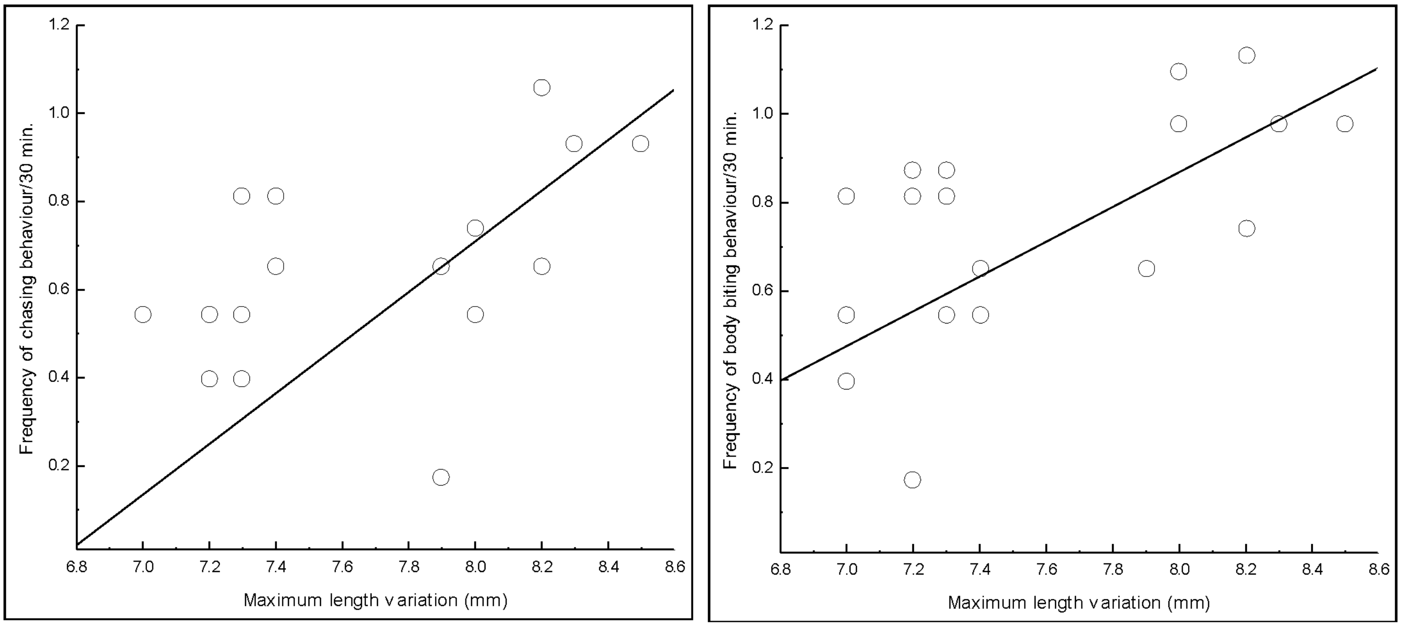
Figure 4: Frequency of a) chasing (y = 8.386 – 0.288x; p = 0.001; r^2 = 35%) and b) tail biting (y = 10.265 – 0.227x; p = 0.04; r^2 = 21%) behaviours fish^{-1} 12 h after feeding as a function of the COV (%) for weight.

Frequency of chasing behaviour $fish^{-1}$ negatively correlated with the COV for weight 12 h after feeding (least-squares linear regression; $p = 0.001$; $r^2 = 35\%$); Similarly, frequency of tail biting behaviour $fish^{-1}$ negatively correlated with the COV for weight 12 h after feeding (least-squares linear regression; $p = 0.04$; $r^2 = 21\%$) (Figure 4). Frequency of chasing behaviour $fish^{-1}$ negatively correlated with the minimum length variation 2 h after feeding (least-squares linear regression; $p = 0.01$; $r^2 = 31\%$); Frequency of chasing behaviours $fish^{-1}$ positively correlated with the minimum length variation 12 h after feeding (least-squares linear regression; $p = 0.01$; $r^2 = 33\%$) (Figure 5).



a) b)

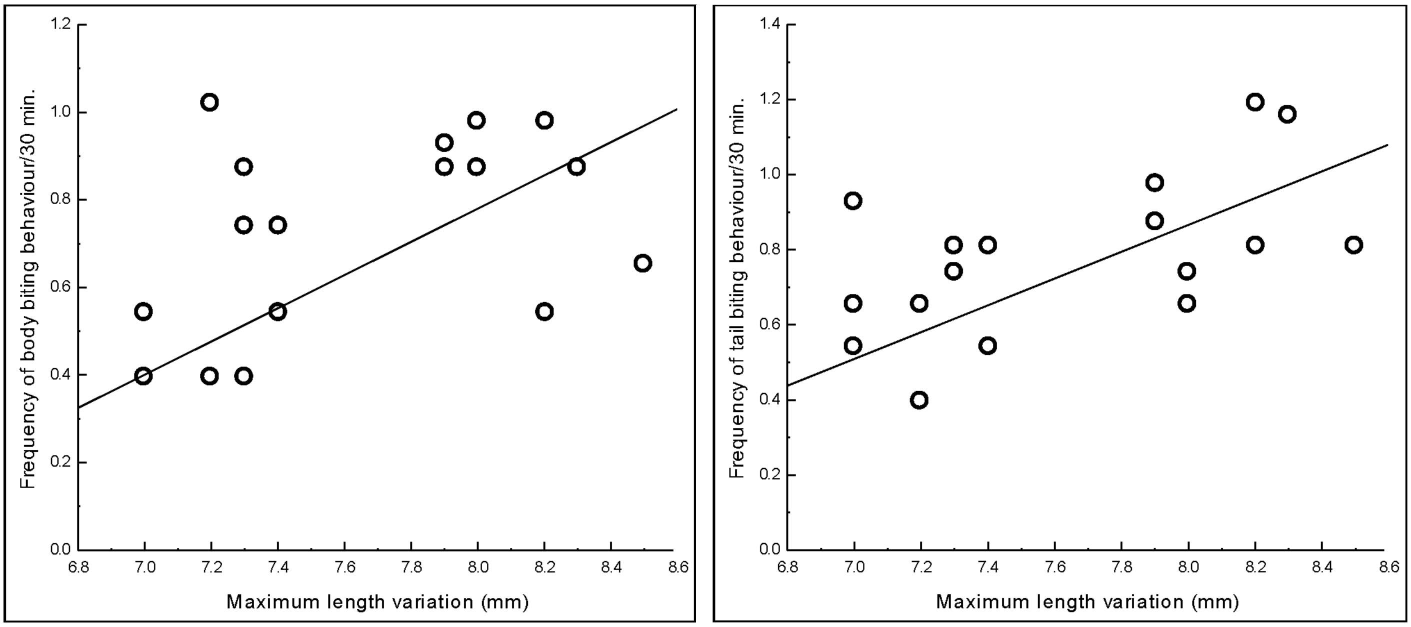
Figure 5: Frequency of a) chasing ($y = 2.949 - 0.394x$; $p = 0.01$; $r^2 = 31\%$) behaviour $fish^{-1}$ 2 h after feeding and b) chasing ($y = 2.309 + 0.439x$; $p = 0.01$; $r^2 = 33\%$) behaviour $fish^{-1}$ 12 h after feeding as a function of minimum length variation (mm).



a) b)

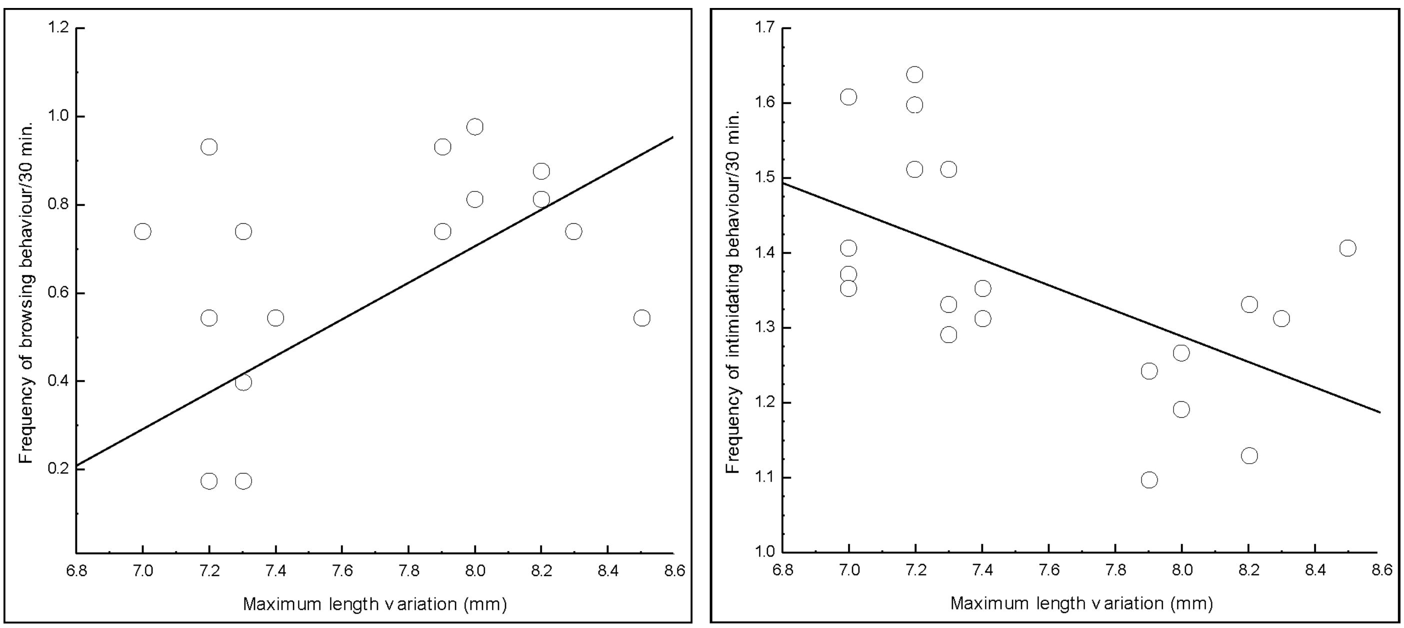
Figure 6: Frequency of a) chasing ($y = 3.888 + 0.575x$; $p = 0.002$; $r^2 = 43\%$) behaviour fish^{-1} 12 h after feeding and b) body biting ($y = 2.271 + 0.392x$; $p = 0.01$; $r^2 = 35\%$) behaviour fish^{-1} 6 h after feeding as a function of maximum length variation (mm).

Frequency of chasing behaviour fish^{-1} positively correlated with the maximum length variation 12 h after feeding (least-squares linear regression; $p = 0.002$; $r^2 = 43\%$); Similarly, frequency of body biting behaviour fish^{-1} positively correlated with the maximum length variation 6 h after feeding (least-squares linear regression; $p = 0.01$; $r^2 = 35\%$) (Figure 6). Frequency of body biting behaviour fish^{-1} correlated with the maximum length variation 12 h after feeding (least-squares linear regression; $p = 0.03$; $r^2 = 24\%$); Similarly, frequency of tail biting behaviour fish^{-1} positively correlated with the maximum length variation 12 h after feeding (least-squares linear regression; $p = 0.01$; $r^2 = 32\%$) (Figure 7).



a) b)

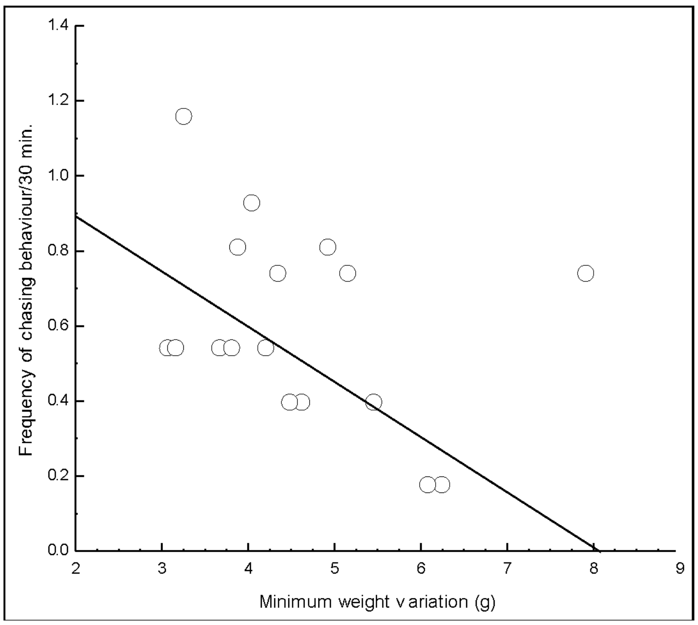
Figure 7: Frequency of a) body biting (y = 2.254 + 0.379x; p = 0.02; r^2 = 26%) and b) tail biting (y = 1.990 + 0.357x; p = 0.01; r^2 = 32%) behaviours fish^{-1} 12 h after feeding as a function of maximum length variation (mm).



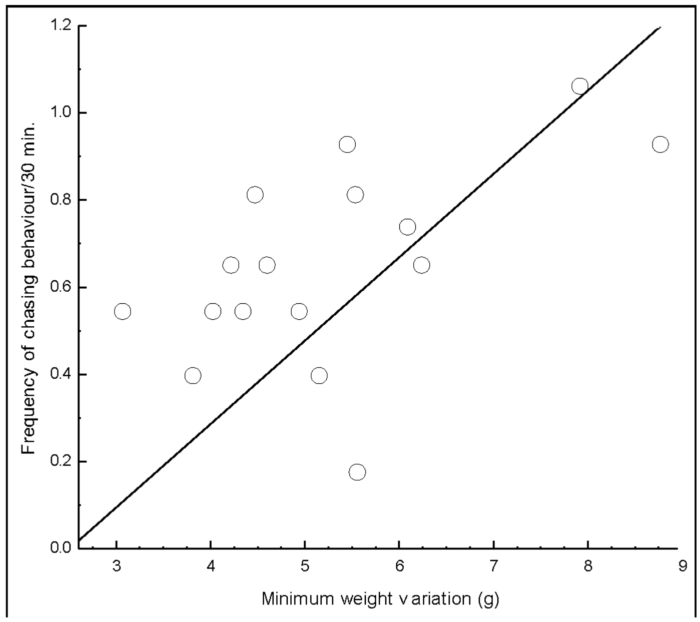
a) b)

Figure 8: Frequency of a) browsing (y = 2.611 + 0.415x; p = 0.03; r^2 = 24%) behaviour fish^{-1} before after feeding and b) intimidating (y = 2.654 - 0.171x; p = 0.01; r^2 = 32%) behaviour fish^{-1} 2 h after feeding as a function of maximum length variation (mm).

Frequency of browsing behaviour fish^{-1} positively correlated with the maximum length variation before feeding (least-squares linear regression; p = 0.02; r^2 = 26%); Frequency of intimidating behaviour fish^{-1} was negatively correlated with the maximum length variation 2 h after feeding (least-squares linear regression; p = 0.01; r^2 = 32%) (Figure 8). Frequency of chasing behaviour fish^{-1} was significantly negatively correlated with the minimum weight variation 2 h after feeding (least-squares linear regression; p = 0.01; r^2 = 30%); Frequency of chasing behaviour fish^{-1} positively correlated with the minimum weight variation 12 h after feeding (least-squares linear regression; p = 0.002; r^2 = 43%) (Figure 9).

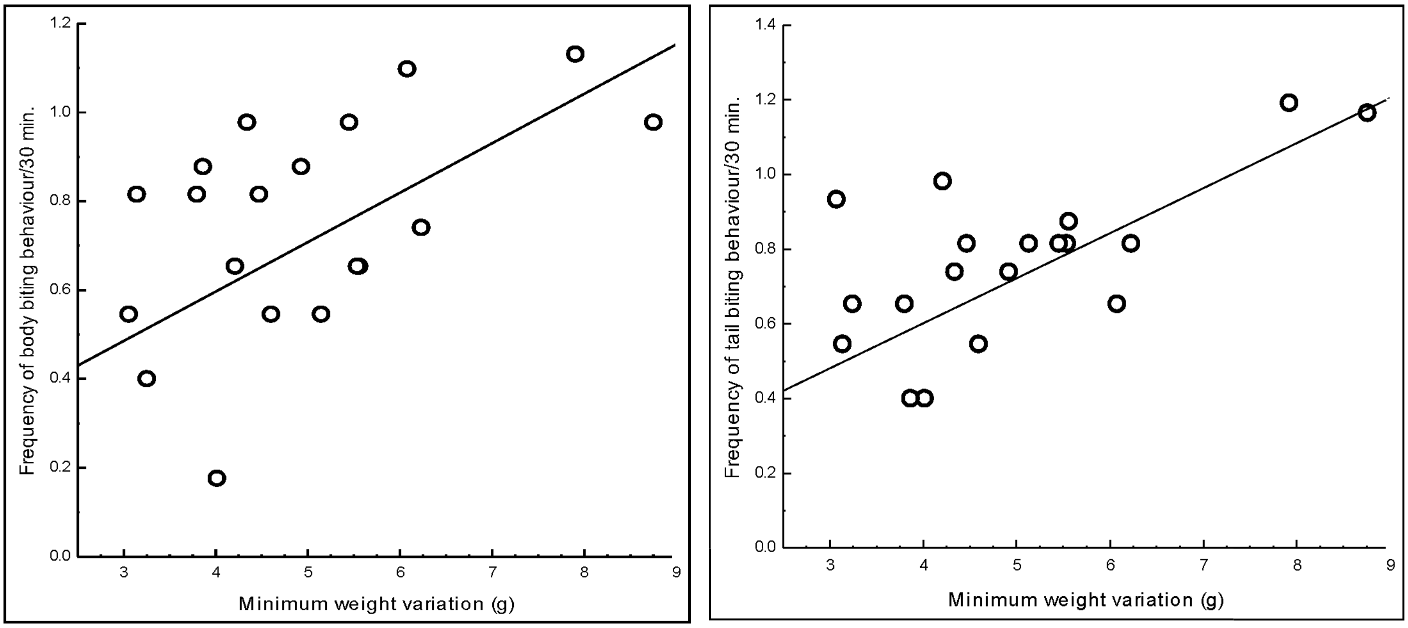


a)



b)

Figure 9: Frequency of a) chasing ($y = 1.187 - 0.147x$; p = 0.01; r^2 = 30%) behaviour fish^{-1} 2 h after feeding and b) chasing ($y = 0.471 + 0.191x$; p = 0.002; r^2 = 43%) behaviour fish^{-1} 12 h after feeding as a function of minimum weight variation (g).



a) b)

Figure 10: Frequency of a) body biting ($y = 0.152 + 0.111x$; $p = 0.02$; $r^2 = 25\%$) behaviour fish^{-1} 6 h after feeding and b) tail biting ($y = 0.119 + 0.121x$; $p = 0.01$; $r^2 = 33\%$) behaviour fish^{-1} 12 h after feeding as a function of minimum weight variation (g).

Frequency of body biting behaviour fish^{-1} positively correlated with the minimum length variation 6 h after feeding (least-squares linear regression; $p = 0.02$; $r^2 = 25\%$); Similarly, frequency of tail biting behaviour fish^{-1} showed a positive correlation with the minimum length variation 12 h after feeding (least-squares linear regression; $p = 0.01$; $r^2 = 33\%$) (Figure 10). Frequency of chasing behaviour fish^{-1} positively correlated with the maximum weight variation 12 h after feeding (least-squares linear regression; $p = 0.01$; $r^2 = 35\%$). Frequency of body biting behaviours fish^{-1} also had positive correlation with the maximum weight variation 6 h after feeding (least-squares linear regression; $p = 0.01$; $r^2 = 32\%$) (Figure 11).

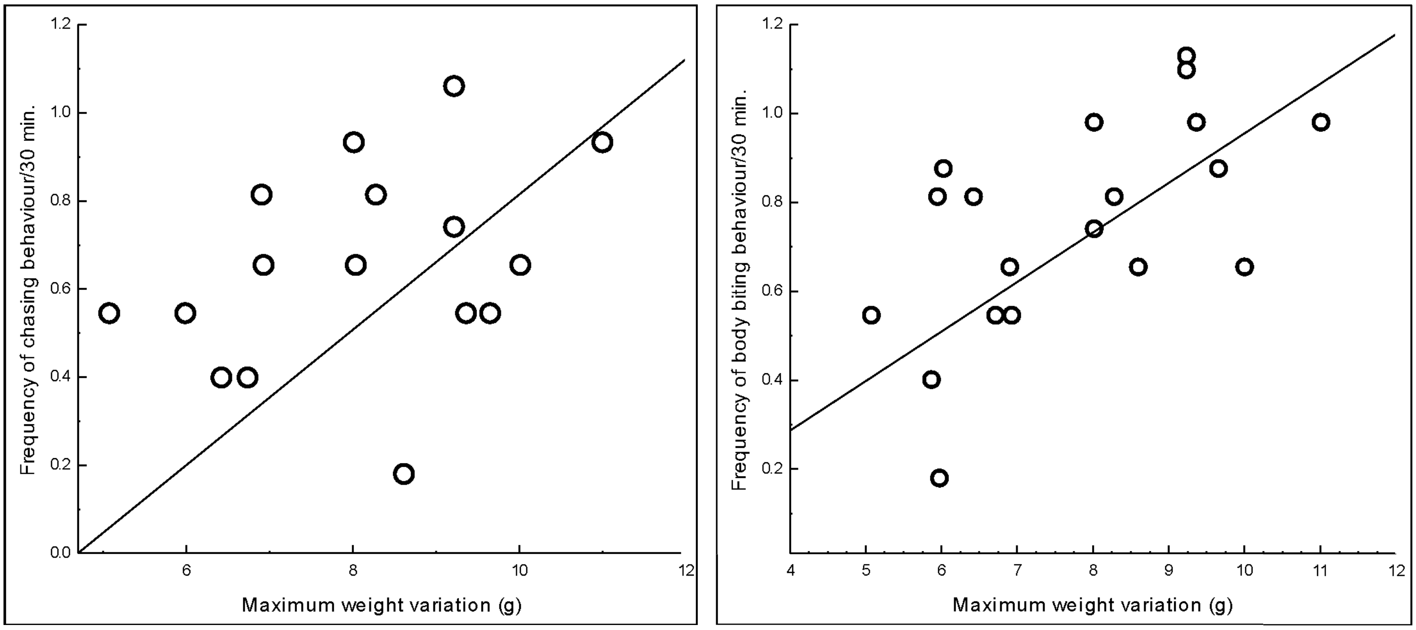
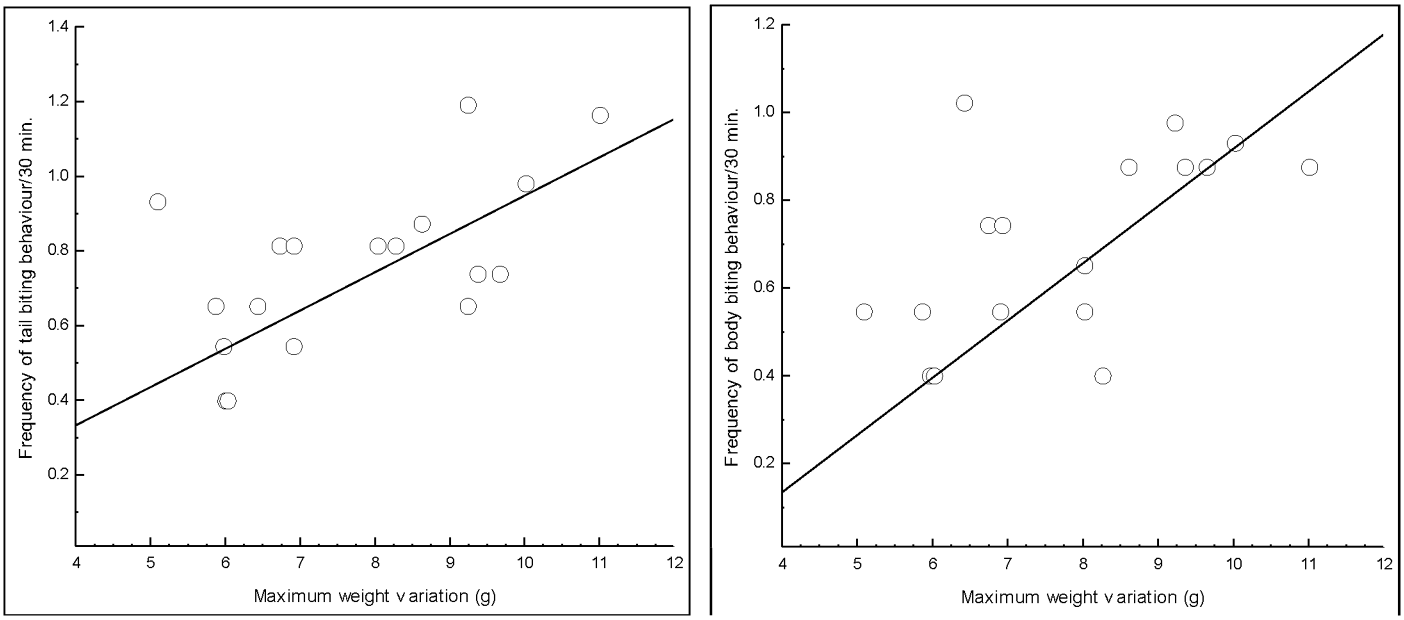


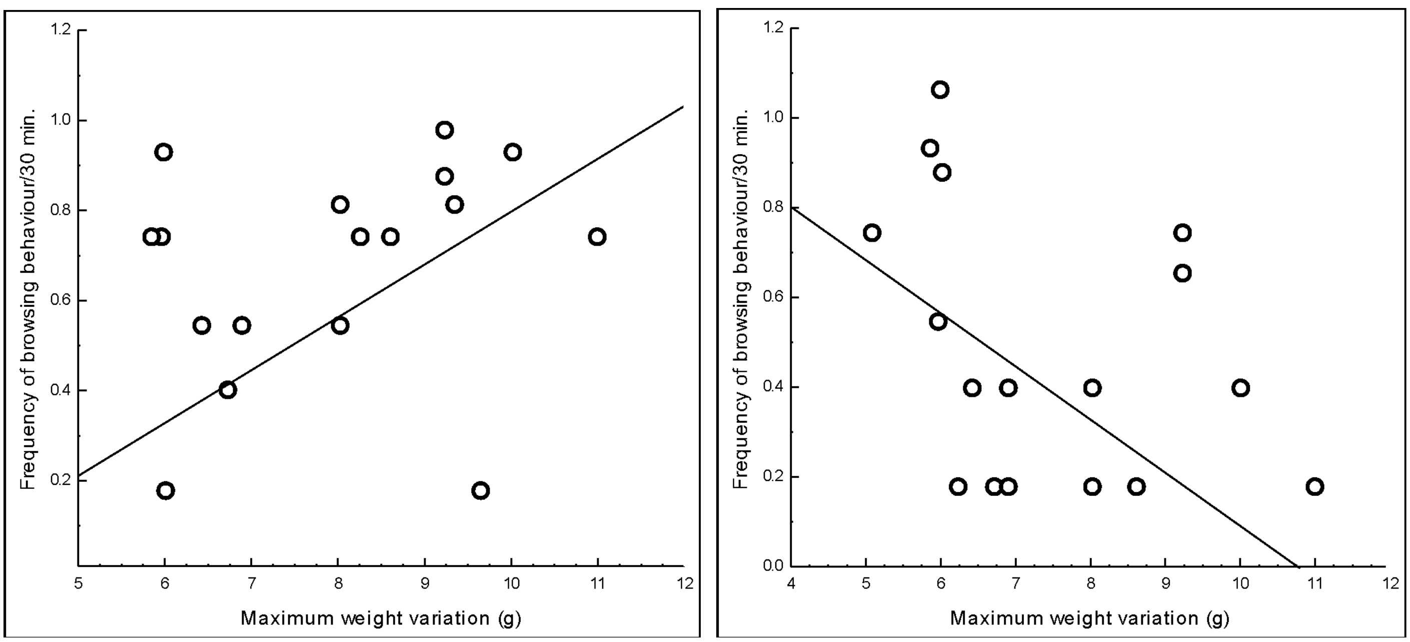
Figure 11: Frequency of a) chasing ($y = 0.721 + 0.154x$; $p = 0.01$; $r^2 = 35\%$) behaviour fish^{-1} 12 h after feeding and b) body biting ($y = 0.160 + 0.112x$; $p = 0.01$; $r^2 = 32\%$) behaviour fish^{-1} 6 h after feeding as a function of maximum weight variation (g).

Frequency of body behaviour fish^{-1} positively correlated with the maximum weight variation 12 h after feeding (least-squares linear regression; $p = 0.01$; $r^2 = 34\%$). Frequency of tail biting behaviour fish^{-1} had a positive correlation with the maximum weight variation 12 h after feeding (least-squares linear regression; $p = 0.01$; $r^2 = 30\%$) (Figure 12).



a) b)

Figure 12: Frequency of a) tail biting ($y = 0.077 + 0.103x$; $p = 0.01$; $r^2 = 30\%$) and b) body biting ($y = 0.388 + 0.131x$; $p = 0.01$; $r^2 = 34\%$) behaviours fish^{-1} 12 h after feeding as a function of maximum weight variation (g).



a) b)

Figure 13: Frequency of browsing ($y = 0.376 + 0.117x$; $p = 0.04$; $r^2 = 22\%$) behaviour fish^{-1} before feeding and b) browsing ($y = 1.276 - 0.119x$; $p = 0.02$; $r^2 = 25\%$) behaviour fish^{-1} 2 h after feeding as a function of maximum weight variation (g).

Frequency of browsing behaviour $fish^{-1}$ positively correlated with the maximum weight variation before feeding (least-squares linear regression; $p = 0.04$; $r^2 = 22\%$); Frequency of browsing behaviour $fish^{-1}$ had negative correlation with the maximum weight variation 2 h after feeding (least-squares linear regression; $p = 0.02$; $r^2 = 25\%$) (Figure 13). Frequency of intimidating behaviours $fish^{-1}$ negatively correlated with the maximum weight variation 2 h after feeding (least-squares linear regression; $p = 0.02$; $r^2 = 25\%$) (Figure 14).

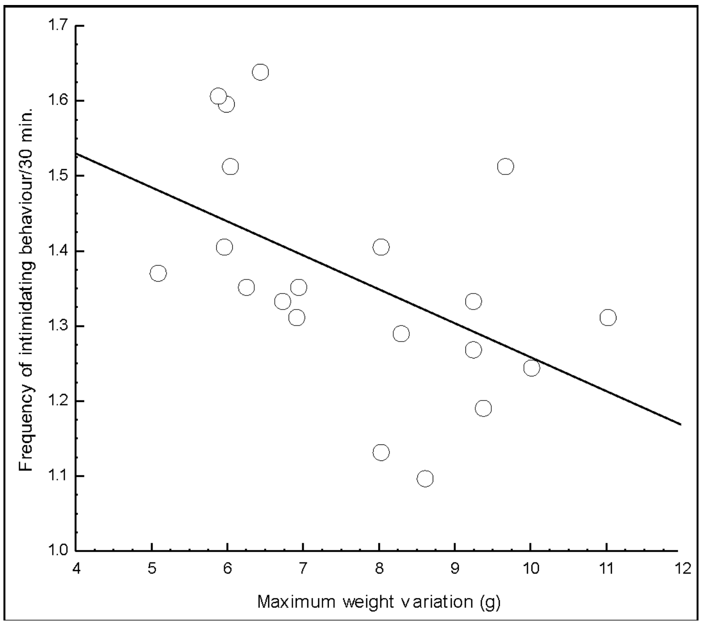
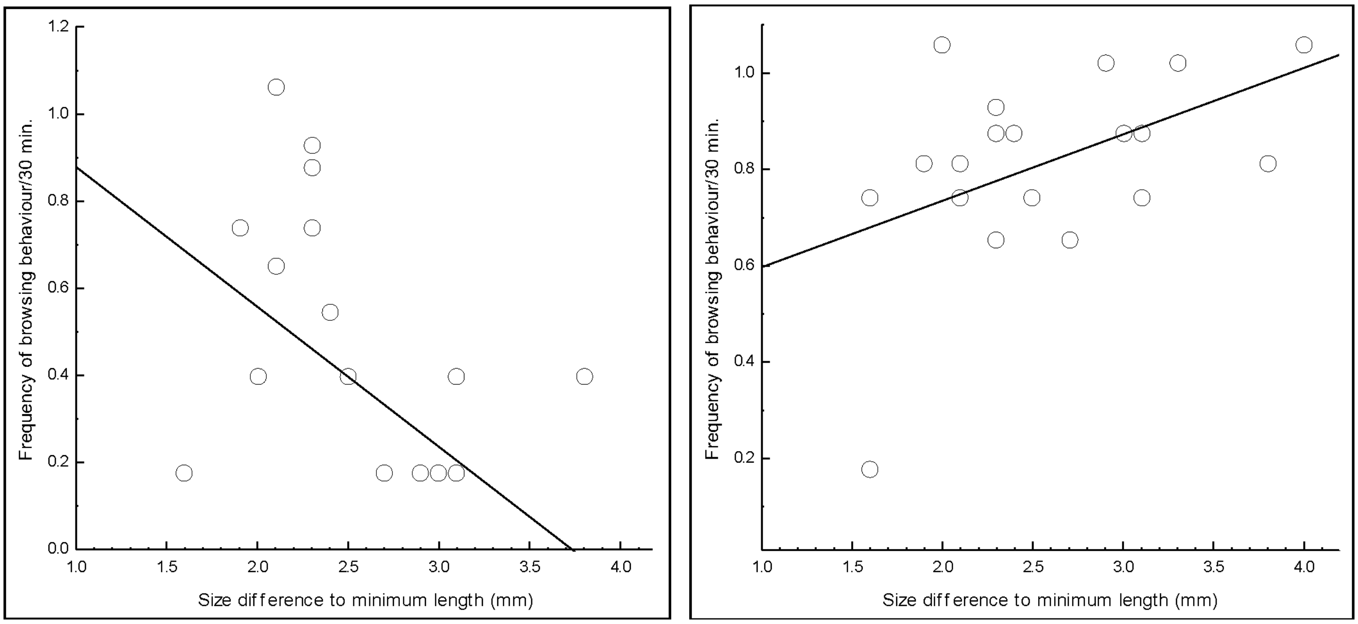


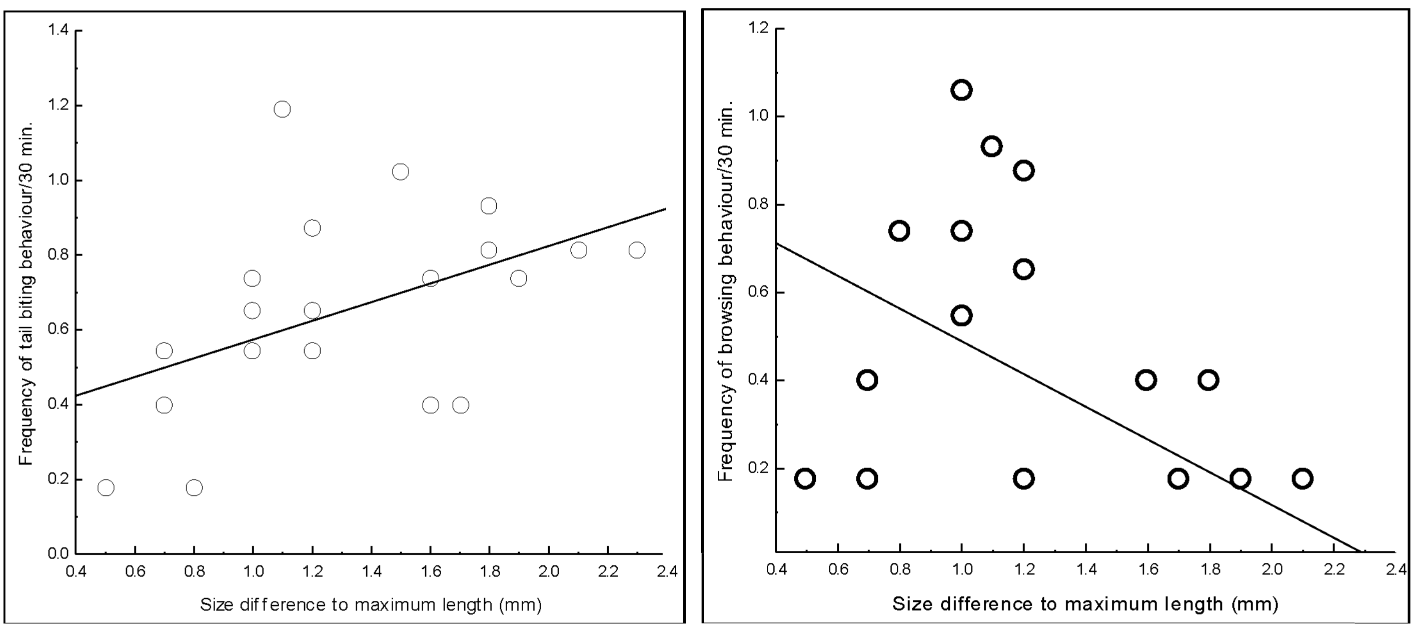
Figure 14: Frequency of intimidating behaviour $fish^{-1}$ 2 h after feeding as a function of maximum weight variation (g) ($y = 1.711 - 0.045x$; $p = 0.02$; $r^2 = 25\%$).

Frequency of browsing behaviour $fish^{-1}$ negatively correlated with the size difference to minimum length before feeding (least-squares linear regression; $p = 0.01$; $r^2 = 29\%$). Frequency of browsing behaviour $fish^{-1}$ positively correlated to size difference to minimum length 12 h after feeding (least-squares linear regression; $p = 0.04$; $r^2 = 22\%$) (Figure 15).



a) b)

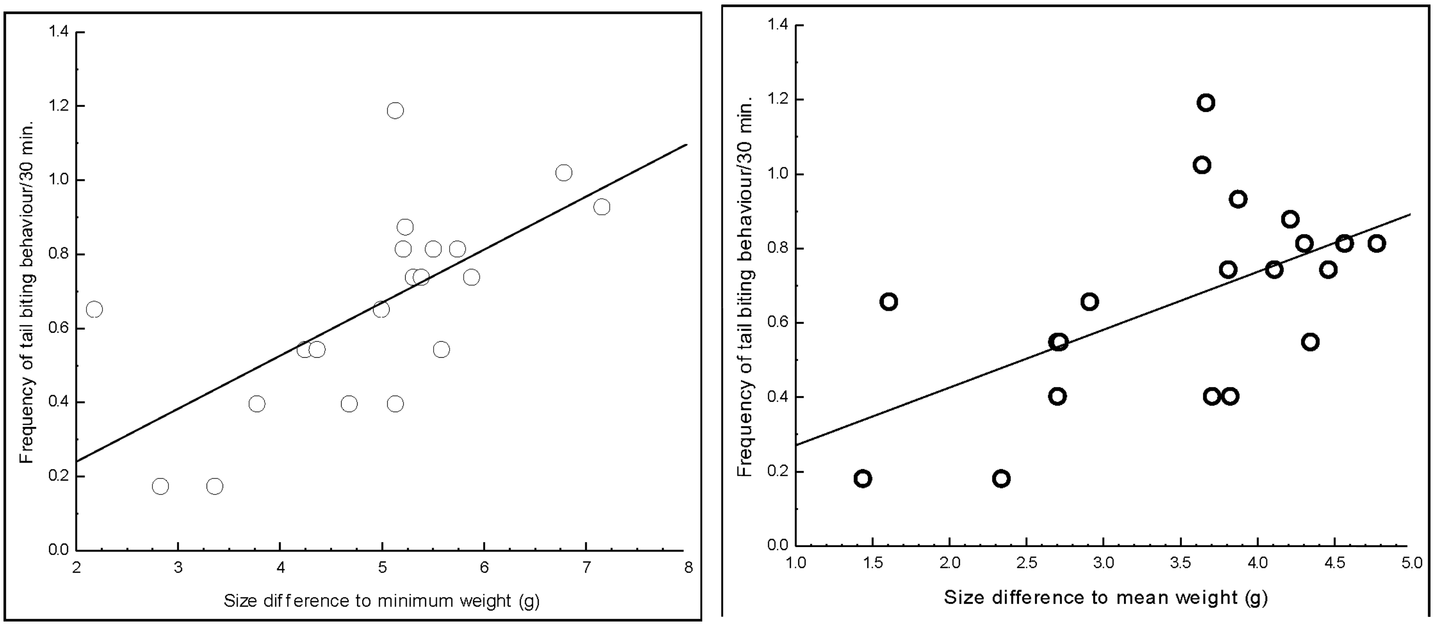
Figure 15: Frequency of a) browsing (y = 1.200 - 0.321x; p = 0.01; r^2 = 29%) behaviour fish^{-1} 2 h after feeding and b) browsing (y = 0.459 + 0.138x; p = 0.04; r^2 = 22%) behaviour fish^{-1} 12 h after feeding as a function of size difference to minimum length (mm).



a) b)

Figure 16: Frequency of a) tail biting (y = 0.324 + 0.250x; p = 0.03; r^2 = 22%) behaviour fish^{-1} before feeding and b) browsing (y = 0.861 - 0.372x; p = 0.04; r^2 = 22%) behaviour fish^{-1} 2 h after feeding as a function of size difference to maximum length (mm).

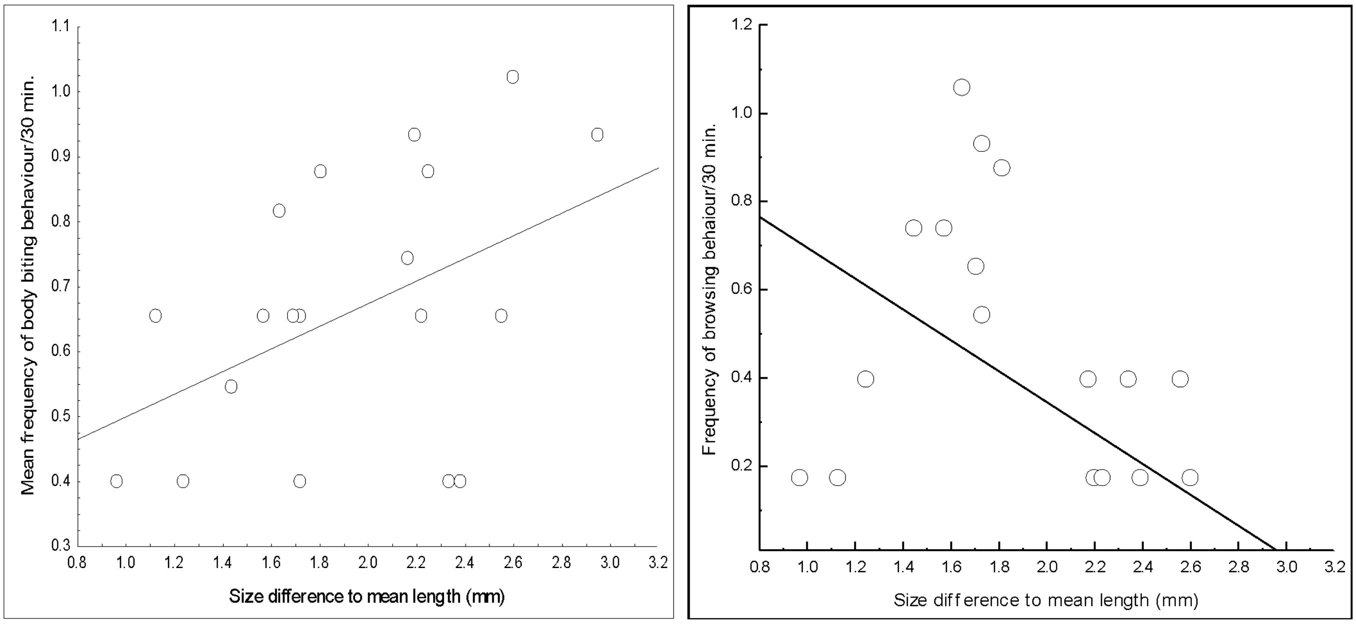
Frequency of tail biting behaviour fish^{-1} positively correlated with the size difference to maximum length before feeding (least-squares linear regression; p = 0.03; r^2 = 22%). Frequency of browsing behaviour fish^{-1} negatively correlated with the size difference to maximum length 2 h after feeding (least-squares linear regression; p = 0.04; r^2 = 22%) (Figure 16).



a) b)

Figure 17: Frequency of a) tail biting (y = 0.047 + 0.143x; p = 0.002; r^2 = 43%) behaviour fish^{-1} before feeding as a function of size difference to minimum weight (g) and b) tail biting (y = 0.115 + 0.156x; p = 0.01; r^2 = 32%) behaviour before feeding as a function of size difference to mean weight (g).

Frequency of tail biting behaviour fish^{-1} before feeding positively correlated with size difference to minimum weight (y = 0.047 + 0.143x; p = 0.002; r^2 = 43%); Frequency of tail biting behaviour fish^{-1} also positively correlated with the size difference to mean weight before feeding (p = 0.01; r^2 = 32%) (Figure 17). Frequency of body biting behaviour fish^{-1} positively correlated with the size difference to mean length 2 h after feeding (least-squares linear regression; p = 0.04; r^2 = 21%); Frequency of browsing behaviour fish^{-1} negatively correlated with the size difference to mean length 2 h after feeding (p = 0.04; r^2 = 22%) (Figure 18).



a) b)

Figure 18: Frequency of a) body biting ($y = 0.326 + 0.174x$; $p = 0.04$; $r^2 = 21\%$) and b) browsing ($y = 1.045 - 0.350x$; $p = 0.04$; $r^2 = 22\%$) behaviours fish^{-1} 2 h after feeding as a function of size difference to mean length (mm).

The majority of the aggressive and browsing behaviours were positively correlated with size variation and differences than those that negatively correlated as time after feeding passed, though these correlations were not all strong. Despite the fact that some aggressive and browsing behaviours correlated with size variation as time after feeding passed, non-significant correlations from linear regression analysis were established ($p < 0.05$) and these results are presented in Table 3.

Table 3: Non-significant correlations between fish behaviours and increasing size variation including its derivatives

COV (%) for length				COV (%) for weight			
Behaviour	**p value**	**y**	**r^2**	**Behaviour**	**p value**	**y**	**r^2**
Body biting 2 h after feeding	0.9198	0.6824 - 0.0027*x	0.0006	Chasing 2 h after feeding	0.0480	0.0176 + 0.0294*x	0.2001
Body biting before feeding	0.0814	1.0188 - 0.0557*x	0.1592	Chasing 6 h after feeding	0.7297	0.5656 + 0.0043*x	0.0068
Body biting 6 h after feeding	0.8540	0.7527 - 0.0084*x	0.0019	Body biting before feeding	0.1102	0.8921 - 0.0146*x	0.1356
Body biting 12 h after feeding	0.8939	0.6601 - 0.0068*x	0.0010	Body biting 2 h after feeding	0.7131	0.6181 + 0.0028*x	0.0077
Tail biting before feeding	0.6984	0.5663 + 0.0139*x	0.0085	Body biting 6 h after feeding	0.4861	0.8443 - 0.0089*x	0.0273
Tail biting 2 h after feeding	0.8317	0.69 - 0.0094*x	0.0026	Body biting 12 h after feeding	0.7311	0.697 - 0.005*x	0.0067
Tail biting 6 h after feeding	0.6488	0.6214 + 0.0192*x	0.0118	Tail biting before feeding	0.3010	0.4868 + 0.0104*x	0.0593
Tail biting 12 h after feeding	0.6410	0.8432 - 0.0201*x	0.0123	Tail biting 2 h after feeding	0.3964	0.4553 + 0.0105*x	0.0402
Browsing before feeding	0.9015	0.4794 + 0.0071*x	0.0009	Tail biting 6 h after feeding	0.8507	0.7852 - 0.0023*x	0.0020
Browsing 2 h after feeding	0.3628	0.6847 - 0.0487*x	0.0462	Tail biting 12 h after feeding	0.1292	1.0069 - 0.018*x	0.1232
Browsing 6 h after feeding	0.9524	0.5684 - 0.0035*x	0.0002	Browsing before feeding	0.5421	0.6876 - 0.0098*x	0.0210
Browsing 12 h after feeding	0.0765	0.5224 + 0.0448*x	0.1641	Browsing 2 h after feeding	0.4548	0.5515 - 0.0114*x	0.0314
Intimidating before feeding	0.7089	1.419 - 0.0054*x	0.0079	Browsing 6 h after feeding	0.4215	0.7637 - 0.0133*x	0.0362
Intimidating 2 h after feeding	0.6409	1.3005 + 0.0095*x	0.0123	Browsing 12 h after feeding	0.2682	0.6833 + 0.0082*x	0.0676

Intimidating 6 h after feeding	0.3933	1.1493 + 0.0218*x	0.0408	Intimidating before feeding	0.8967	1.3749 + 0.0005*x	0.0010
Intimidating 12 h after feeding	0.4259	0.2171 + 0.0052*x	0.0356	Intimidating 2 h after feeding	0.1960	1.2427 + 0.0073*x	0.0910
Chasing before feeding	0.6484	0.2759 + 0.0233*x	0.0118	Intimidating 6 h after feeding	0.8180	1.2649 + 0.0017*x	0.0030
Chasing 2 h after feeding	0.0787	0.1474 + 0.0931*x	0.1618	Intimidating 12 h after feeding	0.9014	0.2473 + 0.0002*x	0.0009
Chasing 6 h after feeding	0.3357	0.3599 + 0.0421*x	0.0516				
Chasing 12 h after feeding	0.2228	0.9292 - 0.0714*x	0.0814				
Minimum length variation				**Maximum length variation**			
Behaviour	**p value**	**y**	**r^2**	**Behaviour**	**p value**	**y**	**r^2**
Chasing before feeding	0.5152	0.2061 + 0.1009*x	0.0239	Chasing before feeding	0.2259	-1.1872 + 0.2137*x	0.0803
Chasing 6 h after feeding	0.9683	0.6031 + 0.0054*x	0.0001	Chasing 2 h after feeding	0.0477	3.2163 - 0.3637*x	0.2006
Body biting before feeding	0.0690	0.4555 + 0.1759*x	0.1721	Chasing 6 h after feeding	0.4251	0.2924 + 0.1228*x	0.0357
Body biting 2 h after feeding	0.9753	0.6806 - 0.0026*x	0.0001	Body biting before feeding	0.4229	0.0508 + 0.093*x	0.0360
Body biting 6 h after feeding	0.0521	0.9042 + 0.2543*x	0.1937	Body biting 2 h after feeding	0.7340	0.4204 + 0.0323*x	0.0066
Body biting 12 h after feeding	0.1259	0.829 + 0.2293*x	0.1252	Tail biting before feeding	0.8150	0.4343 + 0.0296*x	0.0031
Tail biting before feeding	0.9931	0.664 - 0.001*x	0.0000	Tail biting 2 h after feeding	0.2639	1.9157 - 0.1702*x	0.0688
Tail biting 2 h after feeding	0.5836	1.0897 - 0.0732*x	0.0170	Tail biting 6 h after feeding	0.1098	0.9866 + 0.2293*x	0.1359
Tail biting 6 h after feeding	0.4452	0.1343 + 0.0974*x	0.0327	Browsing 2 h after feeding	0.3478	1.6963 - 0.1761*x	0.0491

Tail biting 12 h after feeding	0.0584	0.7767 + 0.2362*x	0.1849	Browsing 6 h after feeding	0.0543	2.3073 + 0.3771*x	0.1905
Browsing before feeding	0.0990	1.2114 + 0.2758*x	0.1440	Browsing 12 h after feeding	0.5375	0.3867 + 0.057*x	0.0215
Browsing 2 h after feeding	0.9248	0.2666 + 0.0155*x	0.0005	Intimidating before feeding	0.3214	1.7583 - 0.0495*x	0.0546
Browsing 6 h after feeding	0.1538	1.0148 + 0.2476*x	0.1097	Intimidating 6 h after feeding	0.6623	0.9945 + 0.0394*x	0.0108
Browsing 12 h after feeding	0.5119	1.1477 - 0.0524*x	0.0243	Intimidating 12 h after feeding	0.5523	0.1485 + 0.0136*x	0.0200
Intimidating before feeding	0.7868	1.4583 - 0.0118*x	0.0042				
Intimidating 2 h after feeding	0.0645	2.0523 - 0.1094*x	0.1773				
Intimidating 6 h after feeding	0.8933	1.3587 - 0.0105*x	0.0010				
Intimidating 12 h after feeding	0.9950	0.2518 - 0.0001*x	0.0000				
Minimum weight variation				**Maximum weight variation**			
Behaviour	**p value**	**y**	**r^2**	**Behaviour**	**p value**	**y**	**r^2**
Chasing before feeding	0.7286	0.3277 + 0.0208*x	0.0069	Chasing before feeding	0.2583	0.0281 + 0.0595*x	0.0704
Chasing 6 h after feeding	0.8604	0.6813 - 0.0091*x	0.0018	Chasing 2 h after feeding	0.1010	1.1662 - 0.0912*x	0.1424
Body biting before feeding	0.0962	0.347 + 0.0623*x	0.1462	Chasing 6 h after feeding	0.8480	0.5687 + 0.0089*x	0.0021
Body biting 2 h after feeding	0.9921	0.666 - 0.0003*x	0.0000	Body biting before feeding	0.4960	0.4713 + 0.0236*x	0.0261
Body biting 12 h after feeding	0.0565	0.0866 + 0.1078*x	0.1876	Body biting 2 h after feeding	0.6538	0.5671 + 0.0127*x	0.0114
Tail biting before feeding	0.9616	0.668 - 0.002*x	0.0001	Tail biting before feeding	0.2451	0.328 + 0.0429*x	0.0743

Tail biting 2 h after feeding	0.4642	0.8119 - 0.0374*x	0.0301	Tail biting 2 h after feeding	0.7147	0.7577 - 0.0168*x	0.0076
Tail biting 6 h after feeding	0.3193	0.5099 + 0.0485*x	0.0551	Tail biting 6 h after feeding	0.1889	0.3119 + 0.0567*x	0.0939
Browsing before feeding	0.1107	0.0223 + 0.1027*x	0.1352	Browsing 6 h after feeding	0.3062	0.069 + 0.062*x	0.0581
Browsing 2 h after feeding	0.5031	0.5705 - 0.042*x	0.0253	Browsing 12 h after feeding	0.5895	0.7034 + 0.0149*x	0.0165
Browsing 6 h after feeding	0.3305	0.222 + 0.0659*x	0.0526	Intimidating before feeding	0.3616	1.4881 - 0.0136*x	0.0464
Browsing 6 h after feeding	0.3305	0.222 + 0.0659*x	0.0526	Intimidating 6 h after feeding	0.5568	1.1715 + 0.0157*x	0.0195
Browsing 12 h after feeding	0.6474	0.8866 - 0.0141*x	0.0119	Intimidating 12 h after feed	0.4825	0.2145 + 0.0048*x	0.0278
Intimidating before feed	0.9062	1.3934 - 0.002*x	0.0008				
Intimidating 2 h after feed	0.0624	1.5707 - 0.0423*x	0.1799				
Intimidating 6 h after feed	0.7514	1.2459 + 0.0095*x	0.0057				
Intimidating 12 h after feed	0.6663	0.235 + 0.0033*x	0.0106				
Difference to minimum length				**Difference to maximum length**			
Behaviour	**p value**	**y**	**r^2**	**Behaviour**	**p value**	**y**	**r^2**
Chasing before feeding	0.3220	0.0865 + 0.1319*x	0.0545	Chasing before feeding	0.3844	0.2234 + 0.1544*x	0.0423
Chasing 2 h after feeding	0.5804	0.6732 - 0.0801*x	0.0173	Chasing 2 h after feeding	0.1042	0.8689 - 0.3025*x	0.1400
Chasing 6 h after feeding	0.4310	0.4003 + 0.0909*x	0.0348	Chasing 6 h after feeding	0.7672	0.5757 + 0.0458*x	0.0050
Chasing 12 h after feeding	0.1953	0.0578 + 0.1989*x	0.0913	Chasing 12 h after feeding	0.0691	0.0246 + 0.3626*x	0.1719

Body biting before feeding	0.8591	0.612 + 0.0156*x	0.0018	Body biting before feeding	0.1368	0.4281 + 0.1681*x	0.1187
Body biting 2 h after feeding	0.0896	0.3624 + 0.1162*x	0.1517	Body biting 2 h after feeding	0.0596	0.438 + 0.1697*x	0.1833
Body biting 6 h after feeding	0.1590	0.2721 + 0.1637*x	0.1071	Body biting 6 h after feeding	0.1239	0.3823 + 0.2363*x	0.1264
Body biting 12 h after feeding	0.1144	0.084 + 0.2044*x	0.1327	Body biting 12 h after feeding	0.0925	0.231 + 0.288*x	0.1492
Tail biting before feeding	0.0797	0.2444 + 0.1591*x	0.1608	Tail biting 2 h after feeding	0.7103	0.7047 - 0.0573*x	0.0079
Tail biting 2 h after feeding	0.5268	0.8184 - 0.0731*x	0.0226	Tail biting 6 h after feeding	0.3800	0.5764 + 0.1285*x	0.0431
Tail biting 6 h after feeding	0.2391	0.4136 + 0.1286*x	0.0761	Tail biting 12 h after feeding	0.0590	0.3491 + 0.2711*x	0.1842
Tail biting 12 h after feeding	0.1058	0.2505 + 0.1772*x	0.1388	Browsing before feeding	0.6785	0.4156 + 0.0826*x	0.0098
Browsing before feeding	0.6262	0.3361 + 0.073*x	0.0135	Browsing 6 h after feeding	0.5713	0.3906 + 0.1159*x	0.0181
Browsing 6 h after feeding	0.5509	0.3065 + 0.0918*x	0.0201	Browsing 12 h after feeding	0.1927	0.6606 + 0.1176*x	0.0924
Intimidating before feeding	0.7665	1.4129 - 0.0113*x	0.0050	Intimidating before feeding	0.7878	1.3655 + 0.0136*x	0.0041
Intimidating 2 h after feeding	0.1861	1.5434 - 0.0694*x	0.0950	Intimidating 2 h after feeding	0.1585	1.4938 - 0.0979*x	0.1073
Intimidating 6 h after feeding	0.5190	1.1796 + 0.0435*x	0.0235	Intimidating 6 h after feeding	0.7920	1.2609 + 0.0237*x	0.0040
Intimidating 12 h after feeding	0.4390	0.2168 + 0.0132*x	0.0336	Intimidating 12 h after feeding	0.6713	0.2381 + 0.0097*x	0.0102
Difference to minimum weight				**Difference to maximum weight**			
Behaviour	**p value**	**y**	**r^2**	**Behaviour**	**p value**	**y**	**r^2**
Chasing before feeding	0.1845	0.0438 + 0.0962*x	0.0957	Chasing before feeding	0.8103	0.3803 + 0.023*x	0.0033

Chasing 2 h after feeding	0.7453	0.5928 - 0.026*x	0.0060	Chasing 2 h after feeding	0.2028	0.74 - 0.1285*x	0.0885
Chasing 6 h after feeding	0.2812	0.3025 + 0.068*x	0.0642	Chasing 6 h after feeding	0.4537	0.5053 + 0.0615*x	0.0316
Chasing 12 h after feeding	0.9507	0.4331 + 0.0054*x	0.0002	Chasing 12 h after feeding	0.9385	0.441 + 0.0086*x	0.0003
Body biting before feeding	0.8311	0.6018 + 0.0103*x	0.0026	Body biting before feeding	0.0902	0.4357 + 0.1013*x	0.1511
Body biting 2 h after feeding	0.1580	0.3995 + 0.0539*x	0.1076	Body biting 2 h after feeding	0.3331	0.5611 + 0.0483*x	0.0521
Body biting 6 h after feeding	0.3567	0.4029 + 0.0599*x	0.0474	Body biting 6 h after feeding	0.7380	0.6371 + 0.0283*x	0.0064
Body biting 12 h after feeding	0.4681	0.3539 + 0.0532*x	0.0296	Body biting 12 h after feeding	0.5795	0.7278 - 0.0525*x	0.0174
Tail biting 2 h after feeding	0.6742	0.4963 + 0.0268*x	0.0100	Tail biting before feeding	0.1412	0.4526 + 0.096*x	0.1163
Tail biting 6 h after feeding	0.4550	0.5244 + 0.0454*x	0.0314	Tail biting 2 h after feeding	0.9756	0.6228 + 0.0025*x	0.0001
Tail biting 12 h after feeding	0.6011	0.5508 + 0.0326*x	0.0155	Tail biting 6 h after feeding	0.8135	0.7081 + 0.0186*x	0.0032
Browsing before feeding	0.1274	0.0749 + 0.1221*x	0.1243	Tail biting 12 h after feeding	0.6566	0.6346 + 0.0358*x	0.0112
Browsing 2 h after feeding	0.2283	0.8193 - 0.0925*x	0.0796	Browsing before feeding	0.3886	0.331 + 0.0911*x	0.0416
Browsing 6 h after feeding	0.5919	0.3215 + 0.0455*x	0.0163	Browsing 2 h after feeding	0.2351	0.1128 + 0.1176*x	0.0774
Browsing 12 h after feeding	0.0799	0.5014 + 0.0643*x	0.1607	Browsing 6 h after feeding	0.6713	0.4458 + 0.0465*x	0.0102
Intimidating before feeding	0.3635	1.476 - 0.0188*x	0.0461	Browsing 12 h after feeding	0.6352	0.7676 + 0.0234*x	0.0128
Intimidating 2 h after feeding	0.5755	1.4442 - 0.0165*x	0.0177	Intimidating before feeding	0.7912	1.3684 + 0.0071*x	0.0040
Intimidating 6 h after feeding	0.8470	1.2572 + 0.0072*x	0.0021	Intimidating 2 h after feeding	0.8192	1.3444 + 0.0087*x	0.0030

Intimidating 12 h after feeding	0.7381	0.2355 + 0.0032*x	0.0064	Intimidating 6 h after feeding	0.7714	1.3224 - 0.014*x	0.0048
				Intimidating 12 h after feeding	0.8984	0.2544 - 0.0016*x	0.0009
Difference to mean length				**Difference to mean weight**			
Behaviour	**p value**	**y**	**r^2**	**Behaviour**	**p value**	**y**	**r^2**
Chasing before feeding	0.3244	0.1071 + 0.1658*x	0.0539	Chasing before feeding	0.7046	0.3063 + 0.0353*x	0.0082
Chasing 2 h after feeding	0.2514	0.8673 - 0.2068*x	0.0724	Chasing 2 h after feeding	0.3810	0.7692 - 0.0871*x	0.0429
Chasing 6 h after feeding	0.4413	0.4183 + 0.1123*x	0.0333	Chasing 6 h after feeding	0.5285	0.4606 + 0.0505*x	0.0224
Chasing 12 h after feeding	0.1027	0.1478 + 0.3122*x	0.1411	Chasing 12 h after feeding	0.9524	0.4821 - 0.0065*x	0.0002
Body biting before feeding	0.2670	0.4174 + 0.1209*x	0.0679	Body biting before feeding	0.2221	0.3994 + 0.0725*x	0.0816
Body biting 6 h after feeding	0.1056	0.2397 + 0.2355*x	0.1389	Body biting 2 h after feeding	0.0888	0.3835 + 0.0805*x	0.1524
Body biting 12 h after feeding	0.1243	0.1257 + 0.2518*x	0.1262	Body biting 6 h after feeding	0.3970	0.4559 + 0.0693*x	0.0402
Tail biting before feeding	0.0518	0.2286 + 0.2208*x	0.1941	Body biting 12 h after feeding	0.7733	0.5223 + 0.0267*x	0.0047
Tail biting 2 h after feeding	0.5982	0.7781 - 0.0771*x	0.0157	Tail biting 2 h after feeding	0.7861	0.5522 + 0.0218*x	0.0042
Tail biting 6 h after feeding	0.2185	0.4183 + 0.1695*x	0.0829	Tail biting 6 h after feeding	0.5604	0.5922 + 0.0446*x	0.0192
Tail biting 12 h after feeding	0.0595	0.2106 + 0.2573*x	0.1836	Tail biting 12 h after feeding	0.4963	0.5258 + 0.0531*x	0.0261
Browsing before feeding	0.6164	0.3414 + 0.0948*x	0.0142	Browsing before feeding	0.4000	0.2235 + 0.0866*x	0.0396
Browsing 6 h after feeding	0.4286	0.247 + 0.1534*x	0.0352	Browsing 2 h after feeding	0.5710	0.5581 - 0.0555*x	0.0182

Browsing 12 h after feeding	0.0535	0.5042 + 0.1611*x	0.1918	Browsing 6 h after feeding	0.9954	0.5431 + 0.0006*x	0.0000
Intimidating before feeding	0.9573	1.3786 + 0.0026*x	0.0002	Browsing 12 h after feeding	0.1248	0.5682 + 0.0714*x	0.1259
Intimidating 2 h after feeding	0.1658	1.5412 - 0.0916*x	0.1039	Intimidating before feeding	0.7864	1.3588 + 0.0071*x	0.0042
Intimidating 6 h after feeding	0.6176	1.2097 + 0.0426*x	0.0141	Intimidating 2 h after feeding	0.9114	1.3486 + 0.0041*x	0.0007
Intimidating 12 h after feeding	0.5024	0.2229 + 0.0145*x	0.0254	Intimidating 6 h after feeding	0.8255	1.2565 + 0.0103*x	0.0028
				Intimidating 12 h after feeding	0.7140	0.2359 + 0.0043*x	0.0076

4. Discussion

Effect of observation time on fish behaviours

Many important culture species are highly cannibalistic at the early stages of development (Baras, 2013). During juvenile stages, dusky kob are aggressive and cannibalism can lead to mortality (O'Sullivan and Ryan, 2001; PIRSA, 2001; Collett, 2007; Timmer and Magellan, 2011). An elevated degree of cannibalism in dusky kob was observed in the second critical phase of development at about 20 days after hatching (DAH), when fish were weaned onto artificial feed (Musson and Kaiser, 2014). Comparable to this recent observation, O'Sullivan and Ryan (2001) suggested occurrence of cannibalism in dusky kob 18 days after hatching. In the present study, the frequency of hatchery-reared juvenile dusky kob browsing and aggressive behaviours consisting of chasing, intimidating, body biting and tail biting behaviours was determined.

Repeated measures ANOVA showed that fish increased browsing (averaging 6.60 ± 0.56) and decreased intimidating aggressive behaviours (averaging 18.60 ± 1.39) per 30 min. 12 h after feeding. Similarly, the increased intimidating behaviours in catfish were observed before feeding in the morning after a long time of no access to food (Kaiser *et al*., 1995a). Increased interactions among fish are likely to take place when fish become hungry (Metcalfe *et al*., 1995; Greaves and Tuene, 2001; Liao *et al*., 2001). Usually, bigger fish intimidate smaller ones (Jobling, 1982; Baras, 1998), for example, in cichlids (Clement *et al*., 2005) and Arctic charr (Øverli *et al*., 1999) and frequent aggression could be linked to hunger (Almaza´n-Rueda *et al*., 2004). The present observations suggest that fish may have become hungry approximately 6-12 h after feeding and increasingly favoured browsing over aggressive behaviours as time post-feeding passed. In comparison, juvenile dusky kob of 2.7 ± 0.5 g $fish^{-1}$ showed empty stomachs after 20 h with about half-empty stomachs within 10-12 h after a single feed to satiation (Ballagh *et al*., 2008). For this reason, a 12-h feeding interval was suggested as the most efficient technique of management to achieve high growth and survival rates of juvenile dusky kob (Ballagh *et al*., 2008). When fed three and four times per day, juvenile dusky kob were fastest in emptying their stomach, with evacuation time averaging 5.5 hours after feeding (Daniel, 2004). Among other culture species, juvenile spotted seatrout *Cynoscion* nebulosus, also a sciaenid, evacuated their gut within 2-4 h after feeding (Wuenschel and Werner, 2004) and juvenile Australian snapper *Pagrus auratus* completely evacuated their gut within 16-20 h with about half the meal emptied within 5 h (Booth *et al*., 2008). Comparable to present observations, spotted seatrout increased their aggression and cannibalism 4-7 h after feeding and the gut passage time could explain this trend of aggressive behaviour (Manley *et al*., 2014).

There were however no significant differences in the frequencies of aggressive (chasing, body biting and tail biting) behaviours fish^{-1} over observation time, as shown by repeated measures ANOVA analysis. There may be other factors that contributed to behaviour trends and shifts observed. For example, fish become used to the times of feeding and are active before feeding, as was also seen in African catfish (Kaiser *et al*., 1995a). Mostly, finfish are fed just before or after evacuating their guts to minimise aggressive behaviours during their early developmental stages (Baras and Jobling, 2002; Manley *et al*., 2014). The relationship between the time required to empty the stomach and the return of appetite exist and stomach fullness in farmed fish influences their hunger and feeding drive (e.g., in rainbow trout, Grove *et al*., 1978). Further, the differences in aggressive behaviours seemed to increase even more with longer feeding intervals (Manley *et al*., 2015). In juvenile dusky kob, feeding frequency and quantity should be carefully considered to avoid negative effects of starvation and ensure improved fish growth and feed conversion (Guy and Smith, 2016). Juvenile dusky kob should thus be fed 4-6 h after each meal per day to maintain less-aggressive behaviours and cannibalism in tanks.

Effect of size variation and its derivatives on fish behaviours

The early development of dusky kob has patterns similar to those of other marine teleost fish (Santamaria *et al*., 2004; Mai *et al*., 2005). Once mouth opening occurs 2-3 DAH (Musson and Kaiser, 2014), fish became active in browsing, searching for food and at this stage, different factors such as food availability and behavioural differences could have influenced how fish find adequate food (Duray *et al*., 1996).

The linear regression analysis showed that size difference variables positively correlated most often with aggressive behaviours and browsing behaviours as time after feeding passed. The next highest frequency was of aggressive and browsing behaviours correlating with maximum weight variation, followed by maximum length variation. Both COV and maximum length variation followed with the minimum length variation least correlating with aggressive and browsing behaviours. Overall, fish showed the highest number of chasing (19 counts) acts, followed by equal numbers of browsing and body attacks (17 counts each) and the least tail biting (11 counts) behaviours positively correlating with size variation as time post feeding passed. Fish size has become the most common factor responsible for aggressive behaviours (Barlow and Ballin, 1976) with increased biting attacks often directed to smaller fish, leading to cannibalism (Fairchild and Howell, 2001; Hatlen *et al*., 2006). Aggressive fish during their early life stages showed type I cannibalism with one fish ingested tail-first when size variability is low (Baras and Jobling, 2002; Baras, 2013). Fish showed negative correlations closer to their last meal, 2 h after feeding and positive correlations longer time after meal, 12 h after feeding between aggressive and browsing behaviours. Concurring with current observations, size difference was the most important variable influencing aggression in cultured fish including sciaenid species like juvenile red drum (Liao and Chang, 2002). Initial size difference among fish is therefore more likely to initiate aggression and consequently lead to cannibalistic interactions (Hecht and Pienaar, 1993; Solomon and Udoji, 2011). Furthermore, size variation among fish positively correlated with their aggressive behaviours and cannibalism (Baras and d'Almeida, 2001; Baras *et al.*, 2000; Kestemont *et al.*, 2003; Skov *et al.*, 2003; Moran, 2007; Solomon and Udoji, 2011). Increased frequencies of aggressive and cannibalistic behaviours as the size differences among fish increased (Miki *et al.*, 2011) were observed in many farmed species including Atlantic cod larvae (Puvanendran *et al.*, 2008), young yellowtail *S. quinqueradiata* (Sakakura and Tsukamoto, 1996) and Nile tilapia *O. niloticus* (Abdel-tawwab *et al.*, 2006). In agreement with present observations, size variation promotes aggressive behaviours in many cultured fish (Baras *et al.*, 2000; Liao and Chang, 2002; Valdimarsson and Metcalfe, 2000; Chang and Liao, 2003; Paulet, 2003; Zakes *et al.*, 2004; Clement *et al.*, 2005; Fessehaye *et al.*, 2006; Solomon and Udoji, 2011).

A few behaviours including chasing (0.2 counts), tail biting (4 counts), intimidating (2.4 counts) and browsing (0.3) behaviours, however, negatively correlated with size variation as time after feeding passed. Interestingly, fish seemed to substitute certain aggressive behaviours, commonly for browsing behaviours, as size variation increased and time after feeding passed. Mostly, fish would alter their aggressive behaviours depending on the availability of food (Limberly, 2002). Moreover, fish showed fewer aggressive and browsing behaviours negatively correlating with size variation, notably, 2 h after feeding which was closer to their last feeding. Similarly, spotted seatrout showed fewest aggressive and cannibalistic behaviours at a 2-h feeding frequency (Manley *et al.*, 2014). The balance between satiation and gut evacuation (Baras and Jobling, 2002) could explain this observation. In addition, aggressive behaviours increased as more time after feeding passed in greater amberjack *Seriola dumerili* (Miki *et al.*, 2011). These patterns of aggressive behaviours may relate to hunger (Almaza´n-Rueda *et al.*, 2004), in addition to increased size variation among fish. For example, increased aggressive behaviours under conditions of low food availability and feeding frequencies (greater amberjack, Miki *et al.*, 2011; spotted seatrout, Manley *et al.*, 2014), often leads to persistent cannibalism (Dou *et al.*, 2000). In carnivorous early juvenile fish, cannibalism is often as an alternative strategy of feeding when food resources become limited (Hecht and Pienaar, 1993; Baras *et al.*, 1999; Liao *et al.*, 2001; Baras and Jobling, 2002; Baras, 2013). Fish may also use aggression and cannibalism as an adaptive feeding intervention during times of low food availability (Rincón and Grossman, 2001) and would attack other fish as the only available alternative prey (Hecht and Pienaar, 1993; Baras and Jobling, 2002). Persistent aggressive acts in several species (Obirikorang *et al.*, 2014) occurred even when catfish were fed to satiation (Kaiser *et al.*, 1995a). Thus, enough feeding and increased feeding frequency alone cannot stop but can reduce the frequency of aggressive behaviours (Jobling, 1985; Wang *et al.*, 1998; Lazo *et al.*, 2000; Manley *et al.*, 2014; Obirikorang *et al.*, 2014). Aggression and cannibalistic behaviours may also continue occurring during the juvenile phase of development if size variation among fish continues to exist (Hecht and Pienaar, 1993; Baras and Jobling, 2002). For this reason, regular size grading of fish can reduce aggressive behaviours and cannibalism (Hecht and Pienaar, 1993; Qin and Fast, 1996; Fessehaye *et al.*, 2006). It is also important to minimise factors leading to high size variation in combination with size-grading techniques to reduce loss of juvenile fish (Qin and Fast, 1996; Fessehaye *et al.*, 2006). Removal of the fish most susceptible to aggression would be an alternative method to reducing aggression as larger fish may dominate in the hatchery tanks (Wankowski and Thorpe, 1979; Metcalfe, 1994; Barki *et al.*, 2000). The correlations between fish behaviours and size variation were, however, not very strong from the linear regressions but due to their significance, the results are reported. The main intention is to demonstrate relevant hypothesis and trends, allowing comparisons with other studies. Fish may also have experienced limited capacity to perform certain behaviours and evidently, some of their aggressive behaviours did not differ significantly between observation times. There may be other factors that contributed to the present observations, which due to limited time and resources, could not be tested and explored further. The non-significant results of fish behaviours correlated with size variation have been organised (Table 3) to provide point of reference in case future studies are run to further explore the present study observations.

In conclusion, the observation time and increasing size variation could explain increased increasing aggressive and browsing behaviours of juvenile dusky kob under culture conditions. Increased aggressive behaviours of juvenile dusky kob correlated more often with increasing size variation than with observation times. Grading the hatchery-reared juvenile dusky kob more often as fish grow and feeding fish no later than 4-6 h between their feedings may reduce aggressive behaviours. Juvenile dusky kob appeared to replace certain aggressive behaviours with others as their needs change, mostly for browsing behaviours, and as time after feeding passed. The current experimental observations provide a foundation for the next experiment to assess the effect of size variation on aggressive behaviours. Chapter 3 reports on the further observations towards exploring the present preliminary findings.

Chapter 3

Observations on the association between size variation and aggressive behaviours of juvenile dusky kob, *Argyrosomus japonicus*

1. Introduction

It is essential to use size-grading to improve growth and survival of smaller individuals in finfish culture, making sufficient resources available for individuals of different sizes (Gunnes, 1976). Aquaculture practice involves this technique also to reduce food wastage and avoid the resultant water quality degradation, which may lead to the development of dominance hierarchies (Davis and Olla, 1987; McCarthy *et al.*, 1992; Jobling and Baardvik, 1994). The increasing size variation usually seen in smaller fish may remain unchanged in large fish (Wickins, 1985; Wallace and Kolbeinshavn, 1988). The maintenance of low size distribution among cultured fish is seemingly the main driving force behind the size-grading technique in aquaculture as larger fish intimidate smaller fish when competing for resources (Munday and Jones, 1998). Cannibalism thus occurs mostly relating to size difference between the largest and smallest individual in a group (Obirikorang *et al.*, 2014). Other factors promoting size differences during early development stages include genetic factors, resource competition and morphological deformities (Kestemont *et al.*, 2003; Baras, 2013).

The manipulation of food consumption has the ability to influence biomass gain by controlling the timing and frequency of feeding in relation to fish size and size variation (Sveier and Lied, 1998). The aggressive behaviour occurs when fish are hungry (Greaves and Tuene, 2001) and the behaviour of fish is influenced by presence of food (Kestemont *et al.*, 2003). Metcalfe *et al.* (1995) reported that fish with a high hunger levels frequently become more active while aggressive and a high rate of aggression could be stressful in fish (Refstie, 1977). A negative relationship between frequent feedings and aggression appeared to be present in the Australian Snapper (*Pagrus auratus*) (Tucker *et al.*, 2006).

Further research is required to make it clear whether size-grading would be a useful strategy to achieve improved fish growth and avoid the impact of high size variation (Barki *et al.*, 2000). Observations from the previous experimental trial established correlations and trends of juvenile dusky kob aggressive and browsing behaviours with increasing size variation, particularly, 12 h after feeding. Additionally, some of these behaviours differed between observation times. The current experimental trial consisted of the observations to explore the relationship between increasing size variation and aggressive behaviours further.

2. Materials and methods

Experimental system, fish and acclimation

Hatchery-reared juveniles of dusky kob, averaging 0.43 ± 0.27 g $fish^{-1}$, were obtained from Oceanwise (Pty) Ltd in East London, South Africa. These experimental fish had smaller average size compared to that of the fish used for the previous experimental trial. The wet weight of each fish was measured with an accuracy of 0.1 g using an electronic digital scale (Denver Instruments, MXX-612, New York, USA). Each of the four observation tanks (14 cm x 13 cm x 10 cm, figure 1) was covered to avoid disturbances to fish and only one small cut was left open to enable observations. A timer switch was set to maintain a 12 h light: 12 h dark regimen throughout the experiment. Salinity was maintained at approximately 35 parts per thousand (ppt) with freshwater added to the system when necessary and was measured by a portable refractometer (Atago S/Mill-E, Tokyo, Japan). Water pH was maintained between 7.4 and 8.5 and water temperature was maintained between 24 and 26 °C. The water temperature and pH levels were measured using a portable electronic probe (Hanna Instruments HI 98128, Rhode Island, USA). Water temperature, salinity and pH were measured twice day^{-1}. The dissolved oxygen (DO) was measured twice a week with a DO meter and was maintained between 80 and 100% of saturation throughout the experiment, using air-stones. Regular water quality measurements were undertaken to test whether ammonia and nitrite concentrations were within limits acceptable for dusky kob. Similar water conditions in both holding and observation tanks were maintained throughout the duration of the experiment. The fish in the holding tank were fed commercial trout pellets three times day^{-1} (08h00, 13h00 and 17h00) to satiation, i.e., when the fish no longer accepted pellets dropped into the tank.

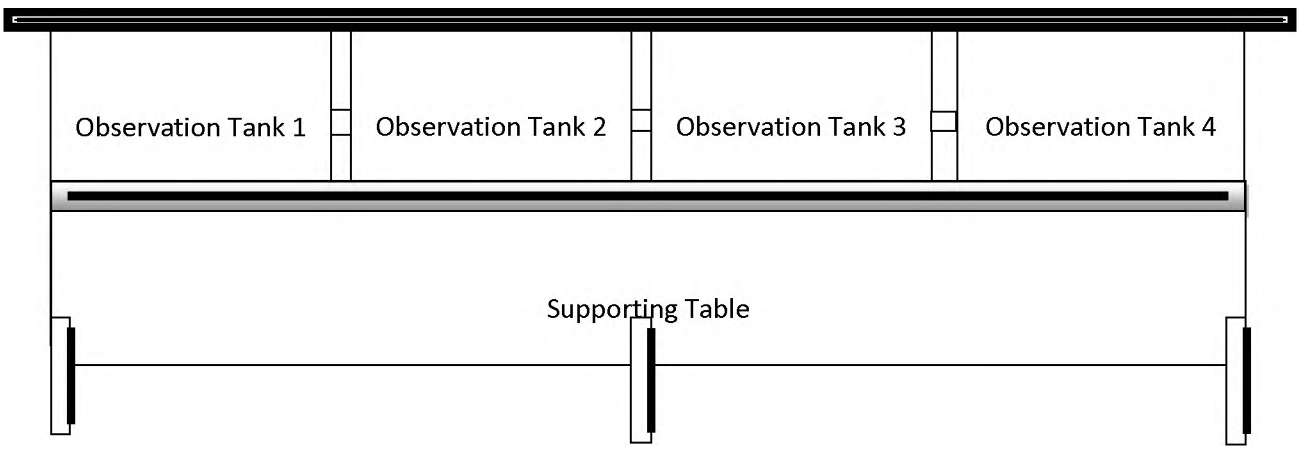


Figure 1: A schematic diagram of four observation tanks in the experimental room

Experimental design and protocols

An individual fish weighing more than the average of all other fish in that tank was chosen as a focal fish. The focal fish was exposed to fish groups of four size variation treatments for observation with the order of presentation randomised. Each group consisted of eight fish. Different values of the COV of non-focal fish were achieved with fish of different sizes. Before the start of each observation, fish were individually weighed to determine the COV for each of the four treatments. The behaviours observed included chasing, body biting, tail biting and intimidating behaviours. The focal fish was only observed once per day, before and 12 h after feeding, respectively. The pelleted food was dropped by hand into the centre equidistant from each side of the observation tank and fish were fed to apparent satiation after the observation in line with observation times. Uneaten food and waste material was removed daily by siphoning it from the bottom of the tank. A countdown timer (stopwatch) with an alarm was used to keep the time per observation. The twenty-four observations were performed on six focal fish.

Data analysis

The relationship between size variation and fish behaviours observed was assessed, taking note of significant correlations ($p < 0.05$) for statistical analyses. The null hypothesis stated that there is no correlation between fish behaviours and size variation including its derivatives. Least-squares linear regression modelling was used to predict frequency of behaviours as a function of size variation. The size variation among non-focal fish was expressed as COV after all fish had been individually weighed. Eight fish in each of observation tanks were used for observations and tank-effect could not be tested. All statistical analyses for the entire dataset were performed using the Statistica software, version 9 (Statsoft, Tulsa, OK, USA).

3. Results

Aggressive behaviours fish^{-1} per 30 min. significantly correlated with size variation (COV) before and 12 h after feeding. However, for certain behaviours, there were no significant correlations with COV-values and these results are presented in Table 1.

Size variation, its derivatives and behaviours

Frequency of chasing behaviour fish^{-1} was significantly positively correlated with the COV-value for weight before feeding (least-squares linear regression; $p = 0.003$; $r^2 = 34\%$) (Figure 2).

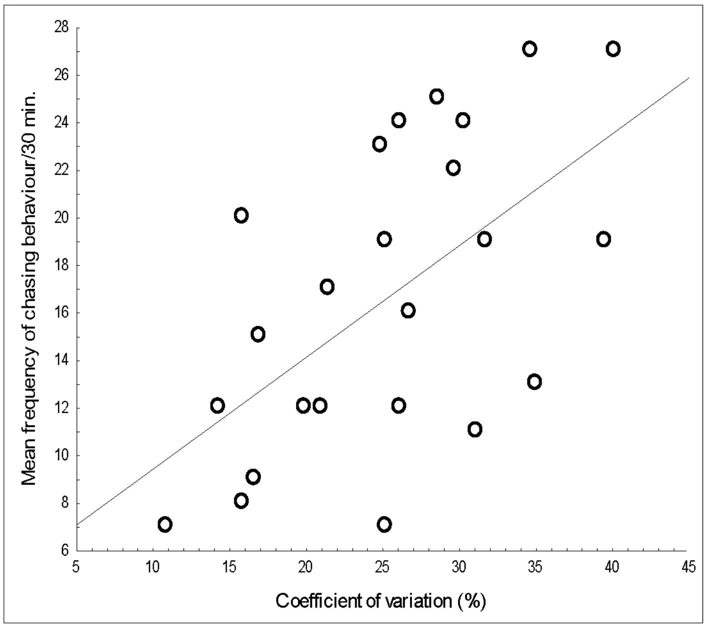
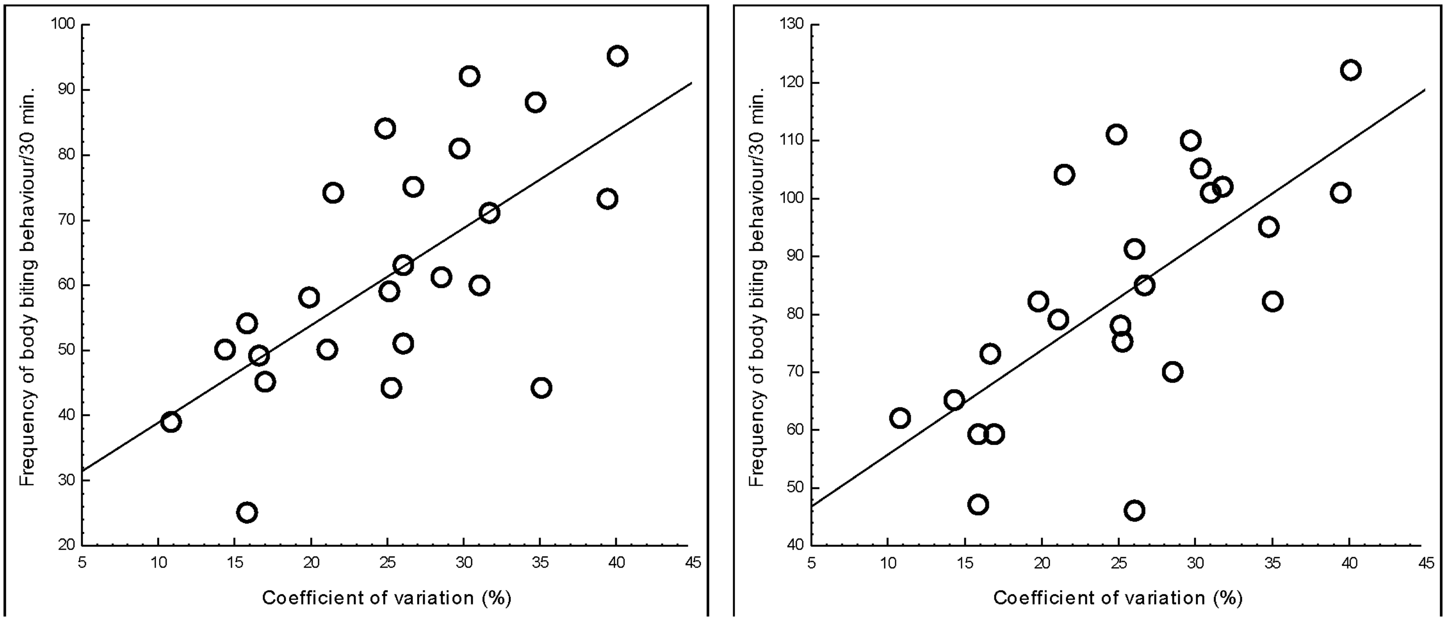


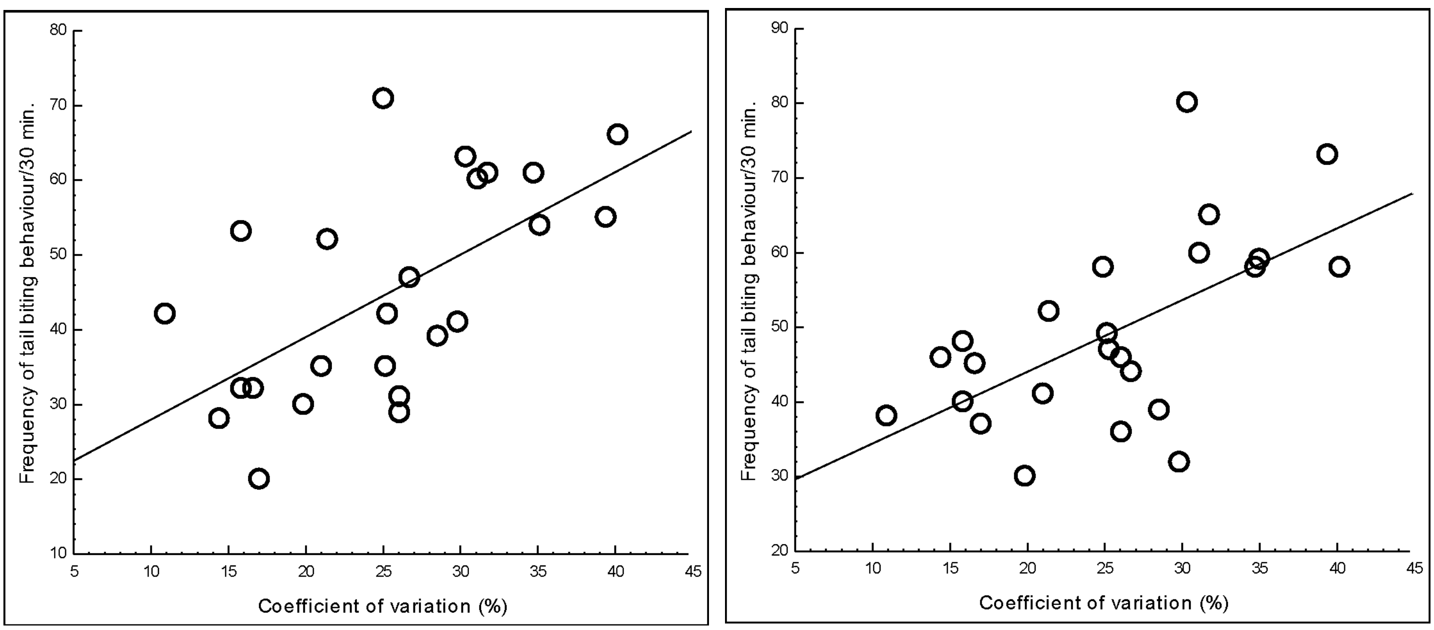
Figure 2: Frequency of chasing ($y = 4.734 + 0.470x$; $p = 0.003$; $r^2 = 34\%$) behaviour $fish^{-1}$ before feeding as a function of the COV (%) for weight.

Frequency of body biting behaviour $fish^{-1}$ had a significant positive correlation with the COV for weight before feeding (least-squares linear regression; $p = 0.001$; $r^2 = 43\%$); Similarly, frequency of body biting behaviour $fish^{-1}$ was significantly positively correlated with the COV for weight 12 h after feeding (least-squares linear regression; $p = 0.0002$; $r^2 = 47\%$) (Figure 3). Frequency of tail biting behaviour $fish^{-1}$ had a significant positive correlation with the COV for weight before feeding (least-squares linear regression; $p = 0.001$; $r^2 = 38$); In the same way, frequency of tail biting behaviour $fish^{-1}$ was significantly positively correlated with the COV for weight 12 h after feeding (least-squares linear regression; $p = 0.002$; $r^2 = 37\%$) (Figure 4).



a) b)

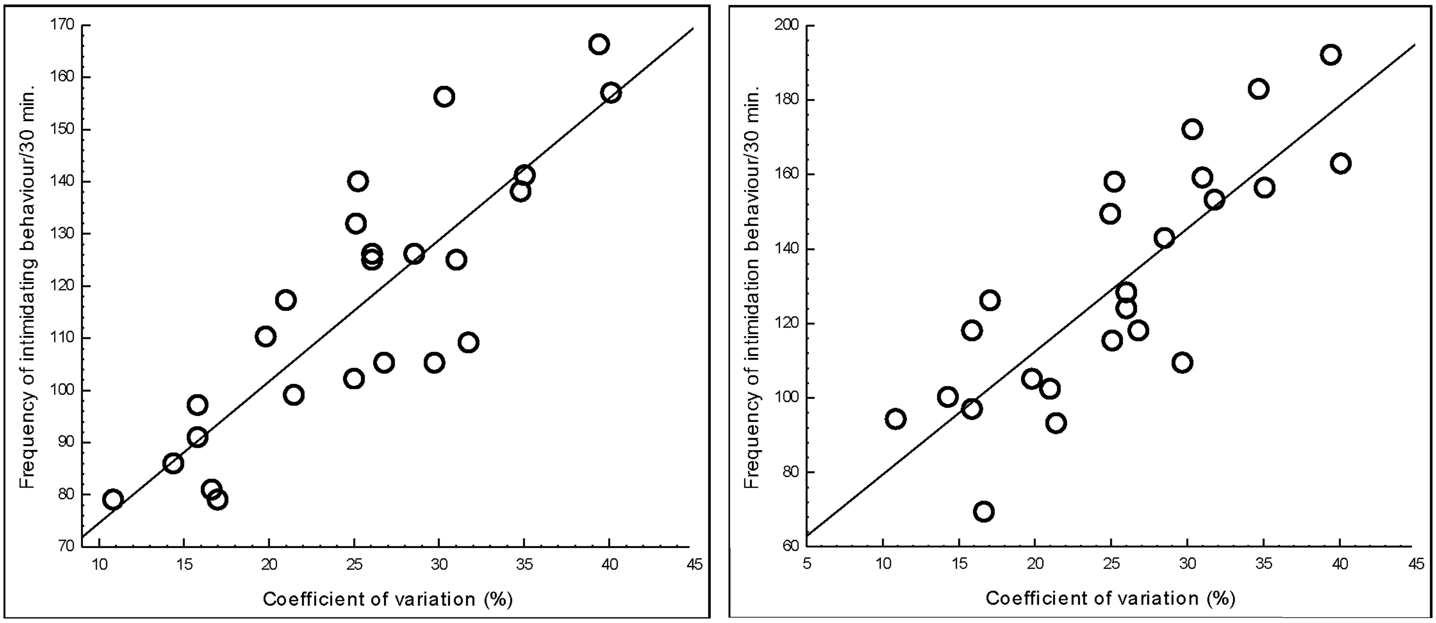
Figure 3: Frequency of a) body biting (y = 23.9676 + 1.4935x; p = 0.001; r^2 = 43%) behaviour fish^{-1} before feeding and of b) body biting (y = 37.729 + 1.803x; p = 0.0002; r^2 = 47%) behaviour 12 h after feeding as a function of the COV (%) for weight.



a) b)

Figure 4: Frequency of a) tail biting (y = 16.961 + 1.103x; p = 0.001; r^2 = 38%) behaviour fish^{-1} before feeding and b) of tail biting (y = 24.839 + 0.960x; p = 0.002; r^2 = 37%) behaviour fish^{-1} 12 h after feeding as a function of the COV (%) for weight.

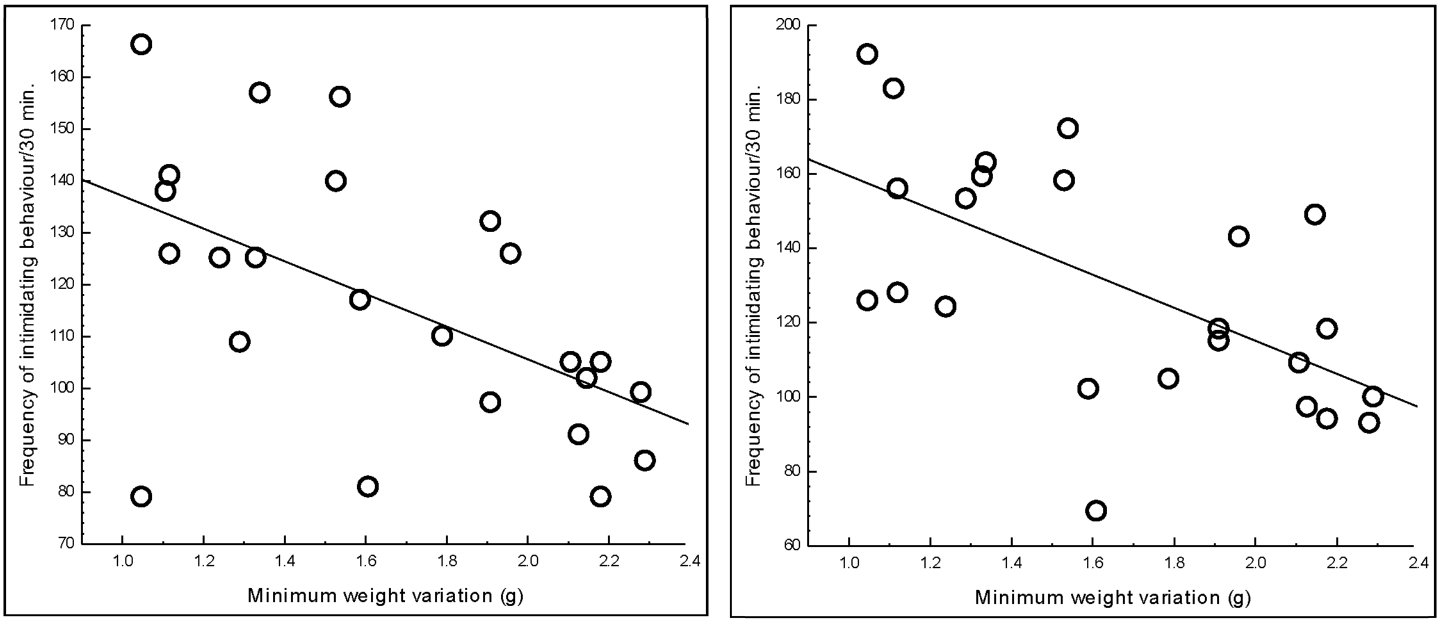
Frequency of intimidating behaviour fish^{-1} was significantly positively correlated with the COV for weight before feeding (least-squares linear regression; $p < 0.0001$; $r^2 = 73\%$); Similarly, frequency of intimidating behaviour fish^{-1} was significantly positively correlated with the COV for weight 12 h after feeding (least-squares linear regression; $p < 0.0001$; $r^2 = 67\%$) (Figure 5).



a) b)

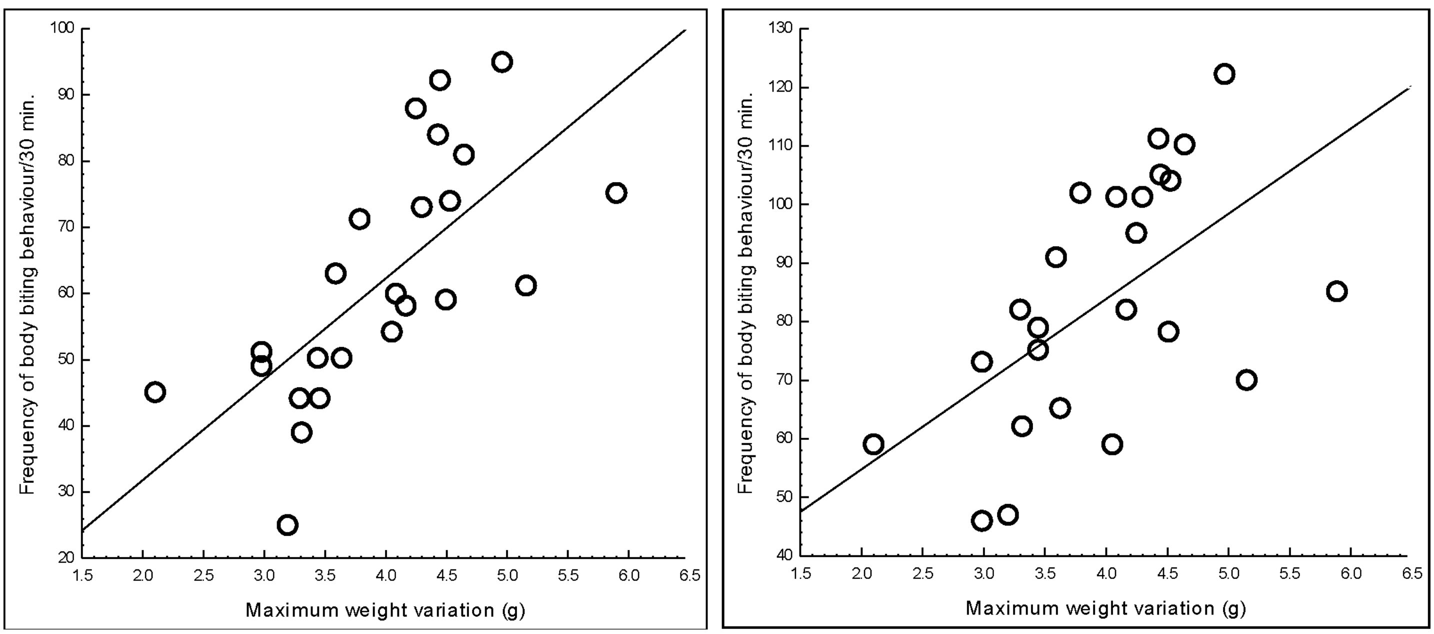
Figure 5: Frequency of a) intimidating ($y = 47.452 + 2.714x$; $p < 0.0001$; $r^2 = 73\%$) behaviour fish^{-1} before feeding and b) of intimidating ($y = 46.366 + 3.305x$; $p < 0.0001$; $r^2 = 67\%$) behaviour fish^{-1} 12 h after feeding as a function of the COV (%) for weight.

Frequency of intimidating behaviour fish^{-1} was significantly negatively correlated with the minimum weight variation before feeding (least-squares linear regression; $p = 0.01$; $r^2 = 30\%$); Similarly, frequency of intimidating behaviour fish^{-1} had a significant negative correlation with the minimum weight variation 12 h after feeding (least-squares linear regression; $p = 0.002$; $r^2 = 37\%$) (Figure 6).



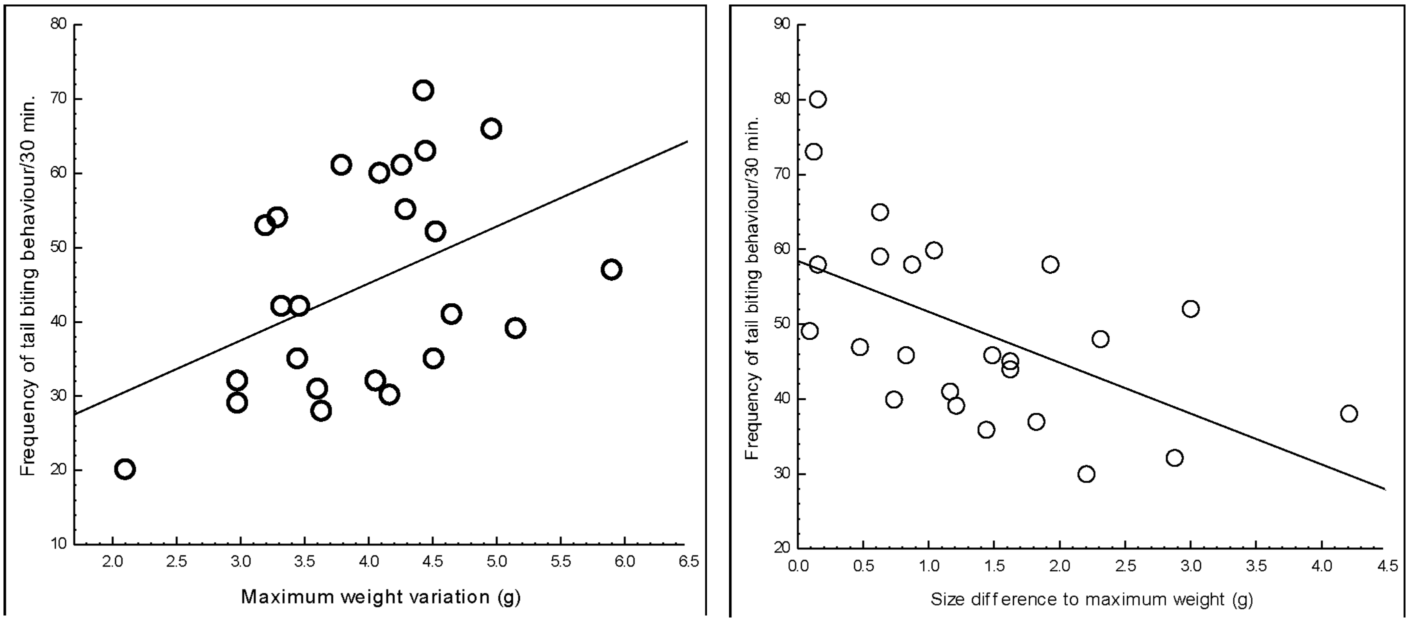
a) b)

Figure 6: Frequency of a) intimidating ($y = 168.582 - 31.499x$; $p = 0.01$; $r^2 = 30\%$) behaviour fish^{-1} before feeding and b) intimidating ($y = 24.839 - 0.960x$; $p = 0.002$; $r^2 = 37\%$) behaviour fish^{-1} 12 h after feeding as a function of minimum weight variation (g).



a) b)

Figure 7: Frequency of a) body biting ($y = 1.331 + 15.239x$; $p = 0.0002$; $r^2 = 48\%$) behaviour fish^{-1} before feeding and b) body biting ($y = 25.721 + 14.543x$; $p = 0.004$; $r^2 = 32\%$) behaviour fish^{-1} 12 h after feeding as a function of maximum weight variation (g).

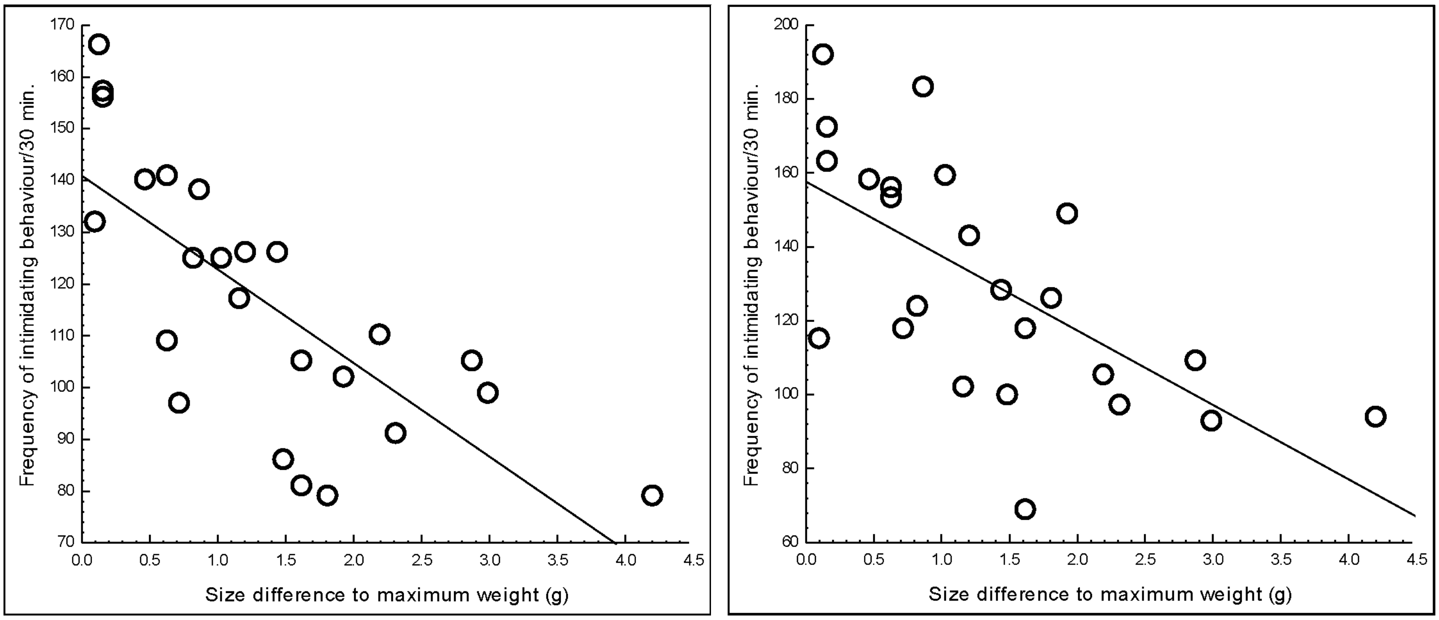


a) b)

Figure 8: Frequency of tail biting ($y = 14.474 + 7.673x$; $p = 0.03$; $r^2 = 20\%$) behaviour fish^{-1} before feeding as a function of maximum weight variation (g) and tail biting ($y = 58.464 - 6.804x$; $p = 0.005$; $r^2 = 31\%$) behaviour fish^{-1} 12 h after feeding as a function of size difference to maximum weight (g).

Frequency of tail biting behaviour fish^{-1} was significantly positively correlated with the maximum weight variation before feeding (least-squares linear regression; $p = 0.03$; $r^2 = 20\%$); Frequency of tail biting behaviour fish^{-1} before feeding was however significantly negatively correlated with the size difference to maximum weight (least-squares linear regression; $p = 0.005$; $r^2 = 31\%$) (Figure 8).

Frequency of intimidating behaviour fish^{-1} was significantly negatively correlated with the size difference to maximum weight before feeding (least-squares linear regression; $p < 0.0001$; $r^2 = 54\%$). Similarly, frequency of intimidating behaviours fish^{-1} had a significant negative correlation with the size difference to maximum weight 12 h after feeding (least-squares linear regression; $p = 0.001$; $r^2 = 42\%$) (Figure 9).



a) b)

Figure 9: Frequency of a) intimidating ($y = 140.944 - 18.090x$; $p < 0.0001$; $r^2 = 54\%$) behaviour fish^{-1} before feeding and b) intimidating ($y = 157.659 - 20.147x$; $p = 0.001$; $r^2 = 42\%$) behaviour fish^{-1} 12 h after feeding as a function of size difference to maximum weight (g).

The results seem to include both positive and negative correlations between fish behaviours and size variation including its derivatives as time after feeding passed. Results also established non-significant correlations of fish behaviours and size variation, COV including its derivatives (Table 1).

Table 1: Non-significant relationships between fish behaviours and increasing size variation and its derivatives

Minimum weight variation				**Maximum weight variation**			
Behaviour	**p value**	**y**	r^2	**Behaviour**	**p value**	**y**	r^2
Chasing before feeding	0.6337	19.1525 - 1.4986*x	0.0105	Tail biting 12 h after feeding	0.2737	35.0306 + 3.5686*x	0.0542
Chasing 12 h after feeding	0.0656	11.3841 + 9.5901*x	0.1458	Browsing before feeding	0.2124	54.5869 + 5.2325*x	0.0698
Body biting before feeding	0.8193	65.2498 - 2.0346*x	0.0024	Browsing 12 h after feeding	0.1293	54.9444 + 6.6422*x	0.1014
Body biting 12 h after feeding	0.6613	90.9933 - 4.5175*x	0.0089	Intimidating before feeding	0.0957	73.7925 + 10.7077*x	0.1210
Tail biting before feeding	0.6431	50.3405 - 3.2447*x	0.0099	Intimidating 12 h after feeding	0.2942	95.6611 + 8.7062*x	0.0499
Tail biting 12 h after feeding	0.0949	65.8662 - 10.0424*x	0.1216				
Browsing before feeding	0.3270	88.2777 - 7.7786*x	0.0437				
Browsing 12 h after feeding	0.8794	83.4582 - 1.281*x	0.0011				
Intimidating before feeding	0.0059	168.5818 - 31.4987*x	0.2968				
Difference to minimum weight				**Difference to maximum weight**			
Behaviour	**p value**	**y**	r^2	**Behaviour**	**p value**	**y**	r^2
Chasing before feeding	0.2380	10.7283 + 1.6161*x	0.0627	Chasing before feeding	0.2201	18.867 - 1.6174*x	0.0675

Body biting 12 h after feeding	0.1353	59.1073 + 6.6382*x	0.0985	Chasing 12 h after feeding	0.8847	26.8371 + 0.3341*x	0.0010
Tail biting before feeding	0.6352	39.572 + 1.4658*x	0.0104	Body biting before feeding	0.4127	66.0687 - 3.0826*x	0.0307
Tail biting 12 h after feeding	0.2823	59.8924 - 2.9076*x	0.0523	Body biting 12 h after feeding	0.3657	88.861 - 3.9407*x	0.0373
Browsing before feeding	0.3058	62.2089 + 3.583*x	0.0476	Tail biting before feeding	0.1573	50.5755 - 4.129*x	0.0888
Browsing 12 h after feeding	0.7575	77.1074 + 1.15*x	0.0044	Browsing before feeding	0.6767	77.3087 - 1.4214*x	0.0081
Intimidating before feeding	0.2721	138.229 - 5.9587*x	0.0545	Browsing 12 h after feeding	0.3380	85.9697 - 3.4081*x	0.0418
Intimidating 12 h after feeding	0.3070	156.0986 - 7.0344*x	0.0474				
COV (%) for weight							
Behaviour	**p value**	**y**	**r^2**				
Browsing before feeding	0.0601	55.1113 + 0.7984*x	0.1515				
Browsing 12 h after feeding	0.1782	65.7588 + 0.6136*x	0.0808				
Difference to mean weight							
Behaviour	**p value**	**y**	**r^2**				
Chasing before feeding	0.7767	15.5382 + 0.4076*x	0.0037				
Chasing 12 h after feeding	0.1239	17.052 + 3.6988*x	0.1042				
Body biting before feeding	0.3479	51.4196 + 3.7767*x	0.0401				
Body biting 12 h after feeding	0.5493	75.7182 + 2.8109*x	0.0165				

Tail biting before feeding	0.9092	45.9689 - 0.365*x	0.0006
Tail biting 12 h after feeding	0.0744	62.6949 - 4.8716*x	0.1375
Browsing before feeding	0.6545	70.8501 + 1.6345*x	0.0093
Browsing 12 h after feeding	0.9322	82.2406 - 0.3277*x	0.0003
Intimidating before feeding	0.0476	146.144 - 10.7682*x	0.1667
Intimidating 12 h after feeding	0.0817	163.7773 - 12.1107*x	0.1314

4. Discussion

Some aggressive behaviours increased with increasing size variation and these include chasing, intimidating, body and tail biting behaviours before and 12 h after feeding. An average of 437 body bites positively correlated more often with size variation, followed by average frequencies of 365 intimidating behaviours and 199 tail bites per 30 min., respectively before and 12 h after feeding. The least shown aggressive behaviours averaged 26 chasing behaviours, positively correlating with size variation on the times specified. The overall trend was that frequency of aggressive behaviours increased with size variation and its derivatives on the specified times. Similarly, juvenile fish showed increased aggressive interactions before feeding (African catfish, Kaiser *et al*., 1995a; Almaza´n-Rueda *et al*., 2004). Increased aggressive behaviours directed to the body side of other fish are a common feature (e.g., in larval walleye, Loadman *et al*., 1986; juvenile vundu, Baras, 1999). Among other behaviours, nipping attacks are also the most common form of aggression in larval spotted sea trout (Manley *et al*., 2015). The high frequency of these biting aggressive behaviours often leads to cannibalism in culture tanks (Hatlen *et al*., 2006). In addition, size variation positively correlated with aggressive behaviours in several fish species (Solomon and Udoji, 2011), such as young largemouth bass (DeAngelis *et al*., 1979), African catfish (Hecht and Pienaar, 1993) and Nile tilapia (Fessehaye *et al*., 2006). The present study suggests that the increased aggressive activities of fish related more often to increased size variation than to time after feeding. On the other hand, many studies suggested that fish with high hunger levels normally show increased aggression (Metcalfe *et al*., 1995; Greaves and Tuene, 2001; Fiogbe and Kestemont, 2003; Almaza´n-Rueda *et al*., 2004), especially during early juvenile stages. Hence, regular feeding intervals and feeding fish almost immediately after gut evacuation may therefore lessen aggressive behaviours (Manley *et al*., 2014). Increased aggression both before and 12 h after feeding could be due to longer time of no access to food and consequently, hunger likely worsens aggressive behaviours (Miki *et al*., 2011). This could be attributed to lack of access to food after a long time (Kaiser *et al*., 1995a).

An average of 311 intimidating behaviours before and after feeding including average of 28 tail bites after feeding negatively correlated with increasing size variation. This may relate to a shift of some behaviours to others depending on the need and capacity of the aggressive fish over time. In the culture of many commercial fish, size-grading resulted in improved growth and survival rate of fish as well as increasing biomass gain by all fish (Gunnes, 1976; Baardvik and Jobling, 1990; Kamstra, 1993). This technique is thus a requirement in fish having wide size variability (Zakes *et al*., 2004). The present results indicate that individual larger fish in groups of variably sized fish qualified more aggressive behaviours than in groups of similarly sized fish. Mostly, fish changed their behaviours in response to size variability of their tank mates and interestingly, fish seemingly can identify the variability in size of a group of fish. Thus, size variation can change the demonstration of aggressive behaviours in fish of the same group (DeAngelis *et al*., 1979). Moreover, the discrepancy in aggressive behaviours between before and after feeding times increases even more with longer feeding intervals (Manley *et al*., 2015).

The results showed that aggression correlated with both COV-values and size differences among fish. Type II cannibalism is more likely to occur, leading to substantial mortalities especially if the size difference widens in juvenile fish, (Baras and Jobling, 2002). It appears that an aggressive fish can change its behaviour as a function of COV values rather than the mean size of other fish before and 12 h after feeding. Seemingly, the more bites an individual aggressive fish delivers, the more chases it can initiate over time resulting in cannibalism among fish of different sizes.

In similar to results obtained from the previous chapter, the correlations were not very strong, but due to their significance, this chapter suggests behaviour trends and related hypotheses. Again, this chapter presented non-significant correlations of fish behaviours and size variation, COV including its derivatives (Table 1) to provide direction for other studies.

The observations of the present study therefore provide a promising opportunity of research for linking behavioural and feeding responses to size variability and time after feeding. They contribute to scientific knowledge about the aggressive behaviours of juvenile dusky kob in relation to changes in size variation through time. Further experimental work was necessary and is presented in Chapter 4 to better understand the aggressive behaviours of the aggressor on smaller fish.

Chapter 4

The effect of aggressor's size on aggressive and browsing behaviours in juvenile dusky kob, *Argyrosomus japonicus*

1. Introduction

The success of a commercial aquaculture operation relies on different parameters within the field of biology, production and economics (Lazo *et al*., 1998). Many limiting factors contribute to the lack of successful larval culture and a common factor is the poor understanding of the effect of feeding and sibling aggression (Lee and Ostrowski, 2001).

The high commercial losses at the early juvenile phase in piscivorous fish are commonly due to intracohort cannibalism (Hecht and Pienaar, 1993; Ruzzante, 1994). This is particular the case when one fish attacks siblings of the same year group (Baras and Jobling, 2002). In certain fish species, size-grading has its limitations as a husbandry practice (Francis and Bengston, 1999) resulting in increased aggressive behaviours (Baardvik and Jobling, 1990). Interstingly, separating small and large fish leads to reduced mortalities associated with cannibalism (Jobling, 1982; Jobling, 1985; Kamstra, 1993). In addition, aggressive behaviours declined as the size difference between individuals increased (Wankowski and Thorpe, 1979, salmonids; Brunkov and Collins, 1998, salamanders; Arctic charr, Baardvik and Jobling, 1990). Normally, fish are size-graded in hatcheries assuming that smaller fish will grow faster, likely due to destruction of social hierarchy (Gunnes, 1976). This rearing technique aims to improve the weight gain by all individuals and to increase their survival rate, with fish obtaining the maximum biomass (Kamstra, 1993; Sunde *et al*., 1998). It is also frequently exercised to make feeding and harvesting operations easier

(Baardvik and Jobling, 1990) as the largest individuals are separated from the smallest fish (Gunnes, 1976), allowing for improved economical production (Saoud *et al.*, 2005).

Information about cannibalistic behaviour and factors moderating it along with environmental improvements are prerequisites for reducing aggression between fish under aquaculture conditions (Baras and Jobling, 2002). In teleost fish, there are two distinct types of cannibalism and these are type I and II. In type I cannibalism, the prey is ingested or partially eaten tail-first (Appelbaum and Kamler, 2000), and in type II cannibalism, the cannibal ingests the whole fish head first (Hecht and Appelbaum, 1988; Baras *et al.*, 2000). For type II cannibalism, a large size difference between predator and prey is needed, but this is not as pronounced for type I cannibalism (Appelbaum and Kamler, 2000). Changes of cannibalism from type I to type II has been evidenced when fish reach a certain age or size and such fish include sharptooth catfish *C. gariepinus* (Hecht and Appelbaum, 1988) and vundu *Heterobranchus longifilis* (Baras, 1999).

Low size variation within fish groups minimised aggressive and cannibalistic behaviours (Liao and Chang, 2002). The variability in size may favour high levels of aggression especially if there is a large size difference among fish (Persson, 1985; Polish, 1988). Fish with larger body size usually become aggressive towards smaller fish (Øverli *et al.*, 1999; Clement *et al.*, 2005) and as a result, they grow faster limiting feeding activity of smaller fish (Jobling, 1985; Metcalfe, 1990; Kooijman *et al.*, 2009).

In dusky kob, high susceptibility to stress under culture conditions frequently involves stress associated with increased aggressive behaviours, which results in cannibalism (O'Sullivan and Ryan, 2001). Previous trials showed that the fish behaved more aggressively in the tanks when size variation among fish increased. Increased aggressive and browsing behaviours, on overall, were observed in groups of fish with high size variation as compared to fish with lower size variation. This chapter aims to find out how the size of focal fish (larger fish) in relation to non-focal fish (smaller fish) affects aggressive and browsing behaviours. Results are expected to contribute to the management of size variation among fish and the use of size-grading technique towards reducing aggressive behaviours.

2. Materials and methods

Experimental system, fish and acclimation

Juvenile dusky kob of an average weight of 30 ± 7.63 g $fish^{-1}$ were obtained from the hatchery-reared stock of Oceanwise (Pty) Ltd located in East London, South Africa. Five hundred and sixty (560) fish were used to conduct twenty observations. The experiment was conducted at Rhodes University research facility in Port Alfred. The tank system was set up prior to the arrival of the fish and consisted of four observation tanks which were part of an indoor partially recirculation water system. Fish were stocked at 10 kg m^{-3}, i.e., 7 fish per 21-L tank at 210 g of fish biomass per tank. This density falls within the range where Pirozzi *et al.* (2008) reported an absence of significant differences in growth rate at crowding densities between 8 and 16 kg m^{-3} when rearing juvenile dusk kob in Australia. Collett (2007) also applied this density in assessing the effect of crowding density on growth, food conversion ratio and survival of dusky kob reared in a closed recirculation system in South Africa. A refractometer was used to measure salinity kept approximately at 35 ppt. Using aquarium heaters, a temperature ranged between 24 and 26 ℃ and pH was kept between 7.2 and 8.5, adding freshwater when necessary. A portable electronic probe was used to measure water temperature and pH daily. Dissolved oxygen (DO) was measured using the DO-meter twice a week and 80 to 100% of saturation was maintained using of aquarium air-stones. Total ammonia to nitrogen and nitrite concentrations ranged between 0.00 to 0.25 mg L^{-1}

N throughout the study. Similar water conditions (temperature, pH and salinity, oxygen) were ensured for fish in the holding and observation tanks. A 12 h light: 12 h dark regimen was maintained throughout the experiment. The pelleted food used was a commercial trout pellet (45% protein; 14% lipid; Aquanutro, Nutroscience Pty Ltd, South Africa). The fish in the holding tank were fed three times (08h00, 13h00 and 17h00) to satiation. The observations were performed and fish were used only once for each observation to obtain the frequency of behaviours. A single focal fish and new group of non-focal fish was chosen for the next observation and each fish was observed once a day 12 h after feeding. The fish in the observation tank were allowed 30 min. to acclimate to the presence of the observer. The five specific behaviours fish^{-1} considered in the previous experiment were observed and these were chasing, browsing, intimidating, body biting and tail biting behaviours. A random selection for thirty fish was individually measured to the nearest 0.1 g to obtain weight fish^{-1} to determine their coefficient of variation (COV).

Experimental design and protocols

Four treatments randomly assigned with fish in the observation tanks were tested to observe aggressive and browsing behaviours. The larger fish was observed as focal fish and non-focal fish were the smaller fish making up each of the treatments with different values of size variation.

Table 1: Description of the treatments used to observe the aggressive and browsing behaviours of dusky kob using focal fish

Treatment	**Description**
Treatment A	non-focal fish group has high COV and a mean weight below that of the focal fish.
Treatment B	non-focal fish group has low COV and a mean weight less than that of the focal fish.
Treatment C	non-focal fish group has high COV and a mean weight equivalent to that of the focal fish.
Treatment D	non-focal fish group has low COV and a mean weight higher than that of the focal fish.

The same focal fish was observed repeatedly in the four treatments and hence, one set of observation lasted for four days, starting from day one up to day four. The treatments were randomly assigned with focal fish for observation. A countdown timer (stopwatch) with an alarm was used to keep the time per observation. The focal fish, considering its weight, was chosen based on the treatments designed. Only aggressive and browsing behaviours of the focal fish were observed. The entire tank was covered to avoid disturbances on fish and a small portion in each observation tank was left open to allow the observer to record the behaviours during observation. Observation tank were cleaned and uneaten food was removed before placing the fish into the tank for observation.

Data analysis

A repeated measures analysis of variance (ANOVA) test was used to test for significant differences between the means of treatments tested. If the F-statistic was significant, a Post hoc Tukey's Multiple Range Test was used to conduct pair-wise comparisons. Error bars in graphs denote standard deviations of mean frequencies. The size variation was determined using the coefficient of variation (COV) values for weight. The COV was the standard deviation as percentage of mean weight x 100. All statistical analyses were performed using Statistica version 9 (Statsoft, Tulsa, OK, USA).

3. Results

Size variation had a significant effect on the aggressive and browsing behaviours fish^{-1} 12 h after feeding. These behaviours were chasing ($p < 0.0001$), browsing (0.0002), intimidating (0.0096), body biting ($p < 0.0001$) and tail biting ($p < 0.0001$) behaviours.

Size variation and behaviours observed

Frequency (22.10 ± 2.41) of chasing behaviour fish^{-1} 12 h after feeding in treatment A and frequency (15.90 ± 1.77) in treatment C did not significantly differ from each other. Frequency in treatment C and frequency (14.10 ± 2.26) in treatment B also did not significantly differ from each other. However, frequency in treatment B and frequency (6.55 ± 1.40) in treatment D were both significantly lower than frequency in treatment A. Average frequency in treatment D was significantly lower than frequencies in treatments C, B and A (Table 2).

Frequency (37.95 ± 3.06) of body biting behaviour fish^{-1} 12 h after feeding in treatment A and frequency (33.75 ± 4.03) in treatment C did not significantly differ to each other. Frequency (25.90 ± 3.30) in treatment B and frequency (19.60 ± 2.13) in treatment D were significantly lower than the frequencies in treatments A and C. Frequency in treatment D was also significantly lower than frequency in treatment B (Table 2).

Table 2: Post-hoc test showing the significant difference between the frequencies of chasing and body biting behaviours fish^{-1} per 30 min. in different treatments. Different superscript letters within the same column indicate significant difference between the average frequencies ($p < 0.05$).

COV treatment	Frequency of chasing behaviour	Frequency of body biting behaviour
A	22.10 ± 2.41^{a}	37.95 ± 3.06^{a}
B	14.10 ± 2.26^{b}	25.90 ± 3.30^{b}
C	15.90 ± 1.77^{ab}	33.75 ± 4.03^{a}
D	6.55 ± 1.40^{c}	19.60 ± 2.13^{c}

Frequency (14.60 ± 1.89) of tail biting behaviour fish^{-1} 12 h after feeding in treatment C and frequency (18.65 ± 2.27) in treatment A did not significantly differ from each other. Frequency in treatment C did not significantly differ to frequency (11.10 ± 1.67) in treatment B. Frequency (7.90 ± 1.62) in treatment D and frequency in treatment B also did not significantly differ from each other. Frequencies in treatment D and B were significantly lower than frequency in treatments A. Frequency in treatment D was also significantly lower than frequency in treatments C (Table 3). Frequencies of intimidating behaviour fish^{-1} 12 h after feeding in the treatments B (52.4 ± 4.6), D (51.1 ± 4.2) and C (60.2 ± 5.8) did not significantly differ from each other. Frequency (66.5 ± 5.2) in treatment A did not significantly differ to frequency in treatment C, however, it was significantly higher than frequencies in treatment B and D (Table 3).

Table 3: Post-hoc test showing the significant difference between the frequencies of tail biting and intimidating behaviours fish^{-1} per 30 min. in different treatments. Different superscript letters within the same column indicate a significant difference between the average frequencies ($p < 0.05$).

COV treatment	**Frequency of tail biting behaviour**	**Frequency of intimidating behaviour**
A	18.65 ± 2.27^{a}	66.50 ± 5.19^{a}
B	11.10 ± 1.67^{bc}	52.35 ± 4.61^{b}
C	14.60 ± 1.89^{ab}	60.20 ± 5.79^{ab}
D	7.90 ± 1.62^{c}	51.10 ± 4.19^{b}

Table 4: Post-hoc test showing the significant difference between the frequencies of browsing behaviour fish^{-1} per 30 min. in different treatments. Different superscript letters within the same column indicate significant difference between the frequencies ($p < 0.05$).

COV treatment	**Frequency of browsing behaviour**
A	53.45 ± 4.43^{a}
B	43.60 ± 3.59^{b}
C	47.25 ± 4.22^{ab}
D	41.20 ± 3.32^{b}

Frequencies of browsing behaviour fish^{-1} per 30 min. 12 h after feeding in treatments B (43.60 ± 3.59), C (47.25 ± 4.22) and D (41.20 ± 3.32) did not significantly differ from each other. Frequency (53.45 ± 4.43) in treatment A was not significantly different to frequency in treatment C but it was significantly higher than the frequencies in treatment B and D (Table 4).

4. Discussion

The increased frequencies of aggressive behaviours per 30 min. occurred in treatment A, sharing similar frequencies in treatment C, compared to other treatments. The increased aggressive behaviours referred to include the highest intimidating behaviours averaging 66.50 ± 5.19, followed by body biting behaviours averaging 37.95 ± 3.06, chasing biting behaviours averaging 22.10 ± 2.41 and tail biting behaviours averaging 18.65 ± 2.27, respectively. The highest frequency of browsing behaviour fish^{-1} per 30 min. was 53.45 ± 4.43, also in treatment A. Treatment A represented a group of non-focal fish having high size variation with their mean weight lower than the mean weight of the focal fish. Treatment C also had non-focal fish having high size variation with their mean weight equivalent to the mean weight of the focal fish. The aggressive behaviours in treatment A were significantly higher than the aggressive behaviours in the treatment D and B, which both had a lower size variation.

Overall, increased aggressive behaviours were observed in high size variation treatments and low aggressive behaviours were observed in lower size variation treatments. Thus, the aggressor showed aggressive behaviours more often towards smaller fish when different sizes (high size variation) existed among them 12 h after feeding. Correspondingly, large fish showed aggressive behaviours towards smaller fish with large size differences existing among fish (Øverli *et al.*, 1999; Clement *et al.*, 2005). Increased chases in groups having increased size variation may be associated with cannibalism attempts, since numerous incidences of cannibalistic behaviours were also observed (Sogard and Olla, 2000). Type I cannibalism where one fish is ingested tail-first (Baras and Jobling, 2002; Baras, 2013) would be represented by the chasing and biting behaviours in juvenile dusky kob. In addition, aggression and cannibalism became more prevalent as size variation increased (DeAngelis *et al.*, 1979; Hecht and Appelbaum, 1988; Hecht and Pienaar, 1993; Baras, 1998; Fessehaye *et al.*, 2006). On the other hand, reduced size variation among fish of the same age could reduce aggressive encounters (Liao and Chang, 2002). The current observations therefore suggest the occurrence of increased aggressive behaviours on the assumption that high size differences and variation exist among fish.

Fish exposed to non-focal fish that had high size variation showed higher frequencies of browsing behaviour than when exposed to fish that had low size variation. This observation is similar to the observation recorded in aggressive behaviours in relation to increased size variation and highest browsing behaviour in treatment A, which again did not significantly differ to treatment C. Other authors reported similar results as fish with high levels of hunger showed aggressive behaviours (Metcalfe *et al.*, 1995; Almaza´n-Rueda *et al.*, 2004). These findings therefore support the suggestion that increased aggressive behaviours could occur if or when large size differences and variation exists among juvenile fish (Hecht and Pienaar, 1993; Baras and Jobling, 2002).

Evidently, aggressor showed more intimidating behaviours as to signal its potential for aggressive behaviours and to intimidate other fish in the observation tanks. The amount of aggressive behaviours of juvenile dusky kob may indicate reality of increased size variation and differences among fish. Furthermore, regular size-grading of fish can be suggested to reduce aggressive behaviours in combination with regular timeous feeding intervals, based on the present observations. These findings thus contribute to better understanding of increased size variation and size-grading effect on aggressive and browsing behaviours of juvenile dusky kob. More studies are, however, necessary to explore this topic further and may present findings as reference point. Data from the tanks used for observation may also need to include testing for a tank-effect on fish behaviours. Research efforts should also aim at advancing what is currently known about the effect of size variation and size-grading on aggressive behaviours of juvenile dusky kob.

Chapter 5

General discussion

The present research determined the effect of time after feeding, considered here as observation time and increasing size variation on the aggressive and browsing behaviours of juvenile dusky kob. This chapter explores how the experiments addressed the fundamental aim of this study and discusses the findings. It also highlights the shortcomings and discrepancies encountered in observing aggressive behaviours and suggestions for future studies.

Statistical analysis indicated that fish increased behaviours 6-12 h after feeding, showing increased browsing activities as time post-feeding passed, from Chapter 2. On average, fish showed fewer aggressive behaviours closer to 2 h after feeding periods and increased aggressive behaviours after feeding. Correspondingly, a significant positive relationship occurred between aggressive behaviours such as chasing, capture and biting of fish and times after feeding (Manley *et al*., 2014). Juveniles of dusky kob also had half-empty stomachs within 10-12 h after single feed to satiation (Ballagh *et al*., 2008). In addition, gastric evacuation time averaged 5.5 hours after feeding fish three and four times per day (Daniel, 2004). In comparison, the sciaenid spotted seatrout increased their aggression and cannibalism 4-7 h after feeding (Manley *et al*., 2014). For this reason, consistently feeding juvenile the fish immediately after gut evacuation can effectively contribute to reducing the aggressive behaviours (Baras and Joblin, 2002; Manley *et al*., 2014).

The results in Chapter 2 showed positive correlations of most aggressive and browsing behaviours with size variation, noticeably 6-12 h after feeding. The results in Chapter 3 further confirmed the existence of significant positive correlations of aggressive behaviours with increased size variation and difference before and 12 h after feeding. Size difference seemed to influence the behaviour of the larger fish to become more aggressive when there is a large size difference between the aggressor and the recipient of the aggression. As a result, aggressive fish can change their behaviour as a function of COV-values rather than the mean size of the other fish under culture setting. These results correspond well with findings of other studies, demonstrating that increased size variation positively correlated with aggressive behaviours and cannibalism (Baras and d'Almeida, 2001; Baras *et al.*, 2000; Kestemont *et al.*, 2003; Skov *et al.*, 2003; Moran, 2007). However, this is the first time this could be shown in juvenile dusky kob. In addition, other authors did not use the coefficient of variation of a group of fish as the independent variable. Using the results from the present study, fish behavioural changes can be related to the need for size grading in this species for the first time. Many studies in different fish species also reported the increased aggressive behaviours with increasing size variation (Solomon and Udoji, 2011).

In fish with high size variation with lower mean weight, the aggressor showed higher frequencies of aggressive and browsing behaviours than in those recorded in fish of lower size variation treatments 12 h after feeding (Chapter 4). As reported in many studies, aggressive behaviours increased as size differences between fish increased (DeAngelis *et al.*, 1979; Hecht and Appelbaum, 1988; Hecht and Pienaar, 1993; Baras, 1998; Fessehaye *et al.*, 2006; Miki *et al.*, 2011). In cases of large size differences, large fish become aggressive towards smaller fish (Øverli *et al.*, 1999; Clement *et al.*, 2005). When this happens, social interactions exist and mostly involve the largest fish acting as the aggressor (Clement *et al.*, 2005), attacking the smaller fish (Jobling, 1982; Baras, 1998; Øverli *et al.*, 1999). In addition, increased aggressive behaviours in non-graded fish possibly are associated with these social interactions among fish of different sizes (Qin *et al.*, 2001). Alternatively, size-dependent aggression and cannibalism can be avoided using consistent size-grading reported in farmed fish (Kamstra, 1993; Goldan *et al.*, 1998; Zakes *et al.*, 2004; Bernatzeder, 2008). For this reason, size-grading should be used in juvenile fish early before fish develop the social hierarchy which involves large dominant fish and smaller subordinate individuals (McIntyre *et al*., 1979; Gilmour *et al*., 2005). Thus, grading fish early before or once they reach the size and age at which they start displaying aggression help avoid the impact of aggression and cannibalism (Gunnes, 1976). Baras *et al*. (2003) suggested that enhanced growth could delay complete cannibalism, allowing size-grading of fish at early stages to reduce mortalities associated with cannibalistic behaviours. In combination with other critical rearing factors, size-grading is essential for the improvement of feed uptake, maximising survival rate and weight gain by all fish (Baardvik and Jobling, 1990; Kamstra, 1993; Sunde *et al*., 1998). Better understanding of factors initiating size variation and rearing conditions is required when using size-grading fish (Paulet, 2003). Innovative technique is also necessary to predict aggressive behaviours in juvenile fish for size-grading practices (Ribeiro and Qin, 2013).

Extra independent variables, referred to as size variation derivatives from the raw data in the experimental methods of the first two chapters, were used to explore the data further. These were minimum weight variation, length variation; maximum weight variation, length variation, size difference to mean length, weight; size difference to maximum weight, length and size difference to minimum weight, length of non-focal fish and the focal fish.

The correlations between fish behaviours and size variation were however not all very strong from the linear regressions from Chapter 2 and Chapter 3 but due to their significance, results are reported to indicate behaviour trends and related hypothesis. The study also presented non-significant correlations of fish behaviours and size variation including its derivatives (Chapter 2, Table 3; Chapter 3, Table 1) mostly to provide point of reference for future studies. Other factors may have been at play and contributed to these experimental observations and results but due to limited time and resources, could not be addressed. Furthermore, some of the experimental methods on behaviours and size variation among f considered here could not be changed as experiments were completed using the available experimental fish. The available time and fish thus limited the experimental observation trials conducted. The number of fish successfully spawned in the Oceanwise (Pty) Ltd hatchery determined the number of experimental fish. Three experiments were all conducted in small aquaria and future research would be necessary to include data and interpretation of results in a larger production-scale setting. Some researchers have managed to conduct behavioural observation trials under both laboratory and commercial conditions. It is however expensive to get all the experimental tools necessary and a number of fish large enough to conduct large-scale trials for commercial culture (Collett, 2007). A combination of laboratory studies and trials in commercial aquaculture settings could identify potential interactions between independent variables (Collett, 2007).

In conclusion, fish seemingly substituted certain aggressive behaviours as size variation increased and time after feeding passed, leading to fewer aggressive behaviours. This may mean that fish had a limited capacity to conduct some of behaviours and as their needs change, they would express certain behaviours including browsing. Fish evidently showed aggressive and browsing behaviours in all trials though certain behaviours did not differ significantly between times considered and as size variation increased. They showed aggressive behaviours as early as in their first two weeks after hatching, averaging 0.43 ± 0.27 g $fish^{-1}$ and that aggression increased as the relative aggressor and victim's size difference increased (shown in Chapter 3). Therefore, full attention is necessary as juvenile dusky kob showed cannibalistic behaviours 18 days after hatching (O'Sullivan and Ryan, 2001) with frequency increasing at about 20 days after hatching (Musson and Kaiser, 2014). As fish grow during the study, increased size variation and differences between fish were clearly noticed as time passed. Comparing the total aggressive behaviours versus size variation in trials, the group of smaller fish used in Chapter 3 showed increasingly higher frequency of aggressive behaviours per 30 min. period. The early days after hatching are seemingly critical and one of previous studies suggested that juvenile dusky kob decreased aggressive behaviours as they grow (O'Sullivan and Ryan, 2001). The size-grading technique in same-age juvenile dusky kob is required to manage the aggressive behaviours associated with increasing size variation. Fish should be size graded early, once they reach the size and age at which they start exercising aggressive behaviours. The gut passage time is essential and the current study suggests the time post-feeding of about 4-6 h per day to maintain less-aggressive behaviours of juvenile dusky kob. The present findings are consistent with previous studies conducted on gut evacuation time explaining the trend of aggressive behaviour and feeding of juvenile fish more than once per day. Furthermore, size variation including size differences and time passed after feeding are determining factors of aggressive behaviours, more likely leading to cannibalism in farmed juvenile dusky kob. It would be interesting to film the observation tank(s) probably from above to observe the aggressive and feeding behaviours of juvenile fish before and after feeding. Such a video would allow accurate assessment of different behaviours, including the fish initiating the aggressive behaviours and the target individuals. The study has thus provided the fundamental groundwork for aquaculturists and future research should reflect on key factors affecting aggressive behaviours of juvenile dusky kob.

General remarks on fish aggressive and non-aggressive behaviours

It is difficult to measure the effect of stress on health of farmed fish but signs of stress and poor environment are possible to detect under farming conditions (Alanärä and Magnhagen, 2001). Non-aggressive behaviour was identified as contact between two fish, which did not lead to aggressive behaviours as fish were usually observed bumping into each another's tail or body side (Kaiser *et al*., 1995a and b). General remarks included aggressive fish targeting and/ or chasing another fish from behind for one or more second, facing another fish in a threatening or intimidating way for one or more second, or biting another fish on its body and / or tail. On the other hand, remarks during the observations of the current present included non-aggressive fish that seemed to have experienced aggression, distancing themselves from other fish or remaining in tank corners or swimming less. Some of these fish immediately avoided contact and darted away from the place of contact in most instances. Fish were swimming at the bottom of the tank, seemingly searching for food. Further observations included some of the smaller fish showing signs of partial or complete loss of their fins or tail, body lesions or bites and a few mortalities were observed in the holding tanks. Observations were only 30-min and hence, the fate of attacked or injured fish is unknown. Paulet (2003) and Bernatzeder (2008) also reported victims of aggression indicated by loss of their fins or tail, fin or body bites, wounds and even mortality. Fish skin lesions and fin damage (Kaiser *et al*., 1995a) including mortality (Koebele, 1985), poor growth and increased susceptibility to infections (Bolnik, 1990) were consequences of aggressive behaviour and cannibalism. Aggression resulted in reduced food conversion efficiency, slower growth and stock losses of fish (Solomon and Udoji, 2011). The total length of juvenile dusky kob was compromised due to cannibalism, thus, fish were size-graded manually every two weeks during the period of experimentation (Bernatzeder, 2008). Palmer (2008) has also observed mortalities due to cannibalism with fish that had been attacked showing partial or total absence of fins and small lesions on the body. In cases where the focal fish successfully captured the prey after chasing, cannibalism was orientated tail-first and fish seemed to manage to escape the attacks.

Description of fish behaviours observed

The juvenile dusky kob behaviours observed were fish browsing at the bottom of the tank, fish attacks including chasing, body or tail biting (nipping) and intimidating behaviours. There behaviours are comparable with behaviour patterns in tanks reported from the other studies (European plaice, Jobling, 1982; vundu, Baras, 1998; Øverli *et al.*, 1999; Arctic charr, Øverli *et al.*, 1999; cichlid species, Clement *et al.*, 2005). Furthermore, similar patterns of aggressive interactions had been studied also in species such as juvenile barramundi *Lates calcarifer* (Puvanendran *et al.*, 2008), young yellowtail *Seriola quinqueradiata* (Sakakura and Tsukamoto, 1996), larval and pre-juvenile Atlantic cod *G. morhua* (Folkvord and Ottera, 1993), and juvenile orange-spotted grouper *Epinephelus coioides* (Takeshita and Soyano, 2009). Common observations included the bigger fish attacking smaller subordinate ones (Øverli *et al.*, 1999; Jobling, 1982; Baras, 1998; Clement *et al.*, 2005). In the present study, aggressive behaviours were recognised as any aggressive acts and intimidation between the two fish where either one fish (focal fish) intimidated, chased or bit another fish on any part of its body (Kaiser *et al.*, 1995a).

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