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Original Article

Seasonal ovarian activity in female climbing perch, *Anabas testudineus* (Bloch, 1792) from the northern and eastern regions of Peninsular Malaysia

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Abstract

The present study was performed to describe the ovarian activity and plasma sex steroid levels in female climbing perch, *Anabas testudineus* in four states, comprised of northern and eastern regions in Peninsular Malaysia, in relation to the water quality parameters. A total of 720 adult-sized female fish were captured from the northern states (Kedah, Perlis) and eastern states, (Kelantan and Terengganu) between April 2013 and March 2014. Significant differences were found in the water quality parameters, gonadosomatic index (GSI) and sex steroid levels among states. The differences in the seasonal gonad cycle (GSI peaked in September to October in Kedah and Perlis, and November to December in Kelantan and Terengganu) and sex steroid levels (17β-estradiol and testosterone levels peaked in August and April, respectively in Kedah and Perlis states; and in December and July, respectively in Kelantan and Terengganu states) in relation to water quality particularly of a lower water temperature, suggests a direct influence of water temperature on the ovarian activity in female climbing perch of Peninsular Malaysia.

Keywords: *Anabas testudineus*, ovarian activity, 17β-estradiol, testosterone, water quality

1. Introduction

Reproduction is crucial for the survival of all living organisms. In poikilothermic vertebrates such as fish, environmental cues play an important role during their reproductive cycles (Ismail, Siraj, Daud, & Harmin, 2011; Saeed, Reza, Bagheer, & Saeed, 2011; Taghizadeh, Imanpoor, & Mehdinejad, 2013), influencing hormonal secretions, gonadal development and courtship behaviour (Peter & Yu, 1997; Pham, Nguyen, Kjorsvik, Nguyen, & Arukwe, 2012; Zohar, Munoz-Cueto, Elizur, & Kah, 2010).

Changes in the water quality affect the secretion of several sex steroid hormones such as 17β-estradiol and testosterone that are linked to ovulation and spawning in fish (Ismail *et al.*, 2011). Gonadosomatic index (GSI), which is an index of gonad size relative to body weight (Wootton, 1991), provides a quantitative assessment to determine the reproductive season (Gutiérrez-Estrada, Pulido-Calvo, & Prenda, 2000). In addition, ovarian activity and sex steroid

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hormones could give first-hand information on the fish reproductive biology of wild broodstocks (Bernal, Aya, De Jesus-Ayson, & Garcia, 2015; Bromage, Porter, & Randall, 2001). The knowledge would be useful in order to regulate the gonadal development and spawning of fishes in the captivity, through manipulation of the identified environmental factors (Donaldson & Hunter, 1983).

Climbing perch, *Anabas testudineus* is a native freshwater fish species of Asia (Froese & Pauly, 2019, Jayaram, 2010; Pal & Chaudhry, 2010). The market price of climbing perch is high in South Asia and Southeast Asia (Chaturvedi, Ambulkar, Singh, & Pandey, 2015; Das, *et al.*, 2009) due to its good flavor, hence has encouraged the wide spread exploitation of the wild population (Bhattacharyya & Homechaudhuri 2009; Hidayat, Carman, & Alimudin, 2016; Mijkherjee, Praharaj, & Das, 2003; Sarkar *et al.*, 2005; Zworykin, 2012). Despite its economic importance, the biggest challenge facing their ecological restoration is insufficient biological information. This study was therefore designed to describe the ovarian activity (histology and gonadal somatic index) and sex steroid levels (17β-estradiol, testosterone) in female climbing perch in the northern and eastern regions in Peninsular Malaysia, in relation to the water quality parameters (water temperature, pH and dissolved oxygen).

2. Materials and Methods

A total of 720 healthy and disease-free adult-sized female climbing perch *(*15 fish per month per state*)* were obtained from the eastern and northern states of Peninsular Malaysia (Figure 1), namely Kelantan (5.2500°N, 102.0000°E), Terengganu (4.7500°N,103.0000°E); Kedah (6.1283N°, 100.3628°E) and Perlis(6.5000°N, 100.3217°E), from April 2013 to March 2014, covering both dry and rainy seasons. Rainy season that is associated with a higher amount of rainfall usually occurs from June to November and July to December, for the northern and eastern states, respectively. Fish (total length 7.0 to 19.0 cm; total weight 20.45 to 78.13 g) were captured using a net or a fishing rod from three representative habitats (irrigation canal, paddy field and swamp) for each state. The irrigation canals selected are of 1.65 ± 0.09 m water depth, with muddy sandy substrate while the both sides of the canals are dominated by water grasses. Paddy fields are comprised of plot of paddy field with a water depth up to 0.40 ± 0.05 m of soft muddy substrate. The swamp areas selected are of estimated ½ acre with water depth of 1.70 ± 0.12 m, of cloudy water and the substrates are mostly muddy, covered by woody debris. A minimum of three replicate of water samples were collected from each of these habitats and measured *in-situ* for temperature, pH and dissolved oxygen by using a hand-held YSI meter (YSI model 556 MPS, USA).

Fish were immobilized in ice-cold water, measured (total length, cm), and weighed (total body weight, g). Females were determined by manual sexing based on the genital papilla (swollen and pinkish) and confirmed by dissection. Individual blood sample was obtained from the caudal peduncle using a heparinized syringe fitted with a 22-gauge needle, kept in an eppendorf tube on ice. The ovaries were removed, weighed and fixed in 10% formalin prior to histological analysis. Samples were transported to Institute of Tropical Aquaculture and Fisheries Research, Universiti Malaysia Terengganu for further analysis. Individual gonadosomatic index (GSI) was calculated using the formula GSI=Wg/Wt*100; where Wg is the weight of the gonads(g); and W, is the total body weight of the individual (g).

Figure 1. Map of the sampling locations

Plasma samples were obtained following Hosseinzade, Imanpoor, and Aghilinejhad (2012) and Ismail *et al.* (2011). 17β-estradiol (E2) and testosterone (T) were quantified using enzyme-linked immunoabsorbent assay (ELISA) of commercial kit (Cayman Chemical Company, United Kingdom) and the absorbance was measured at 404-420 nm using a microplate reader (Spectramax 190 Beckman Coulter, CA) and analyzed (SoftMax Pro software, Molecular Devices).

Paraffin- embedded ovaries were sectioned at 5µm thickness, stained with hematoxylin and eosin (H&E) (Alam, 2012; Okomoda, Koh, Hassan, Amornsakun, & Shahreza, 2018; Solomon, Ugonna, Olufeagba, & Okomoda, 2017; Suchiang & Gupta, 2011) and microphotographed (40× magnification) using a Nikon Eclipse 80i compound microscope, and oocyte developmental stages were identified (Knight, Butler, Smith, & Wager, 2007; Pears, Choat, Mapstone, & Begg, 2007).

The monthly GSI, sex steroids and water quality parameters obtained were pooled for each state, in order to reduce the inter-habitat differences. These data were subjected to a multivariate analysis of variance (MANOVA), at a significance level of $\alpha = 5\%$, followed by Bonferroni correction for post hoc comparisons. One-way repeated measures of ANOVA (rANOVA) were also applied, however, as the sphericity assumption had been violated ($p<0.05$), Greenhouse-Geisser correction was used, prior to pair-wise comparison. Pearson's correlation test was used to explore the relationship between parameters. All statistical analyses were performed with SPSS version 21.

3. Results and Discussion

The ovary of *A. testudineus* is bilobed and elongated, lying posterior-dorsally in the abdominal cavity. The ovaries were light yellow, thin and small before developing into advanced stage (voluminous, bright yellowish with abundant of capillaries) and later reduced to a smaller size of brown reddish, corresponding to immature, maturation and spent phase.

The immature oocytes were small (mean diameter 46.89 ± 5.80 µm), with centrally located nuclei while multiple nucleoli were seen close to the nuclear membrane. The matured oocytes were larger (mean 502.56 ± 5.17 µm), surrounded by zona radiata, filled with yolk globules but no nuclei could be observed. During the spent phase, many of the follicles were empty while few contained maturing and atretic oocytes (Figure 2).

The monthly variation in the GSI is presented in Figure 3. The GSI (%) at the immature phase, maturation phase and spent phase were between 5.15 ± 0.92% to 10.39 ± 1.35 %; 14.70 ± 1.05 to 17.38 ± 0.96, and 3.14 ± 0.28 to 3.49 ± 0.42, respectively. Highest GSI (%) values were obtained in November and December for Terengganu and Kelantan, and in September and October for Kedah and Perlis states, respectively.

The GSI value reported in the current study encompasses the GSI ranges for climbing perch previously reported by Jacob (2005) and Shashi and Akela (1996) in India. Results of MANOVA based on Wilks' λ criterion showed that, the monthly changes in the GSI levels were significantly affected by the monthly changes in water quality parameters (Table 1). Mean GSI were statistically different ($F_{(1.107, 3.321)} = 13.521$, $p=0.029$) with the values were significantly higher in October 2013 compared to May, June and July 2013. Therefore, water quality has significant effect on the oocyte developmental stage as reflected by the GSI values.

In Vietnam, the GSI peaked during May (Hafijunnahar & Hossain, 2016). Higher GSI correlates with a significant increase in oogenesis (Nichole, 2010). We observed that the majority of the oocytes (>90%) were in the immature phase at a higher water temperature while a lower water temperature was associated with the maturation phase. The highest peaks of GSI is probably associated with a higher amount of the rain fall during rainy season (Figure 6) that reflects a colder water temperature while a lower GSI values were observed during hot and dry period.

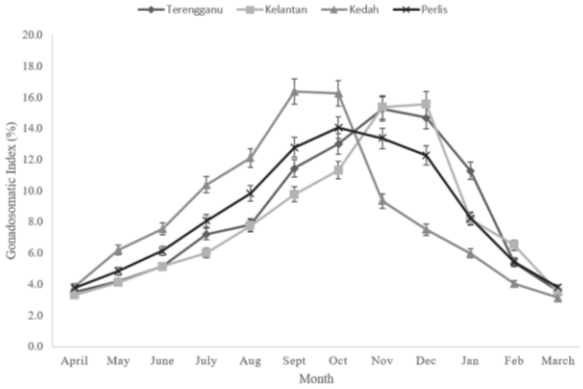
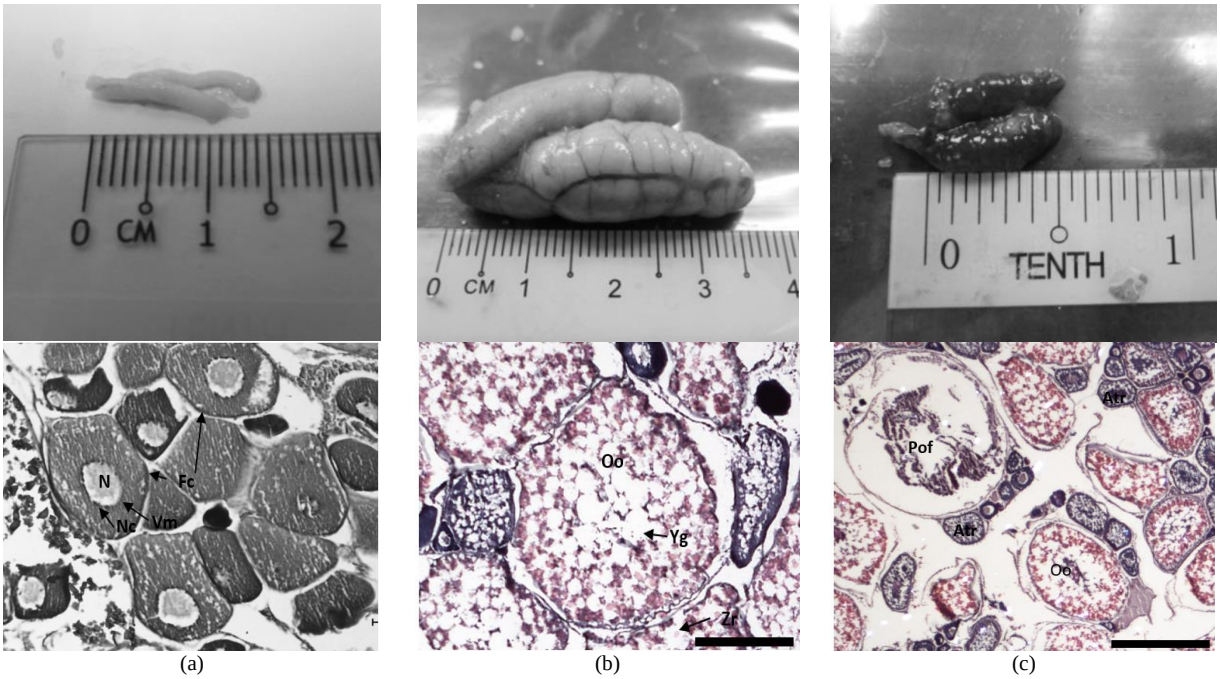


Figure 3. Monthly mean of gonadosomatic index (GSI) in female climbing perch in four sampling states from April 2013 to March 2014



(a) (b) (c)

Figure 2. Ovary of female climbing perch, *Anabas testudineus* at various growth phase (a) Immature phase ovary with early developed oocytes; (b) Maturation phase ovary with large diameter oocyte; (c) Spent phase ovary with empty follicle; Oo=oocyte, N = nucleus, Nc = nucleolus, Fc = follicular cell, Zr = zonaradiata;Yg = yolkglobules, Vm = vitelline membrane; Pof= post ovulatory vesicles; Atr = atretic oocyte; scale bar = 100 µm

Table 1. Results of MANOVA of state and month effects on the water quality based on gonadosomatic index and plasma 17β-estradiol and testosterone in female climbing perch

Parameter	Water quality	Effect	Wilks' λ	F	Hypothesis df	Error df	Sig.	Partial Eta Squared, partial η^2
Gonadosomatic index	Water temperature	State	0.96	0.65	6	190	0.689	0.02
		Month	0.04	32.31	22	190	0.000	0.79
		State*Month	0.08	7.53	66	190	0.000	0.72
	pH	State	0.95	0.81	6	190	0.524	0.03
		Month	0.07	23.24	22	190	0.000	0.73
		State*Month	0.19	3.79	66	190	0.000	0.57
	Dissolved oxygen	State	0.95	0.82	6	190	0.554	0.03
		Month	0.06	25.49	22	190	0.000	0.75
		State*Month	0.19	3.67	66	190	0.000	0.56
17β-estradiol	Water temperature	State	0.81	3.63	6	190	0.002	0.10
		Month	0.04	34.36	22	190	0.000	0.80
		State*Month	0.04	34.36	22	190	0.000	0.80
	pH	State	0.77	4.40	6	190	0.000	0.12
		Month	0.14	14.87	22	190	0.000	0.63
		State*Month	0.17	4.03	66	190	0.000	0.58
	Dissolved oxygen	State	0.79	4.00	6	190	0.001	0.11
		Month	0.13	15.72	22	190	0.000	0.65
		State*Month	0.18	3.95	66	190	0.000	0.58
Testosterone	Water temperature	State	0.80	3.65	6	190	0.002	0.10
		Month	0.05	32.09	22	190	0.000	0.79
		State*Month	0.06	9.44	66	190	0.000	0.77
	pH	State	0.75	5.00	6	190	0.000	0.14
		Month	0.19	11.02	22	190	0.000	0.56
		State*Month	0.21	3.48	66	190	0.000	0.55
	Dissolved oxygen	State	0.79	4.04	6	190	0.001	0.11
		Month	0.20	10.75	22	190	0.000	0.56
		State*Month	0.23	3.16	66	190	0.000	0.52

The highest mean of 17β-estradiol was observed in December and August (Figure 4) while the highest mean of testosterone occurred in July and April (Figure 5) of the eastern and northern states, respectively. The changes in the levels of 17β-estradiol and testosterone were in coherent with the fluctuation in the monthly water quality parameters (Table 1) with the mean 17β-estradiol and testosterone were statistically different ($F(1.243, 13.671) = 25.4088$, $p=0.000$; ($F(1.320, 14.515) = 9.978$, $p=0.004$). Plasma 17β-estradiol fluctuate significantly ($p<0.05$) from April to October 2013 and Dec 2013 to February 2014 while the testosterone levels varies significantly ($p<0.05$) from July to September 2013, and January to February 2014. It is hypothesized that testosterone is a precursor to 17β-estradiol (Chaves-Pozo *et al.*, 2008). Hence, the rise in the level of the former, gradually increases the level of the latter (Ismail *et al.*, 2011). Numerous studies have reported that the ovarian follicular development and gametogenesis is correlated with the sex steroid levels (Rinchard, Kestemont, Kuhn, & Fostier, 1993; Rosenblum *et al.*, 1987; Tamaru *et al.*, 1991). 17β-estradiol stimulates vitellogenesis, so as to produce mature eggs and prepared the female for spawning (Barcellos *et al.*, 2001; Carral *et al.*, 2003; Lee & Yang, 2002). This is in line with our observations that the development and maturation of the oocyte was in synchronization with the changes in 17β-estradiol and testosterone. Similar findings were reported in catfish, *Heteropneustes fossilis* (Lamba, Goswami, & Sundaraj, 1983); gold eye, *Hiodon alosoides* (Pankhurst, Stacey, & Van Der Kraak, 1986); Japanese whiting, *Sillago japonica* (Matsuyama, Adachi, Nagahama, Maruyama, & Matsuura, 1990); Japanese sardine, *Sardines melanostictus*

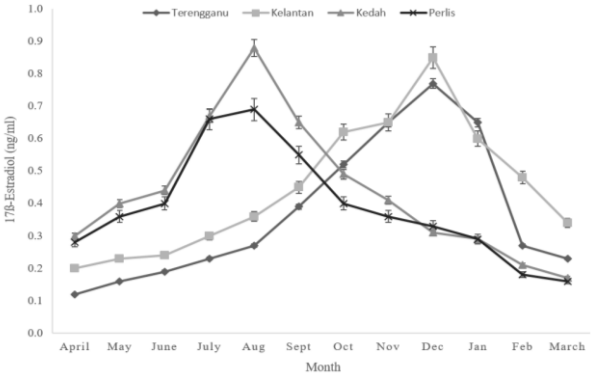


Figure 4. Monthly mean of 17β-estradiol in female climbing perch in four sampling states from April 2013 to March 2014

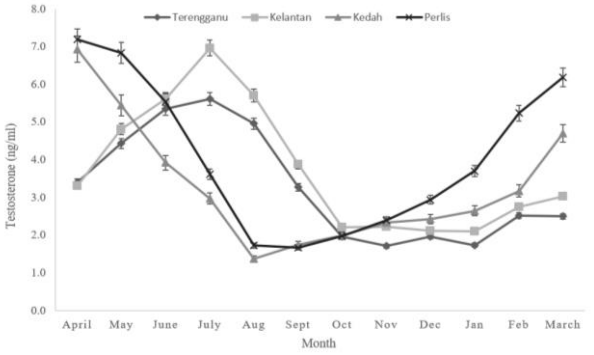


Figure 5. Monthly mean of testosterone in female climbing perch in four sampling states from April 2013 to March 2014

(Matsuyama, Fukuda, Ikeura, Nagahama, & Matsuura, 1991); snook, *Centropomus undecimalis* (Roberts *et al.*, 1999); *Capsian kuntum* (Saeed *et al.*, 2011) and common carp, *Cyprinus carpio* (Taghizadeh *et al.*, 2013).

The monthly mean of water temperature and rainfall, water pH, dissolved oxygen for each state are shown in Figure 6, Figure 7 and Figure 8. In the eastern states, the highest water temperature was recorded in April whereas the lowest water temperature was observed in December. A lower water pH (acidic) was recorded in June while a higher pH (less acidic) was observed in April. The dissolved oxygen was higher in December and February while the lowest was recorded during July. In contrast, a higher water temperature was recorded in February whereas the lowest temperature was observed in September for the northern states. A lower water pH (acidic) recorded in April while a higher water pH (less acidic) was observed in October. The dissolved oxygen was highest during October and lowest during May. The water quality parameters differ significantly ($p<0.05$) among the northern and eastern states with the water temperature varies significantly in June, July, December and January while pH and dissolved oxygen differs significantly in April, August and March; July and February, respectively.

The Pearson correlation coefficient, *r*, (Table 2) indicated that, there is a strong linear relationship between water temperature, 17β-estradiol and GSI ($p<0.05$). The correlation between the GSI with water temperature; and GSI with 17β-estradiol were highly significant ($p<0.05$) with $r > 0.8$ and $r>0.9$, respectively. The GSI showed a negative correlation with water temperature while showing a positive correlation with 17β- estradiol. Environmental cues such as water temperature and daylight dictates the timing of reproductive activity in many tropical (Campos-Mendoza, McAndrew, Coward, & Bromage, 2004; Manosroi, Meng-Umphan, & Manosroi, 2003) and temperate fishes (Estay, Roberto, Nelson, Luis, & Alfredo, 1998; Hoffman, Karin, Deborah, & John, 2008; Katarzyna & Jozef, 2005). The outcome of this study is in contrast with the results reported by Hafijunnahar and Hossain, (2016) that suggest a positive association between the GSI of the climbing perch in Vietnam, with the increased in the water temperature. It is also important to note that from the histological section of the ovaries at the maturation phase and spent phase, few oocytes at different developmental stages were observed *(*data not shown*)*, indicating a possible multiple or asynchronous spawning. Hence, in general, water quality may play some parts if not all, for ovarian activity in climbing perch (Shinsuke, Ito, Kitamura, & Vongvichith, 2009; Sonmoina, Dipanjan, & Kalita, 2011; Uttam, *et al.*, 2005). The ease of accessibility to spawning habitat, availability of food resources and less predation risk associated with a higher amount of rainfall may possibly trigger the ovarian activity of the female climbing perch. In Selangor, a state located in the west region of the Peninsular Malaysia, the breeding season occurs between March to October (Zalina, Saad, Rahim, Christianus, & Harmin, 2012). We suggest that the climbing perch of the northern and eastern states in Peninsular Malaysia is a seasonal spawner as the ovarian activity is higher during September to October for Kedah and Perlis, and between November and December for Kelantan and Terengganu, that correlates with a lower water temperature.

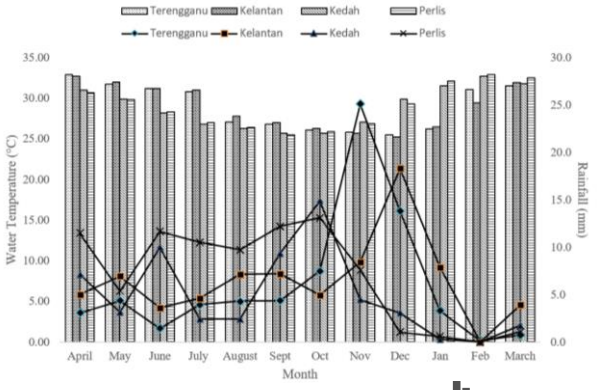


Figure 6. Monthly mean of water temperature (▮) and monthly mean of rainfall (—) in four sampling states from April 2013 to March 2014

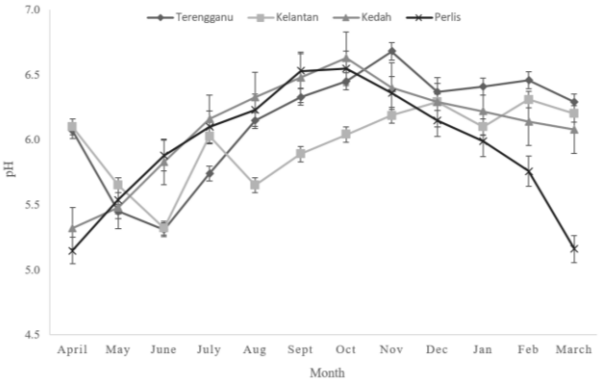


Figure 7. Monthly mean of water pH in four sampling states from April 2013 to March 2014

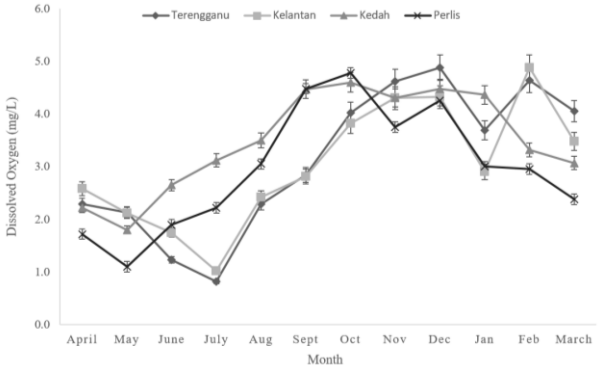


Figure 8. Monthly mean of water dissolved oxygen in four sampling states from April 2013 to March 2014

4. Conclusions

The increased in the ovarian activity of wild female climbing perch, *A. testudineus* of the northern and eastern regions in Peninsular Malaysia, is strongly correlated with a lower water temperature possibly following a higher amount of rainfall. The generated information from this study will provide some guideline for future management, propagation and conservation strategies of wild female climbing perch in Peninsular Malaysia.

Table 2. Pearson correlation coefficient and p-value of gonadosomatic index of female climbing perch in correlation to water quality parameters and sex steroid hormones

	Pearson correlation coefficient, *r*			
	Terengganu	Kelantan	Kedah	Perlis
Water quality				
Water temperature	-0.886 (p= 0.000)	-0.864 (p = 0.000)	-0.884 (p = 0.000)	-0.871 (p = 0.000)
pH	0.535 (p = 0.001)	0.529 (p = 0.001)	0.587 (p = 0.000)	0.461 (p = 0.005)
Dissolved oxygen	0.485 (p = 0.003)	0.397 (p = 0.016)	0.590 (p = 0.044)	0.458 (p = 0.005)
Sex steroid hormones				
17β-estradiol	0.903 (p = 0.000)	0.907 (p = 0.000)	0.918 (p = 0.000)	0.913 (p = 0.000)
Testosterone	-0.451 (p = 0.006)	-0.409 (p = 0.013)	-0.648 (p = 0.000)	-0.587 (p = 0.000)

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