Pertanika J. Trop. Agric. Sc. 43 (1): 65 - 79 (2020)

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Assessment on Reproductive Biology of Asian Swamp Eel, *Monopterus javanensis* La Cepède 1800 in relation to the Impacts of Paddy Practice Management in Kelantan, Malaysia

Ai Yin Sow[1]*, Ahmad Ismail[2], Syaizwan Zahmir Zulkifli[2], Mohammad Noor Amal Azmai[2] and Kamarul Hambali[3]

[1]*Faculty of Agro-Based Industry, Universiti Malaysia Kelantan, Jeli Campus, Locked Bag No. 100, 17600 Jeli, Kelantan, Malaysia*
[2]*Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor*
[3]*Faculty of Earth Science, Universiti Malaysia Kelantan, Jeli Campus Locked Bag No. 100, 17600 Jeli, Kelantan, Malaysia*

ABSTRACT

In order to understand the life history of the Asian swamp eel, *Monopterus javanensis* found in paddy fields, the time of maturation of its gonads was studied by using the gonadal somatic index. The peak gonadal somatic index was first found during the ploughing and seedling seasons in 2011 and 2012. This was accompanied by the yellowish egg sac observation made in the eels during the ploughing and seedling seasons, which was indicative of the mid- and early maturation stages of the gonads. However, the decline in GSI from the growing until the harvesting seasons indicated the poor development of gonads since differentiating the sex of the eels was hard. This could be due to the heavy application of pesticides and fertilisers during the growing season as cadmium present as impurities in the fertilisers, which slowly accumulated in the gonads. The findings highlighted the availability of Asian swamp eels for local eel collectors as part of their income and the complexity of heavy metal bioaccumulation in their gonads for safe eel consumption. Overall, the habitat of the Asian swamp eels may induce the differences in the maturation timing for the species.

Keywords: Asian swamp eel, Kelantan, Malaysia, paddy seasons, reproductive biology

ARTICLE INFO

Article history:
Received: 02 May 2019
Accepted: 15 August 2019
Published: 26 February 2020

E-mail addresses:
gsomaster87@gmail.com (Ai Yin Sow)
aismail@upm.edu.my (Ahmad Ismail)
syaizwan@upm.edu.my (Syaizwan Zahmir Zulkifli)
mnamal@upm.edu.my (Mohammad Noor Amal Azmai)
kamarul@umk.edu.my (Kamarul Hambali)
* Corresponding author

ISSN: 1511-3701
e-ISSN: 2231-8542

INTRODUCTION

Asian swamp eel, *Monopterus javanensis* is widely distributed worldwide and known to be native to Asia (Berra, 2001). It can be found in various aquatic habitats including rivers, lakes, ponds, marshes, swamps, and paddy fields. Due to its unique features, this eel is kept as an aquarium fish (Shafland et al., 2008). The Asian swamp eel's morphological characteristics include a scale-less body, small eyes that are covered by a layer of skin, a cylindrical body with compressed tail tapering to a point, and slate-brown or greenish colour with the ventral portion being lighter in colour and dark spots along the sides (United States Army Corps of Engineers [USACE], 2011). Collins et al. (2002) and Graham (1997) have stated that they are rarely observed as most of the eels are cryptically coloured, active during night time, and often bury themselves in soft sediments (i.e. 80 % muddy and 20 % water areas) (Agromedia, 2000) over a few months. Therefore, chances of their exposure to pollutants are high during paddy seasons. Paddy cultivation consists of several stages, namely, tilling the flooded soils or puddling, transplanting rice, and harvesting (Sahrawat, 2005). The repetitive use of agrochemicals for paddy cultivation has escalated the number of pollutants in its soils. For instance, ploughing the soil can cause pollutants from prior cycles of paddy cultivation to resurface, while the dependency on chemical fertilisers for the seedling and growing seasons adds to the number of pollutants (Sow et al., 2013).

In China, *M. javanensis* has been introduced as one of the rice-fish culture systems in paddy cultivation areas as the system has emerged as an important rural farming system capable of alleviating rural poverty and improving the rural economy (Fang, 2003). Since this species is well-associated with the desirable economic return and thus becomes highly popular among farmers in this study site, the knowledge on gonad development in Asian swamp eels is necessary and required to establish the duration of spawning season, the size and age at their maturity, and the spawning pattern during the paddy seasons. Therefore, this information collectively accounts for eel collectors since this species is vulnerable in Malaysia and may potentially generate income for them. The objective of the present study is to investigate the reproductive biology of Asian swamp eels by using the GSI and basic histological.

MATERIALS AND METHODS

Sampling Location

Monopterus javanensis eels were sampled from paddy fields located at Tumpat, Kelantan (N 06°08.454' E 102°8.430') in Peninsular Malaysia. The paddy plantation has been in operation for a time that is longer compared to this study. A few settlements were located near the study areas, but no heavy industrial activities were reported. In Kelantan, the majority of paddy fields are under the Kemubu Agricultural Development Authority (KADA). A river nearby the paddy fields, which is known as Jal River, supplies the water used by farmers during a shortage of water during the dry season.

Sample Collection

Asian swamp eels were collected by using the *tukil*. The tool is a semi-closed cylindrical tube made from PVC pipe, which is completely sealed at one end and has a spiny entrance at the other end. The sampling of Asian swamp eels was conducted every month from March to October based on the four paddy seasons in 2011 and 2012. Prior to the collection of the Asian swamp eels, the *tukil* was positioned at a selected spot in the paddy field for a day. A certain amount of bait (particularly cooked fish) was inserted in the *tukil*. The trapping of Asian swamp eel was conducted in the late afternoons as they were active at night time to look for food. The trap was checked for the presence of eels the following morning. The Asian swamp eels collected were placed in polythene plastics bags, brought back to the laboratory, and stored in the freezer at the temperature of -20 ºC until the analysis was commenced.

Sample Digestion

The samples were dried in an air-circulating oven for 3-4 days at 60 °C until a constant weight was achieved. Then, each gonad organ was weighed and placed into a digestion tube with the addition of 10 ml of concentrated HNO_3 (AnalaR grade, BDH 69 %), where the reaction was allowed to proceed at room temperature. Three replicates of the samples were digested for each organ in this study. Next, the tubes were placed in a hot-digester apparatus at 40 ºC for 1 hour and the temperature was gradually increased up to 140 ºC for at least 3 hours. After the completion of the digestion procedure, distilled water was added into the tubes at a certain volume (40 ml) and the extracts were next filtered through a Whatman No. 1 (filter speed: medium) filter paper in a funnel. Subsequently, the filtered solution was collected in an acid-washed polythene bottle (Yap et al., 2002a, 2002b). The prepared samples extracted from the gonads of Asian swamp eels were determined for the presence of Cd and Zinc (Zn) by using an air-acetylene flame atomic absorption spectrophotometer (AAS) PerkinElmer Model AAnalyst™ 800 after the filtration. The data were presented in the form of µg/g (dry weight). Multiple-level calibration standards were analysed to generate the calibration curves against which the sample concentrations were calculated. For the accuracy of the results, the r^2 of the calibration curve was in the range of 0.995-0.998. The quality of the method used as the precision check was the Certified Reference Material for fish (DORM-3) from the National Research Council Canada (NRCC). The agreement between the analytical results for the reference material for each metal shows good and acceptable recovery percentage as tabulated in Table 1.

Table 1
Measured result (µg/g dry weight ± SD) of the Certified Reference Material (CRM) for fish with its certified value for Zn and Cd

Metal	Zn[a]	Cd[b]
Measured	46.744±4.74	2.676±0.33
Certified (CRM)	51.3±3.1	2.11±0.15
Recovery	91.12%	126.82%

Note. a: Certified Reference Material (DORM-3), b: Certified Reference Material (PACS-2); SD: Standard deviation

Gonadosomatic Index

A total of 153 Asian swamp eels were collected in 2011 and 2012 for a GSI study. The total length of each eel was measured to the nearest 0.1 cm with the help of an absolute vernier calliper and a measuring tape for the eels with a longer body length. Body weights were recorded to the nearest 0.1 g by using the triple-beam digital weighing balance. After that, the eels were dissected to take out the gonads. Each of the gonads was weighed with the sensitive electric balance. The mean total length (TL) and body weight (BW) were 45.94 cm and 99.79 g for ploughing, 51.83 cm and 166.41 g for seedling, 43.02 cm and 84.32 g for growing, and 46.64 cm and 103.41 g for harvesting seasons in 2011. For 2012, the mean TL and BW were 42.17 cm and 82.83 g for ploughing, 50.28 cm and 144.80 g for seedling, 43.44 cm and 83.61 g for growing, and 44.79 cm and 106.10 g for harvesting seasons, accordingly. The determination of GSI was calculated by using the following formula as described by Devlaming et al. (1982).

Gonads Histological

For histological purposes, a total of 37 Asian swamp eels of undifferentiated sexes were collected during the ploughing, seedling, growing, and harvesting seasons in 2013 and then subjected to the histological procedure. To obtain the gonadal histology, two paraffin-based fixatives are generally and widely used, which are 10 % neutral buffered formalin (McCormick et al., 1989) and Bouin's fluid (Willemse & Van den Berg, 1978). Each eel was measured for its TL and BW randomly cut into four parts and fixed in the 10 % buffered formalin solution. The histological procedures were performed by using the paraffin-embedding method, which consisted of fixing the gonadal tissues, paraffin embedding, sectioning and mounting, and haematoxylin and eosin staining (Bell & Lightner, 1988; Drury & Wallington, 1980). An 8 µm thick section was cut on a rotary microtome and the resulting tissue sections were mounted on a glass treated with dibutylphthalate polystyrene xylene (DPX) mounting medium onto the slides before staining with HE (Harris

$$Gonadosomatic\,Index = \frac{Weight\ of\ gonads}{Weight\ of\ body} \times 100$$

Modified) and eosin (1 % Eosin Y, aqueous). The staining process comprised of a series of steps, which were: 1) soaking the samples in xylene (I) and xylene (II) for 3 minutes, 2) soaking the samples in absolute alcohol (I) and (II), 95 %, 90 %, 80 %, 70 % alcohol and tap water for 3 minutes. After that, the samples were soaked in haematoxylin for 5 minutes, followed by soaking them in tap water for several times. Next, the samples were soaked in eosin again, followed by tap water and 95% absolute alcohol (I) and (II), xylene (I) and (II) for 3 minutes respectively. Afterward, the stained tissues were analysed by using the light microscopy and digital images obtained.

Statistical Analysis

The findings were reported in the form of mean ± standard deviation (SD) values. The Tukey's Post-Hoc HSD test was carried out to identify the significantly different values upon obtaining a significant ANOVA value. Further statistical analyses were conducted with SPSS Statistic Software 21.0 with $p<0.05$ as the significant level.

RESULT

The average concentration of Zn and Cd in the gonads of Asian swamp eels is shown in Table 2. In 2011, the average Zn metal was observed to accumulate the highest in the gonads during the ploughing season (120.00 µg/g), while the average Cd showed higher values during the growing (11.83 µg/g) and harvesting (28.88 µg/g) seasons. Zn showed no significant ($p>0.05$) difference in the gonads across four different paddy seasons in 2012. Similar to 2012, the average Cd was found to be higher in the growing (32.83 µg/g) and harvesting (73.13 µg/g) seasons, and increased threefold from 2011 to 2012. Table 3 shows the observation of Asian swamp eel gonads based on the paddies stages (day) according to four different paddy seasons. In 2011, the GSI peaks of the Asian swamp eels were shown to be higher during the ploughing season (2.28 %), followed by the seedling season (1.94 %). Similarly, the GSI peaks in 2012 were in line with 2011 (Table 3), yielding 1.69 % and 1.58 % for the ploughing and seedling seasons, respectively. The results

Table 2
Zn and Cd concentration in gonads of Monopterus albus *during paddy seasons in 2011 and 2012*

Year	Season	Zn	Cd
2011	Plowing	120.00 ± 88.62^{a}	3.97 ± 3.24^{b}
	Seedling	68.12 ± 16.00^{b}	0.19 ± 0.13^{c}
	Growing	71.84 ± 22.54^{b}	11.83 ± 10.10^{b}
	Harvesting	84.80 ± 13.79^{b}	28.88 ± 8.72^{a}
2012	Plowing	78.18 ± 14.12^{a}	9.93 ± 9.70^{b}
	Seedling	82.66 ± 18.49^{a}	9.61 ± 7.90^{b}
	Growing	86.73 ± 32.65^{a}	32.83 ± 23.89^{a}
	Harvesting	79.51 ± 30.91^{a}	73.13 ± 55.00^{a}

Note. n = 153 samples; Objects indicated with different alphabet $p<0.05$, objects indicated with the same alphabet $p>0.05$

Table 3
Gonadal somatic index (GSI) (mean ± S.D) of Asian swamp eels according to four paddy seasons in 2011 and 2012

Year	Season	Paddies' stage (day)	Gonad weight (g)	GSI (%)	Gonads observation
2011	Plowing	0-30	2.41±2.45	2.28±2.08[a]	Eggs sacs present (eggs in yellowish color) and in mid-maturation stage
	Seedling	30-50	2.48±1.95	1.94±2.14[a]	Eggs sacs present (eggs in yellowish color) and in early maturation stage
	Growing	50-90	0.72±0.94	0.76±0.88[b]	Poorly developed; hard to identify Sexes
	Harvesting	100-120	0.24±0.12	0.25±0.10[c]	Poorly developed; hard to identify sexes
2012	Plowing	0-30	1.24±1.37	1.69±1.81[a]	Eggs sacs present (eggs in yellowish color) and in mid-maturation stage
	Seedling	30-50	2.53±2.43	1.58±1.33[a]	Eggs sacs present (eggs in yellowish color) and in early maturation stage
	Growing	50-90	1.00±1.53	0.85±0.99[b]	Poorly developed; hard to identify Sexes
	Harvesting	100-120	1.13±1.32	0.77±0.64[b]	Poorly developed; hard to identify sexes

Note. n = 153 samples; Objects indicated with different alphabet $p<0.05$, objects indicated with the same alphabet $p>0.05$

also demonstrated yellowish egg sacs identified through the dissection of the eels, indicating the peak period of maturity found during the ploughing and seedling seasons. Consequently, it also indicated the availability of mature eels for the eel collectors, which may indirectly increase the source of their income. Nonetheless, an abrupt decline in GSI was seen from 0.76 % during the growing season to 0.25 % during the harvesting season in 2011. For 2012, a gradual decrease in GSI was observed from 0.85 % in the growing season to 0.77 % in the harvesting season. Figure 1

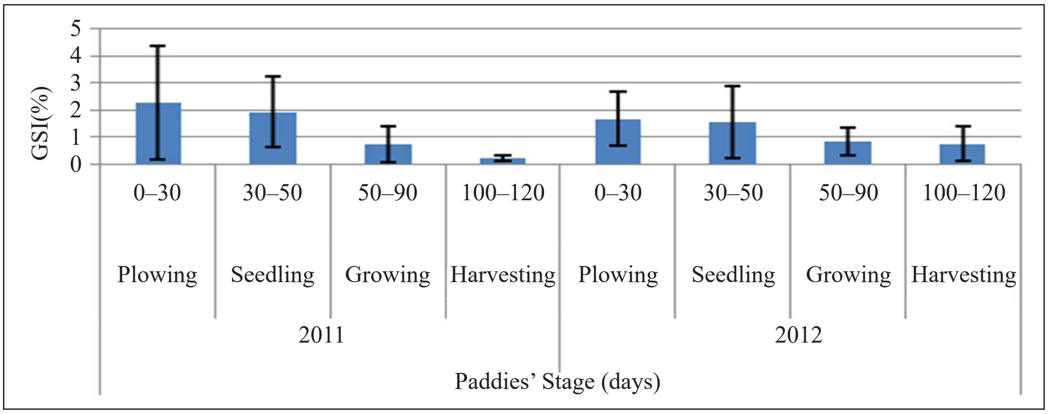

Figure 1. Gonads development based on paddies'stage (days) and GSI (%) in 2011 and 2012

indicates that the maturity of Asian swamp eel gonads begins to decrease from the ploughing until the harvesting season. The error bar in Figure 1 is omitted due to the big variable detected in this study, which is due to the ungrouping of Asian swamp eel sizes according to the BL and BW throughout the four paddy seasons.

DISCUSSION

High or minimal loaded heavy metals have been reported to affect both the quality and quantity of the gametes and endocrine systems, which eventually disrupt the gametogenesis processes (Jezierska & Witeska, 2001). This occurs either indirectly via the accumulation of heavy metals in reproductive organs or directly by attacking the free gametes released into the water system (Ebrahimi & Taherianfard, 2011). Different tissues of the fish species have dissimilar bioaccumulation capacities (Alhashemi et al., 2012), rendering it important to analyse other tissues, such as the gonads (Has-Schön et al., 2008; Jarić et al., 2011). Higher average Cd was found in the gonads of Asian swamp eels in 2011 and 2012 compared to the study conducted by Chi et al. (2007), who reported 0.017 µg/g in *Cyprinus carpio* and 0.010 µg/g in *Cyprinus auratus* from the Taihu Lake in China. The presence of the highest Zn average in the gonads in this study is also supported by the study conducted by Chi et al. (2007). According to Olsson et al. (1989), Zn is an essential element involved in the development of both sperm and ovary.

GSI is one of the important parameters of fish biology, which gives a detailed idea regarding the fish reproduction and reproductive status of the species and helps in ascertaining the breeding period of fish (Mohan & Jhajhria, 2001; Muchlisin et al., 2011; Murua & Saborido-Rey, 2003). In this study, the maturity of Asian swamp eel eggs may be due to the presence of high nutrition as a result of the heavy application of agrochemical fertilisers (Yin et al., 2012) and the abundance of diet (i.e. other smaller living aquatic organisms). It may also be attributable to the irrigation process into paddy fields during the ploughing season, which increases the food availability for these Asian swamp eels located in the paddy field areas. Based on Ali's (1993) study on catfish, *Clarias macrocephalus* on rice fields of Perak, the increase in irrigated water levels in the paddy field and rainfall seemed to be the spawning cues. As stated by Kumar et al. (2013), the presence of humid and waterlogged environments in the rice ecologies causes organic matter decomposition for varied biological organisms (i.e. microorganisms, diatoms, phyto and zooplankton, micro invertebrates and benthic macroinvertebrates). This maintains the soil fertility by releasing nutrients and sediment bio-revolving process. In addition, flooded paddy fields make the nutrients available for farmers and diversify biological organisms that are directly or indirectly performing important ecosystem services (Melo et al., 2015).

A unique characteristic of the Asian swamp eel is its ability to burrow deep into the mud and remain in contact with the water table (Liem & Inger, 1987; Sterba, 1983), which may be another reason for the maturity of gonads. Cheng et al. (2003) and Hill and Watson (2007) had reported that the clay contents of the soils represent the nutritional source for eel's growth. Meanwhile, the timing of maturation for the eel may not be associated with its age or growth, but rather the need to be prepared for certain physiological conditions (Arai & Chino, 2013). They had also reported that the deposition of high Zn in the gonads might play an important physiological role during gonadal maturation. This was observed in this study with the higher Zn accumulation in gonads ranging between 68.12-120 µg/g in 2011 and 78.18-86.73 µg/g for four paddy seasons.

Furthermore, poor development of gonads and difficulties in identifying the sexes were observed in this study, while the GSI decreased during the growing and harvesting seasons for both years. In the current study, the growing stages of paddy were divided into three stages, with the major focus placed on the fertilising process. In stage one, compound fertilisers were introduced, while at stage two, urea fertilisers were used. Meanwhile, urea and compound fertilisers were both used for the growing stage of the paddy cycle. In stage three, foliar fertilisers were applied to the paddies by the farmers. As stated by Yin et al. (2012), heavy application of fertilisers in paddy cultivation areas is done to ensure the quality of rice produced. The presence of heavy metals, especially Cd in the paddy fields through paddy practice management can contribute to the poor development of Asian swamp eel gonads by decreasing the size and number of oocytes in this study. Cd is one of the most toxic heavy metals and a non-essential element widely found in agricultural management practices such as fertilisers and pesticides, otherwise present as impurities (Satarug et al., 2003). El-Ebiary et al. (2013) had concluded that a high level of Cd directly affects the gonad structure through vacuolisation disorders (i.e. abnormally-shaped ova with a high amount of vacuoles and a detached follicular wall). Changes in the ovary of *Heteropneustes fossilis* (stinging catfish) had been observed and reported by Sharma et al. (2011), and of *Carassius gibelio* B. (Prussian carp) by Drag-Kozak et al. (2018). Hwang et al. (2000) had shown that Cd inhibited the synthesis of vitellogenesis in the hepatocytes of the rainbow trout, whereas Szczerbik et al. (2006) had suggested that it acts mainly at the level of the ovary in the Prussian carps. Meanwhile, Brown et al. (1994) had reported that oogenesis was delayed in the brown trout and inhibited in the rainbow trout after exposure to this metal. In addition, Cd has been revealed to disrupt the reproductive function in female fishes by reducing their ovary weight and limiting their development (Szczerbik et al., 2006). Therefore, it can be suggested that Cd affects the quantity and quality of gametes (Annabi et al., 2012).

The histological observation of the female gonads of Asian swamp eel (Figure 2) is done to ascertain the pattern of oocyte maturation and identify its breeding season. The oocyte maturation can be separated into five stages: 1) oogonia, 2) primary growth or peri-nucleolar stage, 3) cortical alveoli stage, 4) vitellogenesis, and 5) ovulated oocyte, based on the size of oocytes (Table 4). In the present study, it was observed that the Asian swamp eels collected were exclusively in their oogonia and primary growth stage of maturity during the harvesting and seedling seasons, respectively. However, it is not easy to distinguish the newly formed oocytes from oogonia under microscopy (Ravaglia & Maggese, 2002). Therefore, an examination of the histological sections stained with H&E is helpful to distinguish the germinal cells as the cytoplasm becomes basophilic when oogenesis begins (Uribe et al., 2012). As illustrated in Figure 3, the primary oocyte

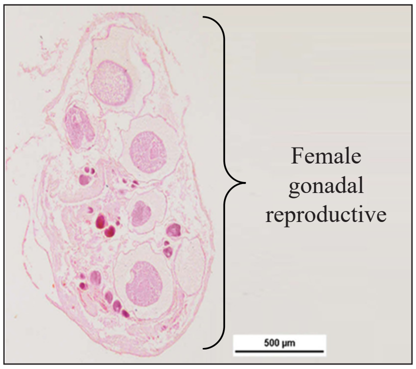

Figure 2. Female gonadal reproductive system with magnification size of 400

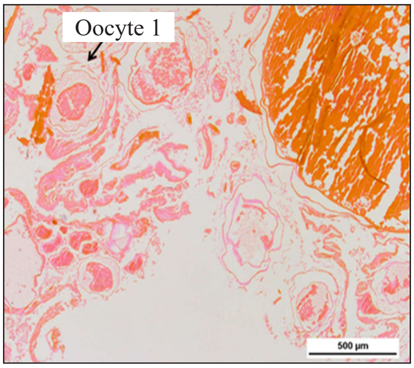

Figure 3. Oocyte 1 (primary growth) of Asian swamp eel with magnification size of 400

Table 4
Counted sizes (µm) of female ovarian development of 37 individuals during different paddy seasons

Season	Oogonia		Oocyte 1		Oocyte 2		Oocyte 3		Oocyte 4	
	No	Size (µm)	No	Size (µm)	No	Size (µm)	No	Size (µm)	No	Size (µm)
Plowing	0	-	0	-	4	728.16-920.37 (809.4)	29	404.77-848 (667.12)	24	667.29-2440.54 (1472.7)
Seedling	0	-	1	214.11	0	-	7	330.37-663.63 (486.68)	15	742.67-2105.34 (1482.1)
Growing	0	-	0	-	0	-	17	375.16-888.85 (655.71)	10	683.27-1692.76 (986.27)
Harvesting	3	16.37-88.78 (53.81)	0	-	2	577.12-623.87 (600.5)	14	312.47-724.23 (539.22)	11	326.39-2030.21 (934.5)

Note. n = 37 samples. Oocyte 1: primary gorwth or perinucleolar stage; oocyte 2: cortical alveoli stage; oocyte 3: vitellogenesis; oocyte 4: ovulated oocyte or matured; value in parentheses (average) appears slightly irregular in shape with an abundant cytoplasm of diameter about 214.11µm. The irregular shape of the oocyte in the primary growth stage is probably due to the stress generated by the expanding oocytes around them. Oocytes of the cortical alveoli stage then appeared only during the harvesting until ploughing seasons, whereby their diameter increased noticeably compared to those of the primary growth stage. The sizes of the cortical alveoli stage oocytes range from 728.16 to 920.37 µm during the ploughing season (Table 4), which is slightly larger compared to the harvesting season (577.12-623.87 µm). The presence of oocytes in vitellogenesis was not restricted to any season but their quantity was the highest in the ploughing season, followed by the growing season. In vitellogenesis, a clear shrinkage of oocytes is seen (Table 4); this scenario does not parallel results reported in the literature. A histological study of the gonads of Asian spiny eel, *Mastacembelus armatus* has shown an increase in oocyte diameter when it enters the vitellogenesis stage (Ali et al., 2016). Besides, the oocyte diameter of *Synbranchus marmoratus* has increased with correspondence to its ovarian stage (Ravaglia & Maggese, 2002). Meanwhile, the late-maturation stages occur when the oocyte is almost fully grown and ready for ovulation. As illustrated in Figure 4, the matured oocytes are usually recognised by the presence of colour pigments contained within the lipid droplets. Furthermore, at this stage, a decrease in the nucleo-cytoplasmic ratio of the oocytes (Figure 4) is witnessed upon comparison with the primary stage oocytes (Figure 3). The early maturation of male Asian swamp eel gonads is illustrated in Figure 5. During the early maturation stage, testicular maturation begins by elongation due to the mitotic division of both Sertoli cells (SC) and spermatogonia (SG). Moreover, the lobules commonly have discontinuous lumina (Figures 5 and 6), which grows in length during the early maturation stage. A continuous germinal epithelium (GE) is seen within the lobules,

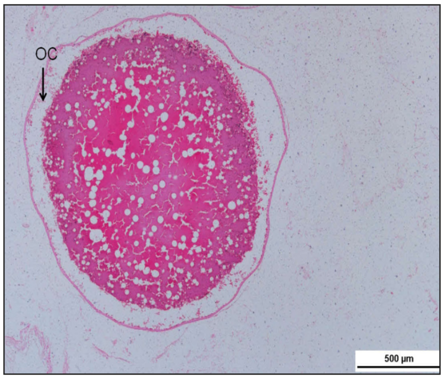

Figure 4. Late maturation of oocytes (OC) 4 (matured) cell of Asian swamp eel with magnification size of 400

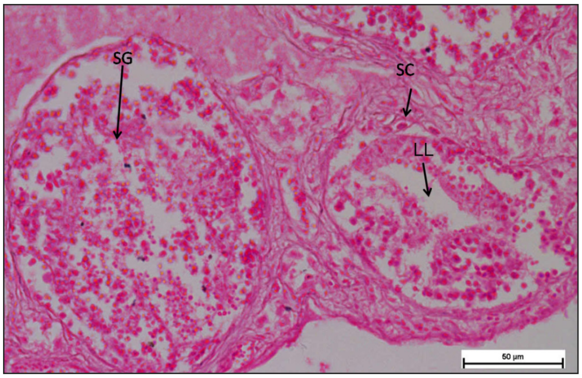

Figure 5. Mid-maturation of male Asian swamp eel with the presence of spermatogonia (SG) and Sertoli cell (SC) with the magnification size of 400. Lobule lumen (LL) is discontinuous

which consists of conspicuous SC where the processes surround a cluster of SG. In contrast, the mid-maturation class of male gonadal reproductive system (Figure 6) is characterised by the presence of GE. A discontinuous GE is composed of the regions of SC associated with germ cells. As mid-maturation progresses, the sperm matures, which is followed by the spermiation (i.e. release of mature sperm into lonular lumina) (Nostro et al., 2003). From the slides observed, sex reversal of Asian swamp eel is identified through the presence of spermatogonia (SG) and mature oocyte (MO) (Figure 7). Sex reversal of Asian swamp eel (Figure 8) is indicated by the presence of vitellogenic oocyte (VOC) and Sertoli cells (SC).

Figure 6. Early-maturation of male Asian swamp eel with the magnification size of 400. A continuous germinal epithelium which consisiting of Sertoli cell (SC) and spermatogonia (SG) within spermatocycsts. Lobules (L) is discontinuous

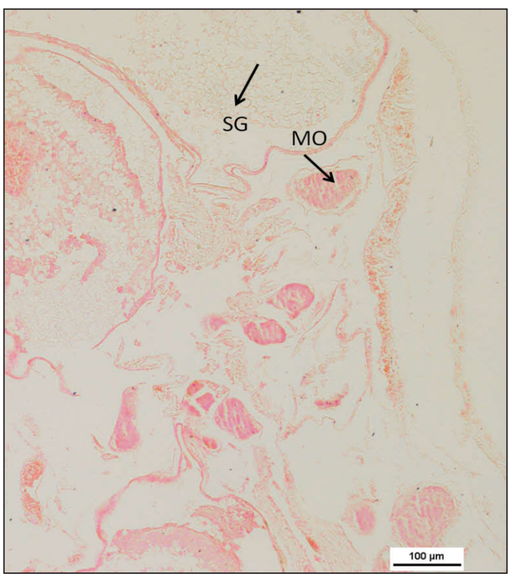

Figure 7. Sex reversal of Asian swamp eelwith magnification of 1000. SG: Spermatogonia, MO: Matured oocyte

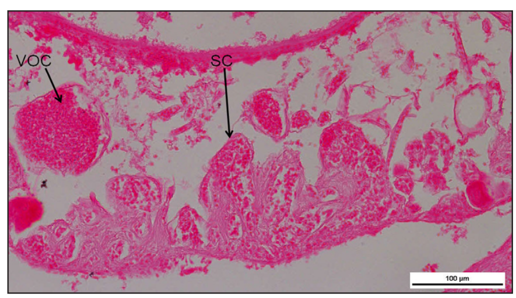

Figure 8. Sex reversal of Asian swamp eel which consisting of vitellgenic oocytes (VOC) and Sertoli cell (SC). The elongation of each testis lobules indicated the sex reversal of Asian swamp eel has begun. Magnification size of 200

CONCLUSION

Although the late-maturation oocytes appeared throughout all four paddy seasons, their number peaked in the ploughing season. This suggests that the ploughing season offers a better environment for the Asian swamp eels to breed. In contrast, the ploughing and seedling seasons revealed bigger-sized late-maturation stage oocytes compared to the late-maturation oocytes formed during the growing and harvesting seasons. The diameter of oocytes at the late-maturation stage is frequently associated with mortality rate, whereby larger oocytes contain more yolk to enhance the probability of successful hatching. In short, the data collected in Table 4 and GSI calculation, it can be concluded that the ploughing season offers the best environment for Asian swamp eels to breed and a higher chance for the eggs to hatch successfully.

CONFLICT OF INTEREST

Authors declared there is no conflict interest.

FUNDING STATEMENT

This study was jointly supported by the Research University Grant Scheme, RUGS (Project No: 03-01-11-1155RU) with the grant provided by Universiti Putra Malaysia as well as the Fundamental Research Grant Scheme (FRGS) (Project No: FRGS/1/11/ST/UPM/02/12) awarded by the Malaysia Ministry of Higher Education (MOHE).

REFERENCES

Agromedia, R. (2000). *Penternakan belut di halaman rumah* [Husbandry of eels in the yard]. Kuala Lumpur, Malaysia: Synergy Media.

Alhashemi, A. H., Karbassi, A., Kiabi, B. H., Monavari, S. M., & Sekhavatjou, M. S. (2012). Bioaccumulation of trace elements in different tissues of three commonly available fish species regarding their gender, gonadosomatic index, and condition factor in a wetland ecosystem. *Environmental Monitoring and Assessment*, *184*(4), 1865-1878.

Ali, A. B. (1993). Aspects of the fecundity of the feral catfish, *Clarias macrocephalus* (*Günther*), population obtained from the rice fields used for rice-fish farming in Malaysia. *Hydrobiologia*, *254*(2), 81-89.

Ali, M. R., Rahman, M. M., Sarder, M. R. I., & Mollah, M. F. A. (2016). Histological study of the developing gonads of endangered freshwater spiny eel, *Mastacembelus armatus* during the reproductive cycle. *Journal Bangladesh Agricultural University*, *14*(2), 261-269.

Annabi, A., Kessabi, K., Kerkeni, A., Said, K., & Messaoudi, I. (2012). Influence of cadmium exposure on growth and fecundity of freshwater mosquitofish *Gambusia affinis*: *In situ* and *in vivo* studies. *Biological Trace Element Research*, *148*(3), 345-355.

Arai, T., & Chino, N. (2013). Timing of maturation of a tropical eel, *Anguilla bicolor bicolor* in Malaysia. *Journal of Applied Ichthyology*, *29*(1), 271-273.

Bell, T. A., & Lightner, D. V. (1988). *A handbook of normal penaeid shrimp histology*. Baton Rouge, USA: World Aquaculture Society.

Berra, T. M. (2001). *Freshwater fish distribution*. Cambridge, USA: Academic Press.

Brown, V., Shurben, D., Miller, W., & Crane, M. (1994). Cadmium toxicity to rainbow trout *Oncorhynchus mykiss* Walbaum and brown trout *Salmo trutta* L. over extended exposure periods. *Ecotoxicology and Environmental Safety*, *29*(1), 38-46.

Cheng, H., Guo, Y., Yu, Q., & Zhou, R. (2003). The rice field eel as a model system for vertebrate sexual development. *Cytogenetic and Genome Research*, *101*(3-4), 274-277.

Chi, Q. Q., Zhu, G. W., & Langdon, A. (2007). Bioaccumulation of heavy metals in fishes from Taihu Lake, China. *Journal of Environmental Sciences*, *19*(12), 1500-1504.

Collins, T. M., Trexler, J. C., Nico, L. G., & Rawlings, T. A. (2002). Genetic diversity in a morphologically conservative invasive taxon:

Multiple introductions of swamp eels to the southeastern United States. *Conservation Biology*, *16*(4), 1024-1035.

Devlaming, V., Grossman, G., & Chapman, F. (1982). On the use of the gonosomatic index. *Comparative Biochemistry and Physiology Part A: Physiology*, *73*(1), 31-39.

Drąg-Kozak, E., Socha, M., Gosiewski, G., Łuszczek-Trojnar, E., Chyb, J., & Popek, W. (2018). Protective effect of melatonin on cadmium-induced changes in some maturation and reproductive parameters of female Prussian carp (*Carassius gibelio* B.). *Environmental Science and Pollution Research*, *25*(10), 9915-9927.

Drury, R. A. B., & Wallington, E. A. (1980). Preparation and fixation of tissues. In *Carleton's histological technique* (5th ed., pp. 41-54). Oxford, United Kingdom: Oxford University Press.

Ebrahimi, M., & Taherianfard, M. (2011). The effects of heavy metals exposure on reproductive systems of cyprinid fish from Kor River. *Iranian Journal of Fisheries Sciences*, *10*(1), 13-26.

El-Ebiary, E., Wahbi, O., & El-Greisy, Z. (2013). Influence of dietary cadmium on sexual maturity and reproduction of red tilapia. *The Egyptian Journal of Aquatic Research*, *39*(4), 313-317.

Fang, X. Z. (2003). Rice-fish culture in China. *Aquaculture Asia*, *8*(4), 44-46.

Graham, J. B. (1997). *Air-breathing fishes: Evolution, diversity, and adaptation*. Amsterdam, Netherlands: Elsevier.

Has-Schön, E., Bogut, I., Rajković, V., Bogut, S., Čačić, M., & Horvatić, J. (2008). Heavy metal distribution in tissues of six fish species included in human diet, inhabiting freshwaters of the Nature Park "Hutovo Blato" (Bosnia and Herzegovina). *Archives of Environmental Contamination and Toxicology*, *54*(1), 75-83.

Hill, J. E., & Watson, C. A. (2007). Diet of the nonindigenous Asian swamp eel in tropical ornamental aquaculture ponds in West-Central Florida. *North American Journal of Aquaculture*, *69*(2), 139-146.

Hwang, U. G., Kagawa, N., & Mugiya, Y. (2000). Aluminium and cadmium inhibit vitellogenin and its mRNA induction by estradiol-17 β in the primary culture of hepatocytes in the rainbow trout *Oncorhynchus mykiss*. *General and Comparative Endocrinology*, *119*(1), 69-76.

Jarić, I., Višnjić-Jeftić, Ž., Cvijanović, G., Gačić, Z., Jovanović, L., Skorić, S., & Lenhardt, M. (2011). Determination of differential heavy metal and trace element accumulation in liver, gills, intestine and muscle of sterlet (*Acipenser ruthenus*) from the Danube River in Serbia by ICP-OES. *Microchemical Journal*, *98*(1), 77-81.

Jezierska, B., & Witeska, M. (2001). *Metal toxicity to fish*. Siedlce, Poland: Wydawnictwo Akademii Podlaskiej.

Kumar, A., Colton, M. B. M., Springer, M., & Trama, F. A. (2013). Macroinvertebrate communities as bioindicators of water quality in conventional and organic irrigated rice fields in Guanacaste, Costa Rica. *Ecological Indicators*, *29*, 68-78.

Liem, K. F., & Inger, R. F. (1987). Functional design of the air ventilation apparatus and overland excursions of teleosts. *Fieldiana Zoology*, *37*, 1-29.

McCormick, J. H., Stokes, G. N., & Hermanutz, R. O. (1989). Oocyte atresia and reproductive success in fathead minnows (*Pimephales promelas*) exposed to acidified hardwater environments. *Archives of Environmental Contamination and Toxicology*, *18*(1-2), 207-214.

Melo, S., Stenert, C., Dalzochio, M. S., & Maltchik, L. (2015). Development of a multimetric index based on aquatic macroinvertebrate communities to assess water quality of rice fields in southern Brazil. *Hydrobiologia*, *742*(1), 1-14.

Mohan, D., & Jhajhria, A. (2001). Comparative growth and production studies of carps in Shekhawat pond Jodhpur. *Journal Nate*, *13*(2), 123-130.

Muchlisin, Z. A., Musman, M., Fadli, N., & Siti-Azizah, M. N. (2011). Fecundity and spawning frequency of *Rasbora tawarensis* (Pisces: Cyprinidae) an endemic species from Lake Laut Tawar, Aceh, Indonesia. *AACL Bioflux*, *4*(3), 273-279.

Murua, H., & Saborido-Rey, F. (2003). Female reproductive strategies of marine fish species of the North Atlantic. *Journal of Northwest Atlantic Fishery Science*, *33*, 23-31.

Nostro, F. L., Grier, H., Andreone, L., & Guerrero, G. A. (2003). Involvement of the gonadal germinal epithelium during sex reversal and seasonal testicular cycling in the protogynous swamp eel, *Synbranchus marmoratus* Bloch 1975 (Teleostei, Synbranchidae). *Journal of Morphology*, *257*(1), 107-126.

Olsson, P., Zafarullah, M., & Gedamu, L. (1989). A role of metallothionein in zinc regulation after oestradiol induction of vitellogenin synthesis in rainbow trout, *Salmo gairdneri*. *Biochemical Journal*, *257*(2), 555-559.

Ravaglia, M. A., & Maggese, M. C. (2002). Oogenesis in the swamp eel *Synbranchus marmoratus* (Bloch, 1795) (Teleostei, Synbranchidae). Ovarian anatomy, stages of oocyte development and micropyle structure. *Biocell*, *26*(3), 325-337.

Sahrawat, K. (2005). Fertility and organic matter in submerged rice soils. *Current Science*, *88*(5), 735-739.

Satarug, S., Baker, J. R., Urbenjapol, S., Haswell-Elkins, M., Reilly, P. E., Williams, D. J., & Moore, M. R. (2003). A global perspective on cadmium pollution and toxicity in non-occupationally exposed population. *Toxicology Letters*, *137*(1-2), 65-83.

Shafland, P. L., Gestring, K. B., & Stanford, M. S. (2008). Florida's exotic freshwater fishes: 2007. *Florida Scientist*, *71*(3), 220-245.

Sharma, S., Manhor, S., Qureshi, T., Kaur, P., & Dar, B. (2011). Histological studies on the cadmium chloride exposed air-breathing fish, *Heteropneustes fossilis* (Bloch) with special reference to ovaries. *International Journal of Environmental Sciences*, *2*(2), 411.

Sow, A. Y., Ismail, A., & Zulkifli, S. Z. (2013). An assessment of heavy metal bioaccumulation in Asian swamp eel, *Monopterus albus*, during plowing stages of a paddy cycle. *Bulletin of Environmental Contamination and Toxicology*, *91*(1), 6-12.

Sterba, G. (1983). *The aquarium encyclopedia*. Cambridge, USA: The MIT Press.

Szczerbik, P., Mikołajczyk, T., Sokołowska-Mikołajczyk, M., Socha, M., Chyb, J., & Epler, P. (2006). Influence of long-term exposure to dietary cadmium on growth, maturation and reproduction of goldfish (subspecies: Prussian carp *Carassius auratus gibelio* B.). *Aquatic Toxicology*, *77*(2), 126-135.

United States Army Corps of Engineers. (2011). *Monopterus albus*. Retrieved April 16, 2019, from http://el.erdc.usace.army.mil/ansrp/monopterus_albus.pdf

Uribe, M. C., Grier, H. J., & Parenti, L. R. (2012). Ovarian structure and oogenesis of the oviparous goodeids *Crenichthys baileyi* (Gilbert, 1893) and *Empetrichthys latos* Miller, 1948 (Teleostei, Cyprinodontiformes). *Journal of Morphology*, *273*(4), 371–387.

Willemse, J. J., & Van den Berg, P. G. (1978). Growth of striated muscle fibres in the *M. lateralis* of the European eel *Anguilla anguilla* (L.) (Pisces, Teleostei). *Journal of Anatomy*, *125*(Pt 3), 447-460.

Yap, C. K., Ismail, A., Tan, S. G., & Omar, H. (2002a). Correlations between speciation of Cd, Cu Pb and Zn in sediment and their concentrations in total soft tissue of green-lipped mussel *Perna viridis* from the west coast of Peninsular Malaysia. *Environment International*, *28*(1-2), 117-126.

Yap, C. K., Ismail, A., Tan, S. G., & Omar, H. (2002b). Concentration of Cu and Pb in the offshore and intertidal sediments of the west coast of Peninsular Malaysia. *Environmental International*, *28*(6), 467-479.

Yin, S. A., Ismail, A., & Zulkifli, S. Z. (2012). Heavy metals uptake by Asian swamp eel, *Monopterus albus* from paddy fields of Kelantan, Peninsular Malaysia: Preliminary study. *Tropical Life Sciences Research*, *23*(2), 27.

Zuiew, B. (1793). Biga Mvraenarvm, novae species descriptae [*'Biga Mvraenarvm'*, the new species described]. *Nova Acta Academiae Scientiarum Imperialis Petropolitanae*, *7*, 296-301.