Gastric emptying and food consumption of *Scatophagus argus*

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Abstract. The present study was carried out to investigate the stomach volume, gastric motility, gastric emptying time and gastric emptying rate of the spotted scat fish, *Scatophagus argus* in laboratory conditions. Stomach volume was examined using freshly caught of various fish sizes by ligatured stomachs at the tips of the burette. Stomach volume is correlated with maximum food intake (S_{max}) and it can estimate the maximum stomach distension by allometric model i.e volume = $0.0000089W^{2.93}$. Gastric emptying time (GET) was estimated by feeding the various sizes of fish until satiation at different time since feeding using radio-opaque barium sulphate ($BaSO_4$) paste injected in the wet shrimp in proportion to the body weight. X-Radiography observation on the gastric motility showed the fish (200 g) that fed to maximum satiation meal (circa 11 g) completely emptied their stomach within 30-36 hrs. To evaluate the GET from x-ray, fish were fed with white shrimp without $BaSO_4$ until satiation and were serially dissected at series of time. Satiation feed (S_{max}) were fitted by allometric model i.e $S_{max} = 0.07292W^{0.97}$, where the scat fish consume more than the satiation feeding model (b>0.67). Food consumption digestion study of scat fish shows that the fish of various sizes emptying their stomach at the same phase (0.3 g hr^{-1}), with normalised gastric emptying rate (GER) around 0.0024-0.0026 g^{-1} hr^{-1} gbw^{-1}. The slow rate of GET and GER may contribute to the slow growth rate of scat fish. The results of the present study will provide the first baseline information on the stomach volume, gastric emptying of scats fish in captivity.

Key Words: gastric empying, stomach volume, X-radiography, gastric emptying time (GET), gastric emptying rate (GER).

Introduction. Spotted scats have turned into an imperative nourishment fish in peninsular Malaysia particularly to local coastal community. In some nation in Southeast Asia, for example, Philippines, the spotted scat has turned into one of the critical protein sources and it has presented as one of the aquaculture fish (Barry & Fast 1988). In line with the fast-growing aquaculture industry, it is important for the nutritionists and feed manufacturers to have a knowledge on food consumption, gastric emptying time and gastric emptying rate in fishes. Intensive studied in fish gastric emptying or evacuation rate is an essential component for both field and laboratory studies apprehensive with fish feeding rates, energy budgets, and trophic dynamics of aquatic systems (Bajkov 1935; Hunt 1960; Eggers 1977; Elliot & Persson 1978; Forrester et al 1994; Sweka et al 2004). This is important to elucidate the behaviour and response of fishes toward feeds type given which helps in understanding the physiological response of fishes on food intake and digestion in either wild or cultured fish's species. It is necessary to know the duration of digestion which always determined under laboratory conditions. Digestive process may differ in different fish species, such as digestive anatomy and digestive physiology which could serve as a basis for estimating general parameters of digestion.

The essential factors which influencing the duration of digestion in fishes include temperature and salinity (Windell et al 1978; Albert 1995; Bromley et al 1997; Darbyson et al 2003; Andersen & Beyer 2008), feeding level and meal size (Henken et al 1985; Gongnet et al 1987), age and holding density (Hastings 1969; Windell et al 1978) and dietary component (Beamish & Thomas 1984; Hanley 1987; Krogdahl et al 1999). Determination of food passage time in the digestive tract in fish includes dissection, monitoring feces production, stomach suction, artificial food, and X-radiography. X-radiography technique provides great advantages in studying non-osseous structures, like elucidating aspects of functional activity in fish, besides being a non-invasive method of enormous convenience. Previous researchers have conducted comparative studies by using $BaSO_4$ as a contrast medium to trace the gastric motility of a single meal by applying X-radiography technique in fishes (Edwards 1971, 1973; Jobling et al 1977; Grove et al 1978; Mazlan et al 2002; De et al 2014; Das et al 2014; Mazumder et al 2015). In-depth study on food consumption and gastric emptying time in fishes are important to elucidate the behavior and response of fishes toward feeds type given. Therefore, the aim of this study was to determine and estimate the food consumption, digestion time and digestion rate of spotted scat, *Scatophagus argus* using x-radiography technique. This aids in comprehension of the physiological response of fishes on food intake and digestion in either wild or cultured fishes species. The outcome results are critical parameter for the determination of fish nutrition management in aquaculture.

Material and Method. A total of 83 live fish were collected in January-December 2013 using cast net, scooped net and hand line at the nearby cages in Sungai Terus, Pekan, Pahang. Samples were separated into two groups (live samples n = 63 and dead samples n = 20) and transported to the Marine Science laboratory of Universiti Kebangsaan Malaysia. Live fish was held in a recirculation system at 26ºC in 700 L aerated holding tanks and fed white shrimp (*Penaeus* sp.) for at least 4 weeks prior to start the experiment.

Estimation of stomach volume. In the laboratory, the pre-selected dead samples (n = 20) of various sizes were sampled to estimate the stomach volume. The stomach and intestines were carefully removed by cutting out the body cavity of each fish. Stomach content was pressed out gently using fingers and a cotton string was used to tie around the anterior of the oesophagus to a burette and at the posterior around the pyloric sphincter. The maximum stomach distension (maximum stomach volume) was measured as the total volume of water required to dilate the stomach until it burst.

Gastric emptying study. A total of 9 live fish of similar size were used for gastric motility study by using x-radiographic technique. Prior to start the experiment of gastric motility, healthy fish were transferred to the experimental tank (n = 3 fish/tank) and deemed to accept white shrimp over two-week period. A small amount of barium sulphate ($BaSO_4$) paste (radio opaque tracer) were prepared (concentration 1 g $BaSO_4$: 5 mL distilled water) and 0.1-0.15 mL g^{-1} wet weight was injected into the muscle of white shrimp using a hypodermic syringe. The shrimp with injected radio opaque paste were kept frozen prior to the start of the experiment. All experimental fish were deprived of food for 120 hr and then offered pre-weighted radio-opaque labelled shrimp to satiation. In this case, all fish feeding voluntarily to satiation were assumed filled with radio opaque paste at least 2/3 of the total stomach volume. The exact amounts of food eaten were recorded for each fish. At a selected time after feeding (4, 6, 8, 10, 12, 14, 16, 18, 20, 24, 28, 32, 36, 45 and 50 hr), the fish were anesthetized using 0.22 mL L^{-1} of TRANSMORE® (NIKA) for 10-15 minutes and at once X-rayed with a microradiograhic unit (M60, Softex, Tokyo, Japan) to trace the movement of food along the gut (Al-Aradi 1986; Mazlan & Grove 2003; Das et al 2014). The experiment was repeated with the remaining stock of live fishes. The temperature was maintained and fixed at 26°C.

A total of 30 live fish were used for gastric emptying and food consumption study. Prior to start the experiment of gastric motility, healthy fish were transferred to the experimental tank (n = 3 fish/tank) and deemed to accept white shrimp without inert marker over two-week period. All experimental fish were deprived of food for 120 hr and then offered pre-weighted shrimp to satiation. The exact amounts of food eaten were recorded for each fish. At a selected time after feeding the fish were killed by a blow to the head and digestive tract opened to remove contents of each fish and wet weight of content was taken to the nearest 0.001 g. Curves of the change in contents with time were fitted to the data using the maximum voluntary meal size as a standard (Fletcher et al 1984; Mazlan 2001). The square root model (Andersen 1998, 1999, 2001; Andersen & Beyer 2008; Mazlan 2001) used to describe gastric emptying of the meal;

$$[S_t = S_0(1-S_0^{(a-1)}\rho(1-a)t)^{(1-a)-1})+\xi]$$

where S_t is the total stomach content at time t after ingestion of a meal of size, S_0 is stomach content at time zero and ξ is the random error term. Model parameters for a maximum satiation meal (when S_0 = maximum satiation amount Smax and the rate constant, ρ gastric emptying rate (GER), and gastric emptying coefficient a were estimated by non-linear iterative method using Levenberg-Marquardt algorithm.

Statistical analysis*.* Gastric emptying curve a non-linear regression equation was fitted using Microcal OriginTM version 6 software and the parameters to be estimated were named and declared. The non-linear procedure first examined the starting value specifications of the parameters and evaluated the Chi square (χ^2) value at each combination of values to determine the best values to start the iterative algorithm. The programme uses the Levenberg-Marquardt iterative method (Mazlan 2001).

Results

Estimation of stomach volume*.* Prior to start the experiment of gastric emptying and food consumption of spotted scat, estimation of stomach fullness by estimating stomach volume was conducted (Figure 1). Stomach volume or stomach capacity is correlated with maximum amount of food intake (S_{max}) based on fish size between individuals. It also helps to estimate the maximum of stomach distention of each fish by allometric model i.e volume = $0.0000089W^{2.93}$.

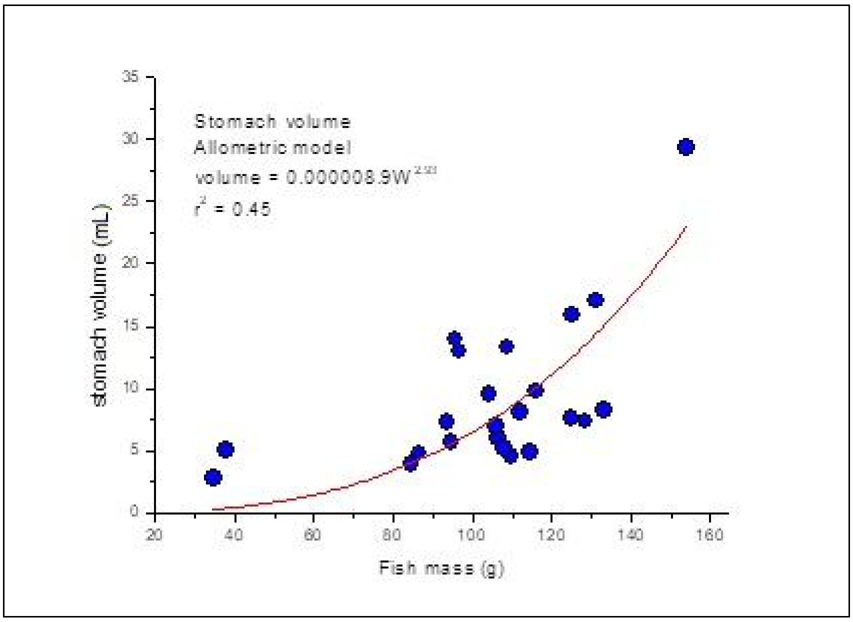

Figure 1. Relation between stomach volume (mL) and fish mass (g wet wt.) of spotted scat were fitted using allometric model.

Gastric emptying study*.* The movement of food items in digestive tract after different time intervals since feeding are described in Figure 2. The figure clearly shows the stomach is still fully filled with radio opaque paste after 2 hrs since feeding. The x-radiographic micrograph shows that within 4 hour after feeding, the stomach is still full with the residuum and a small portion of food bolus/chime has seen entered the anterior intestine; 8-12 hour since feeding, the food has entered the middle intestine and small portion also has reached the rectum. After 16 hour since feeding, approximately 10-20% of the original meal still remains in the stomach and at 24 hour, less than 5% of the original meals remains in the stomach. Finally, within 32-36 hour since feeding, the food item have completely left the stomach. Voluntary satiation feeding in these fish ranges between 8-13% of their body weights. However, not all fish eat the same amounts.

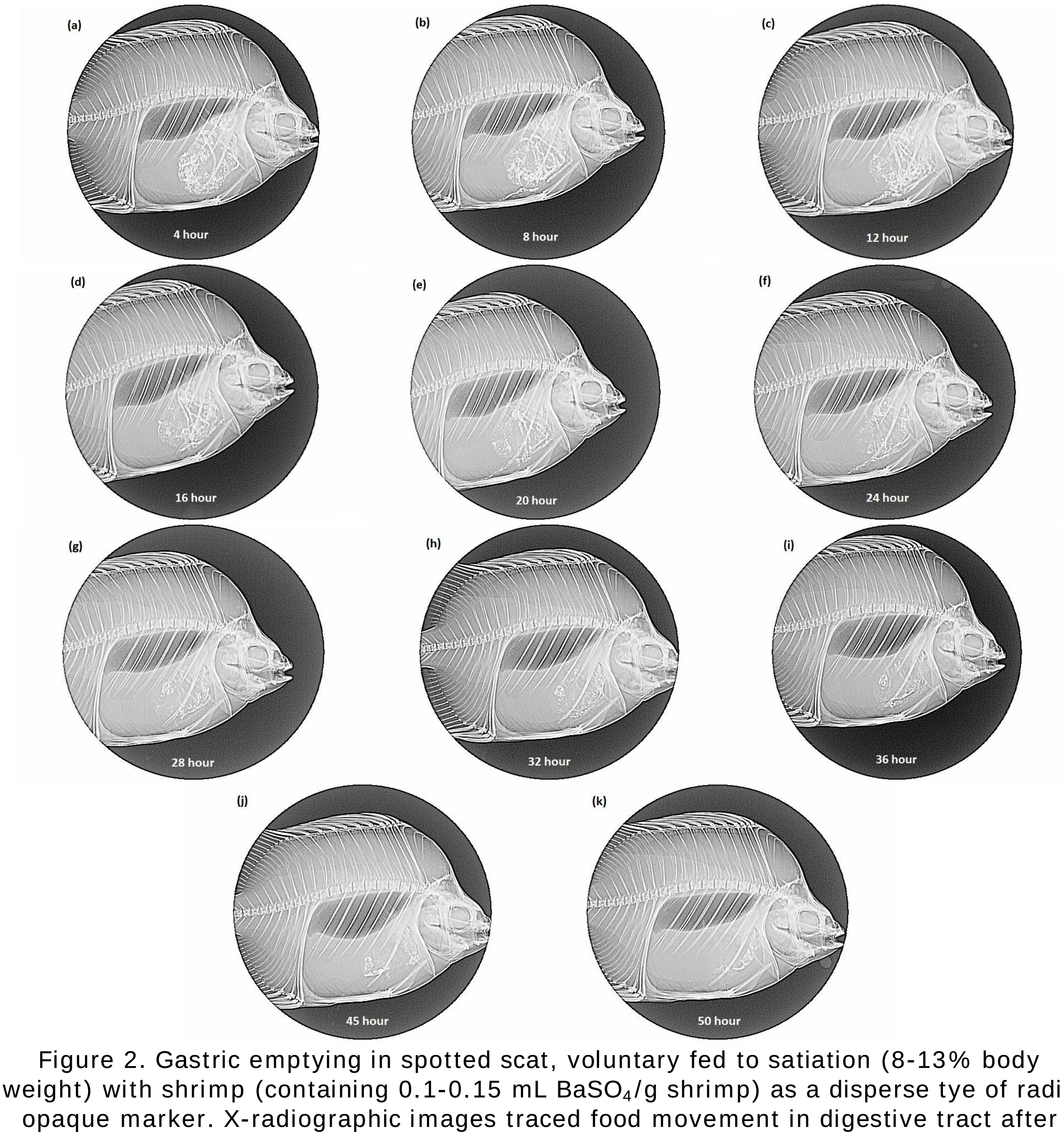

Figure 2. Gastric emptying in spotted scat, voluntary fed to satiation (8-13% body weight) with shrimp (containing 0.1-0.15 mL $BaSO_4$/g shrimp) as a disperse tye of radio opaque marker. X-radiographic images traced food movement in digestive tract after different times since feeding.

Modelling of satiation feeding is shown in Figure 3 to estimate the feed intake capability in fish of various size. According to the satiation feeding model by Mazlan et al (2002), satiation amount (S_{max}) increased allometrically with fish weight, $S_{max} = aW^b$, where b is 2/3 or 0.67. Based on the modelling of satiation feeding on scat fish shows that the b value is 0.97 which is more than the modelling of whiting fish by Mazlan et al (2002). Based on our stomach volume model earlier, the stomach volume of scat fish is higher than S_{max}.

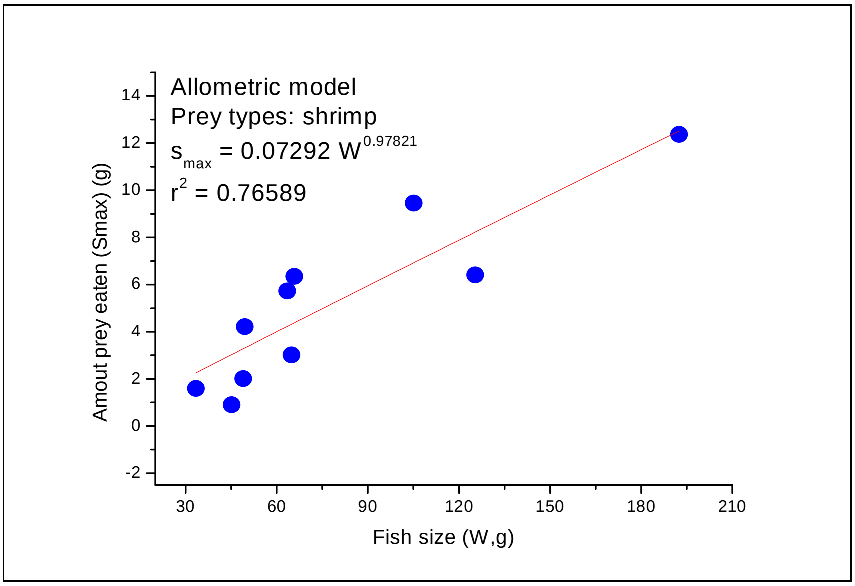

Figure 3. Relationship between amount of satiation meal (S_{max} g wet wt.) and fish size (W, g wet wt.) of spotted scat were fitted using allometric model.

Stomach residuum were collected at 1, 2 , 3, 24 and 48 hours since feeding to estimate gastric emptying rate of various fish size (Figure 4). All fishes were fed until satiation, however not all fish consumed the same amount. In accordance to gastric emptying rate (GER) to fish size, fish with body weight of 49.04 g at 1 hour time since feeding (TSF) have the highest GER = 0.029 g^{-1} hr^{-1} g bw^{-1}. Followed by 49.56 g fish at 2 hour TSF with GER = 0.006 $^{-1}$ hr^{-1} g bw^{-1}. Although fish at 3 hour TSF is bigger in size, 125.45 g the GER was 0.002 $^{-1}$ hr^{-1} g bw^{-1}. Meanwhile fish at 24 hour TSF ranging from 63.6 to 192.6 g have an average GER of 0.0028 $^{-1}$ hr^{-1} g bw^{-1}. Fish at 48 hour TSF ranging between 102.9 to 116.4 g with GER ranging 0.002-0.003 $^{-1}$ hr^{-1} g bw^{-1}. Estimation of gastric emptying rate of spotted scat is shown in Figure 4 were GER = 0.0024-0.0026 $^{-1}$ hr^{-1} g bw^{-1}. The GER of spotted scat shows GER decreases as time increases. The white shrimp prey has been de-shelled and pre-weighted to a small size shrimp paste due to the small fish mouth. This help the fish to eat the pre-weight prey wholly without break it apart.

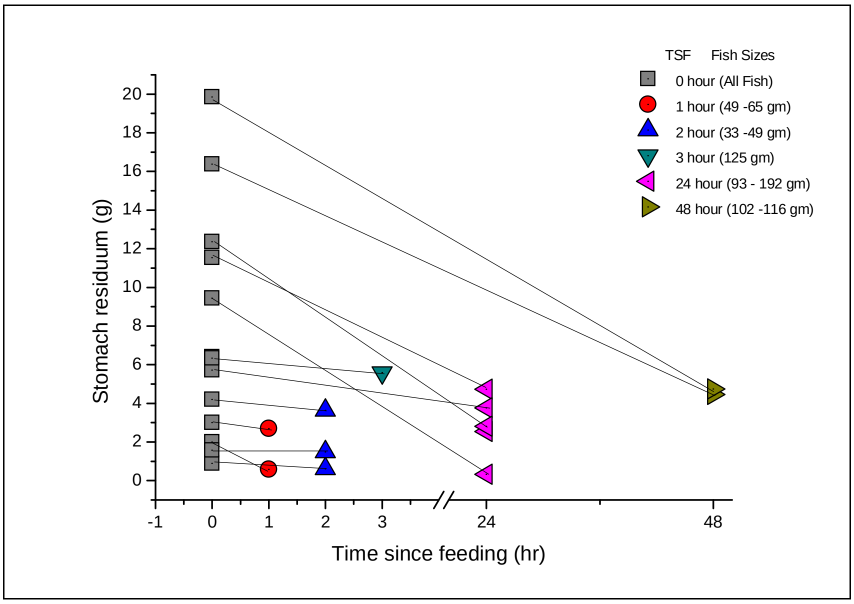

Figure 4. Relation between stomach residuum and time since feeding using different size of fish with different individual for each hour. The estimated gastric emptying rate is 0.0024-0.0026 $^{-1}$ hr^{-1} g bw^{-1}.

Modelling of gastric emptying time and stomach residuum (S_t) at stated time (t) after maximum satiation amount (S_{max}) was plotted graphically by non-linear regression using square root model (Andersen 1998, 1999, 2001; Andersen & Beyer 2008; Mazlan 2001) (Figure 5). The maximum meal size ($S_0/_{max}$) for spotted scat used in this modelling was 25 g and the gastric emptying parameter (ρ) was 0.14 g hr^{-1} and gastric emptying coefficient (α) was 0.5.

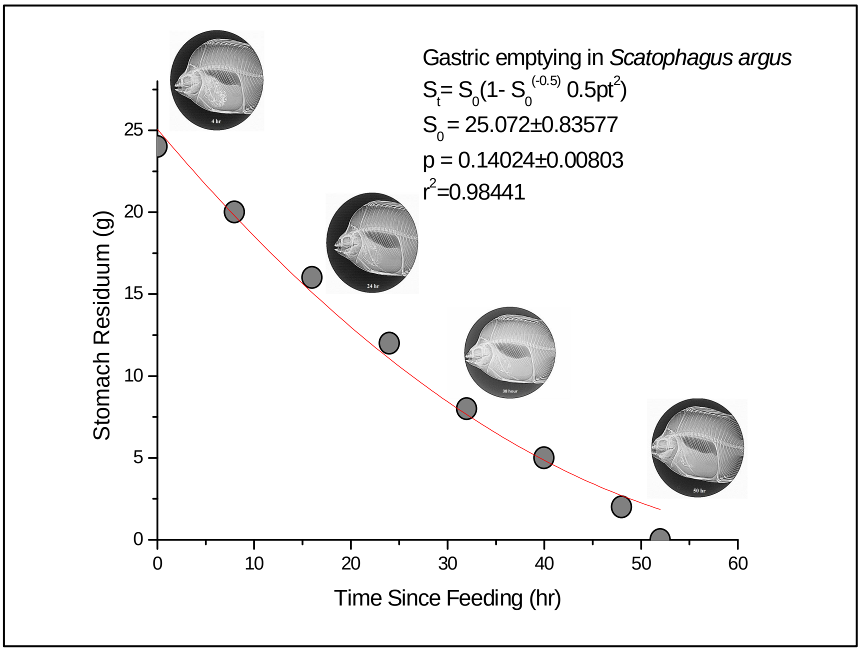

Figure 5. Gastric emptying curve of spotted scat feeding on pre-weighted white shrimp fitted using square root model [$S_t = S_0(1\text{-}S_0^{(\alpha-1)}\rho(1\text{-}\alpha)t)^{(1-\alpha)-1})+\xi$] where S_0 is the maximum meal size at time zero, ρ is gastric emptying rate (GER) and α is gastric emptying coefficient.

Discussion. Stomach volume of a fish is correlated with the amount of food intake. The relationship of stomach volume with food consumption has been suggested by Koskela et al (1997), Pirhonen & Forsman (1998) and Ogata & Shearer (2000). Although the studies on direct measurements of stomach volume in other fish species has been conducted by Ogata & Shearer (2000) and Nikki et al (2004), documented information regarding direct measurements of stomach volume in spotted scat is still lacking. Estimation of maximum food intake in fish can be achieved by measuring the maximum volume of the stomach. In the present study, stomach volume is calculated by measuring the amount of water that can be injected into an empty stomach until it burts. The results of present study indicate that the stomach volume and the amount of ingested food as estimated using x-ray method show a significant positive relationship in spotted scat. Similar results have also been reported in rainbow trout (Nikki et al 2004; Phirhonen & Koskela 2005). The estimation of maximum stomach capacity has been widely studied in various species of fish, with most researcher agreed that the maximum amount eaten in a meal (S_{max}) increases with body weight (W). Stomach volume gives an estimation of maximum stomach capacity suggested by Mazlan et al (2002), who seems to underestimate the satiation amounts for spotted scat in their study. Based on the model of maximum stomach capacity suggested by Mazlan et al (2002), maximum stomach satiation is 2/3 of the stomach capacity, but in our findings, it is more than suggested which is 0.97821. Similar results were reported in clownfish (*Amphiprion ocellaris*) (Ling & Ghaffar 2014) where the maximum stomach satiation was also higher. Spotted scat is an opportunistic feeder in which the tendency to consume more food is higher and approaching the maximum volume model. Being an omnivorous feeder, the tendency of the stomach to distend more enables the scat fish to consume more food while feeding. Such an example, spotted scat prefers algae in their diet, which allows their stomach to be compact and compressed. The shape of the stomach also plays an important role in fish digestion and food packaging in the stomach. Spotted scat has a U-shape stomach which aid in the high surface area of food absorption and lowered the gastric emptying time where it is essential in resiliency of the fish survival in limited food supply. Higher accuracy in estimating stomach volume can be achieved when measuring the stomach content where the fish have higher stomach fullness. These direct measurements of estimation of stomach volume will provide information on the capabilities of the stomach and the maximum satiation feed which aid in estimation of gastric evacuation using X-ray technique and estimation gastric emptying rate and gastric emptying time in spotted scat.

Based on the x-radiographic observations, the $BaSO_4$ marker passed through the digestive tract at the same rate as the food being digested, although traces of $BaSO_4$ could been observed at the stomach cavity at 45-50 hour but not in the intestines. The disappearance of $BaSo_4$ from x-ray images shows that this method is suitable to tract the gastric emptying in scat fish. Mazlan et al (2002) stated that the use of $BaSO_4$ paste within the natural food may affect the digestion rate of the fish but this method was useful without sacrificing the fish. Gastrectomy observation of *S. argus* showed that the fish (200 g) completely evacuated the stomach within 32-36 hours (GET) post feeding after been starved for 3 days. This is the first report of gastric motility in spotted scat in Malaysia and elsewhere. In nature, spotted scat often swallowed whole prey or nibble before swallowed if the prey is bigger than the mouth. White shrimp were used in this experiment and were easier to inject with $BaSO_4$. In order to minimize the loss of the marker in the water, the fish were fed with 0.2 g small bite size frozen shrimp sprat until satiation. Voluntary satiation feeding in these fish ranges between 8-13% of their body weights. However, not all fish eat the same amounts.

In the present study shows that the GER of smaller fish 45-50 g (0.003-0.006 g^{-1} hr^{-1}), were slightly higher than bigger fish 116-192 g (0.002-0.003 g^{-1} hr^{-1}) (Figure 4). Jobling et al (1977) and Flowerdew & Grove (1979) stated that the smaller fish processes a given relative meal more quickly than bigger fish. Similar findings were reported by Gillum et al (2012) for red drum fish (*Sciaenops ocellatus*), where the fish were fed with the same feed and the gastric evacuation of smaller red drum were slightly faster than larger red drum. Compared to the findings of other studies which used crustacean prey, many authors have observed a lag in evacuation due to the cuticle exoskeleton (Bromley 1991; Andersen 1999; Temming & Herrmann 2003). However, in this study the prey were deshelled first before feeding due to the small fish mouth. A slow rate of gastric emptying may contribute to the low consumption of food and slow growth of scat fish. Food consumption digestion study of scat fish shows that the fish of various sizes emptying their stomach at the same phase (0.3 g hr^{-1}), with normalised GER around 0.0024-0.0026 g^{-1} hr^{-1} gbw^{-1}, to explain that the digestion in this fish species is independent of fish size. In comparison of sacrificed scat fish with x-ray observations, similar rates of gastric emptying of food with and without $BaSO_4$ proves that $BaSO_4$ does not influence the digestion rate. The results of the present study will provide important baseline information for development of aquaculture of scats fish in captivity.

Conclusions. This study has provided the first information on gastric motility, gastric emptying time, gastric emptying rate and the effect of meal size to pepsin activity in the stomach of *S. argus*. This important information is useful in fish farming, management and conservation for *S. argus* in Malaysia and other regions.

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