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EFFECTS OF SEASONAL VARIATIONS AND POND AGE ON TRACE ELEMENTS AND THEIR CORRELATIONS WITH PLANKTON PRODUCTIVITY IN COMMERCIAL FRESHWATER CRAYFISH (CHERAX CAINII AUSTIN, 2002) EARTHEN PONDS.

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i. EFFECTS OF SEASONAL VARIATIONS AND POND AGE ON TRACE ELEMENTS AND THEIR CORRELATIONS WITH PLANKTON PRODUCTIVITY IN COMMERCIAL FRESHWATER CRAYFISH (*CHERAX CAINII* AUSTIN, 2002) EARTHEN PONDS.

ii. TRACE ELEMENTS, PLANKTON PRODUCTIVITY IN PONDS

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V. Abstract

Seasonal variations can affect the concentration of trace elements, and the change in their concentrations can affect the natural productivity of freshwater aquaculture ponds. Hence, we studied the seasonal variations of the 12 pre-selected trace elements (Co, Cu, Fe, Mn, Zn, Se, Ca, Mg, P, S, Al, Si) and their relationships with primary and secondary productivity in two aged ponds, stocked with three different life stages of marron (*Cherax cainii*), for a period of one year. Trace element analysis were performed by using (Agilent, ICP-OES). Except Co and Se, all trace elements, primary and secondary productivity were influenced by seasonal variation. The pond age significantly influenced the concentrations of some trace elements. On a seasonal basis, trace elements were positively correlated with the plankton abundance, species diversity, wet and dry plankton weights. Seasonal variations and pond age affected the dissolved concentrations of trace elements and plankton productivity.

Keywords: Trace elements; Phytoplankton; Zooplankton; ICP-OES; Seasonal variations, Semi-intensive marron ponds.

vi. INTRODUCTION

In the south-west of Western Australia marron (*Cherax cainii*, Austin, 2002) is a commercially important species for aquaculture with high market value. Like other aquaculture industries, crayfish farming is also dependent on good water quality (Ackefors, 2000). With other optimum ranged physico-chemical water parameters such as temperature, DO, pH, and ammonia, crayfish have a high requirement for certain minerals (Merrick & Lambert 1991). These trace elements are necessary for crayfish growth, although some are growth inhibitory (Maguire, 1979). In the aquatic environment trace elements are added via natural processes such as, precipitation input, soil degradation, rock disintegration, atmospheric deposition and anthropogenic activities such as mining, urban, agricultural and industrial activities (Arain et al., 2008; Ahmed at al., 2011; Reimer, 1999; Khatri & Tyagi, 2015). Many aquatic organisms assimilate dissolved trace elements directly from the environment (Zhang et al., 2018), including phytoplankton (Baeyens et al., 1998; Hassler et al., 2004), while zooplankton can acquire them through aqueous and dietary ingestion (Yu & Wang, 2002).

Several researchers have explained the importance of trace elements in plankton growth and their role in photosynthesis (Anderson et al., 1978; Egna & Boyd, 1997; Goldman, 2010; Kenneth et al., 1991; Zhihong et al., 2010; Twining & Baines, 2013). Trace element concentration can influence the plankton productivity in aquatic ecosystems (Goldman, 2010; Shi et al., 2013; Jeziorski et al., 2008; Giordano et al., 2005; Giordano & Prioretti, 2016; Ikem & Adisa, 2011; Wallen, 1979). Moreover, their fluctuations can affect the phytoplankton (Zhang et al., 2013; Downs et al., 2008). There is a lack of public and scientific information on the concentrations of trace elements in water, their seasonal variations and effects on plankton productivity in freshwater crayfish ponds. Within a farm, ponds are often treated similarly throughout the season, yet huge differences result in the water colour and yield (personal communication with farmers). These variations in production are not well understood (Abu Hena et al., 2018). Studies that define the relationships among the physical and chemical characteristics of water and plankton in freshwater crayfish ponds are scarce, as is the information regarding the impacts of pond age on water chemistry and plankton productivity. A better understanding regarding the impacts of seasonal variations on trace elements, and their effect on different aspects of the planktons can help to increase the natural productivity of the freshwater crayfish ponds and in turn improve the growth rate of the cultured animal. As marron are largely detritivores and polytrophic in feeding habit (Morrissy, 1979), and natural food produced in ponds may be considered to provide most of marrons micro-nutritional requirements (Morrissy, 1979; Fotedar et al., 2015). With the objective of filling this research gap we investigated the seasonal variations of 12 pre-selected trace elements, plankton abundance, species diversity, their wet and dry weight and the seasonal correlations between trace elements and plankton productivity during four different seasons and in two different aged commercial marron culture ponds.

Material and methods

Sampling site

A commercial marron farm was selected in collaboration with the Marron Grower Association (MGA) of Western Australia (WA) and South West Catchment Council to analyse the dissolved concentration of selected trace elements and their correlations with the natural productivity over the period of one year. The commercial farm, with a capacity of 60 ponds for marron culture is located in the south-west of WA near Manjimup (34°18’75” S, 116°06’61” E). A subsample of 28 ponds was identified based on their location and time of pond construction and utilization. Of these, 12 were defined as old ponds and 16 as new ponds. Each pond had an area of 1000 m^2 with a water depth of approximately 1.5m. Ponds were stocked with three different life stages of marron: nine ponds were stocked with juveniles (3500), nine ponds with brooders (250 male and 800 females with new born juveniles) and the 10 ponds with grow-out: monosex with 40 to 90g adult marron (n~3750), 95 to 130g adult marron +1000 juveniles (n~2700**)** and 135 to 180g adult marron +1000 juveniles (n~2111). This stocking protocol was a commercial protocol followed by the farmer. Marron were stocked in ponds six months before the commencement of sampling.

Sampling and analysis

Water parameters

Sampling was performed seasonally, once every three months, from January to December 2016. Physical and chemical parameters including pH, dissolved oxygen, turbidity, and temperature were recorded on site. For pH an Ecoscan pH 5 meter (Eutech) was used. Turbidity was measured with a secchi disc, with clear ponds defined as having 1.5m visibility. For dissolved oxygen and temperature an Oxyguard digital dissolved oxygen meter (Handy Polaris 2) was used. The water samples for trace element and plankton analysis were collected in 100mL volume containers in two replicated and transported in cold storage to the Curtin Aquatic Research Laboratory (CARL), Curtin University, Bentley, Australia. The water samples for trace elements and plankton studies were filtered and preserved within 24 hours and analysed within a week.

Trace elements

The dissolved concentrations of trace elements in pond water (pre-filtered with 0.45µm Millipore filters to eliminate the suspended particles) were analysed by using Inductively Coupled Plasma Optical Emission Spectrometry (Agilent, ICP-OES, spike recovery limit 80-120%) and standard methods described in (APHA, 2012) to the different detection limits of <0.01mg L^{-1} (Al), <0.005 mg L^{-1} (Ca), <0.002 mg L^{-1} (Co), <0.001 mg L^{-1} (Cu), <0.002 mg L^{-1} (Fe), <0.005 mg L^{-1} (Mg), <0.0002 mg L^{-1} (Mn), <0.02 mg L^{-1} (P), <0.05 mg L^{-1} (S), <0.02 mg L^{-1} (Se), <0.02 mg L^{-1} (Si) and <0.002 mg L^{-1} for (Zn) at Murdoch University, Perth, Australia.

Plankton sampling

Upon collection the phytoplankton samples were preserved with acid Lugol's iodine, and preserved plankton samples were settled and viewed under a compound microscope at 400X. The plankton abundance (no/mL) was estimated using a haemocytometer. The zooplankton samples were filtered and preserved with ethanol, settled and viewed under the dissection microscope to assess the zooplankton abundance (no/mL). The phytoplankton and zooplankton species were identified to the lowest possible taxonomic level using a keys from the manual by Ingram et al., (1997) and a book by Lund (1995). To find the wet weight of phytoplankton and zooplankton, a pump vacuum rocker 300 (SPARMAX TC-501) and Whatman filter papers (GF/C type) having a millipore diameter of 0.47µm were used. The empty filter papers were weighed individually (W_0) and the plankton samples (100ml volume) from the pond were filtered while avoiding contamination to get the wet weight (W_1). Dry weight was obtained by placing the same filter papers with the sample in crucibles which were kept in an oven at 60°C overnight. The next day the crucible was removed from the oven and placed immediately into the desiccator for half an hour, and then the filter paper reweighted until a constant weight (W_2) was achieved. The phytoplankton and zooplankton abundance were calculated by using the equations adapted from Ingram et al., (1997) & Nugroho & Fotedar (2013).

Phytoplankton abundance (ind. L^{-1}) = ((No.X1000)/ (Volume of grid (0.1 mm^3)/No. of grid squares counted) X (Conc. Vol. /1 mL))/Tot. Vol.

Zooplankton abundance (ind. L^{-1}) = (No. X (Conc. vol./Sub. Vol.))/Tot. vol.

Where,

No. = Mean number of individuals counted, Tot. Vol. = Total volume of water (L) collected from pond, Conc. Vol. = Volume of water (mL) containing concentrated zooplankton after sieving (100ml), Sub. Vol. = Sub sample of water (mL) from concentrated volume in which plankton is counted (1mL).

The plankton weights were calculated by the following equations;

Wet weight= $W_1 - W_0$

Dry weight = $W_2 - W_3$

Where, W_0 is wet filter paper weight, W_1 wet filter paper and plankton weight, W_2 is dry filter paper and plankton weight and W_3 is dry filter paper weight.

Animal ethics statement

Animal ethics approval was not required for the experiment.

Analysis of data

Data were analysed using SPSS version 23 and are expressed as means and standard errors. One-way analysis of variance (ANOVA) with LSD post hoc tests were used to determine the significant difference in trace element distribution and plankton parameters between the seasons. Independent t-tests were used to find the significant difference in dissolved trace element concentrations and plankton productivity between old and new ponds. Means of individual trace elements in 12 old aged ponds were compared with the mean of individual trace element in 16 new aged ponds for each season. Kruskal-Wallis test was used when data did not conform to the assumptions of normality or homogeneity of variance. The data were transformed to $\log_{10}$ (x+1) to obtain the consecutive distributions. The correlations were analysed with Pearson and linear regression. Tests were considered statistically significant at $p<0.05$. Multivariate general linear model tests were used to determine the interactions and value $p<0.05$ were considered as significant.

Results

Trace element fluctuations in pond water over the seasons

The dissolved concentration of trace elements in pond water were analysed for four seasons. When two different aged ponds (old and new) were pooled together there was a relatively high variation in the average dissolved concentrations of some of the trace elements from summer to spring. Mg, Cu and Fe showed the greatest variations over the four seasons (Table 1). Co and Se were below detectable levels and Ca and Mg showed the highest concentrations in summer and autumn. There was a significant ($p<0.05$) interaction between seasons and pond age for S and Cu only.

Fluctuations in primary and secondary productivity of the ponds over the seasons

The plankton abundance was significantly higher during the autumn than other seasons (Table 2). The higher phytoplankton species diversity was found in autumn and spring. Throughout the study time, Chlorophyta (green algae) such as, *Closterium* spp., *Scenedesmus* spp., *Spirogyra* sp., *Volvox* spp., *Pandorina* sp., *Pediastrum* sp., *Chlorella* spp.; Euglenoid *Euglena* spp.; Bacillariophyta *Navicula* sp., *Aulacoseira* sp.; and unidentified dinoflagellate species, were found in the pond water samples. Three species of Rotifera: *Branchionus plicalitis*; *Keratella quadrata* and *Keratella cochlearis,* Cladoceran *Daphnia* sp.; *Moina* sp; and Calanoid copepods (both nauplii and adult), were recorded in the pond water samples.

Difference in trace element concentrations between pond age

Mn, Si and S varied significantly between pond age over one year, wherein S was higher in new ponds and Mn and Si were lower in new ponds (Table 3). The trace elements concentration was the same in all ponds irrespective of stocked life stages of marron.

The dissolved trace elements and their safe concentrations to aquatic life

The dissolved concentrations of pre-selected 12 trace elements were compared with their safe concentrations for aquaculture species over the long-term exposure (Table 4). The mean concentrations of trace elements were higher than the mentioned safe concentration. But they were not harmful for the pond biota as the animals are harvested once a year.

Trace elements and productivity in marron ponds

The correlations between the trace elements, primary and secondary productivity in different seasons were evaluated based on pond age and different life stages of stocked marron (Table 5). Zooplankton abundance and phytoplankton abundance showed a positive correlation during summer. Ca, Mg and Zn were negatively correlated with the natural productivity of the ponds.

Physical and chemical parameters

The maximum average temperature of pond water (25.8±0.2 °C) was recorded during summer while the minimum value was recorded during the winter (10.2±0.2 °C) (Table 6). The turbidity of 1.5m deep ponds were essentially very low, with the bottom being clearly visible.

DISCUSSION

Seasonality had a significant effect on most of the pre-selected trace elements, seasonal changes can affect trace element concentrations in the aquatic environment (Akçay et al., 2003; Turgut, 2003). Out of the 12 trace elements studied, only Se and Co were below the detection limits while the other 10 trace elements showed seasonal fluctuations (Table 1). The seasonal fluctuations of trace elements can largely be credited to environmental factors, including temperature, evaporation and rainfall and plankton dynamics (Adhikari & Ayyappan, 2004; Dickson,1975; Gibson et al., 2000; Hecky et al., 1986; Kimball, 1973; Sierp & Qin, 2001; Zhang et al., 2013). Farming practices, such as liming, water sources, feeding, fertilization, harvesting, cleaning the pond bottom soil at harvest and overnight drying may also have an effect (Adhikari, 2003; Boyd, 1997; Borg et al., 2001; Christos et al., 2018; El-Saharty, 2014; Abu Hena et al., 2018; Shaked et al., 2004). Furthermore, the fluctuations in plankton abundance, and phytoplankton species diversity (Table 2) can relate to changes in the concentrations of trace elements, sunlight hours, and temperature (Calijuri et al., 2002; Sommer et al., 2012). Also as shown by Van Nguyen & Wood, (1979) & Papst et al., (1980). These authors suggested that the phytoplankton communities collapse with nutrient depletion, as algal production is dependent on nutrients, and environmental changes such as low water temperature and cloudy weather. The south-west of WA has four distinct seasons. The summer and winter can be considered extreme weathers, whereas autumn and spring can be grouped together in terms of temperature regimes. In south-west of WA, where the majority of marron farms in Australia are located, also experiences winter rain and high evaporation during the summer months.

There appeared to be the build-up of macronutrients, such as P, over the culture period, before decreasing after the refilling of ponds. The build-up of uneaten feed on the pond bottom can cause increases in P concentration, for example, in tiger shrimp (*Penaeus monodon*) culture ponds (Malaysia), the cause for the increase in P concentration was the settling of suspended particles and uneaten feed during the culture period, with feed being the key source of P (Abu Hena et al., 2018). The highest concentration of P, Si, Zn and Cu occurred during autumn, heating up of upper sediments by overlying water in summer clarified the seasonal pattern of dissolved Si flux from sediments which increased during the autumn, in shallow lakes in central Canada (Hecky et al.,1986). High temperatures, pH and increased invertebrate activities promotes the dissolution of diatom silica in summer, which contributes to high dissolved Si levels by autumn (Dickson, 1975; Gibson et al., 2000). Research suggests that an increase in silicate concentration corresponds approximately with that in phosphate concentration (Heron, 1961). Contrary to our findings, El-Saharty (2014), found a reduction in phosphate concentration during the autumn, due to an increase in uptake of phosphate by phytoplankton. P was decreased due to the presence of phytoplankton in a study by Sierp & Qin (2001). P was positively correlated to plankton abundance, species diversity, phytoplankton wet weight and zooplankton dry weight.

In the aquatic environment, Ca and Mg are naturally available and can also be added through feed. There is a least information available on the seasonality of Mg in freshwater ponds compared to other studied elements. Over the seasons, the Mg concentration was lower than the Ca concentration and a decrease in Ca may have caused the decrease in Mg, as in water Mg is often associated with Ca and its concentration generally remains lower than Ca (Venkatasubramani & Meenambal, 2007). There is a least possibility of trapped elements getting discharged into the water in winter, a decline in exchangeable Ca concentration from soil can cause a decrease in the Ca concentration in water (Jeziorski et al., 2008). Harvesting and refilling of the ponds may have reduced the concentration also the Ca absorption by marron, as aquatic crustaceans obtain most of their required Ca from the surrounding water (Robertson, 2002).

Irrespective of pond age and life stage of marron, no trace elements exhibited any relationship with natural productivity, possibly due to the high inbuilt variability caused by farm management procedures and the high variability between ponds of different age, location and life stage cultured. However, when ponds were categorised into two age groups and three life stages, natural productivity showed significant relationships with various trace elements (Table 5). Zn and Cu showed a similar pattern in correlations with the natural productivity, likely due to its synergistic behaviour with Cu to inhibit photosynthesis in plants and phytoplankton species (Goldman, 2010). Both trace elements showed positive and negative correlations with the natural productivity. Cu plays a dual role in the metabolism of plants or algae, as a micronutrient and a toxicant (El-Shobashi, 1991; Downs et al., 2008). Cu was positively correlated to phytoplankton abundance, but negatively correlated to the wet weight of phytoplankton, relating to its higher concentration during autumn, as it inhibits algae cell division and photosynthesis at high levels (Goldman, 2010). Cu concentration during the autumn may be high enough to be toxic for some plants (Riley, 1939). High levels of free Cu and low levels of Fe are harmful for phytoplankton (Sunda et al., 1981) and can reduce growth and reproduction in plants (El-Saharty, 2014).

High Fe, Al and Mn concentration in water can be harmful for the crayfish farming, in Sweden the crayfish ponds with >0.5 mg^{-1} Fe and Mn were forced to reduce the concetration in supplying water (Ackefors, 2000). In current study Fe and Mn concetratioon were ranged between 0.03 to 0.1 mg^{-1}and 0.002 to 0.005 mg^{-1} respectively. Fe showed a positive correlation with the phytoplankton abundance during summer. It's average concentration fluctuated greatly, and increased during winter and spring, which could have been due to the Fe leaching from pond bottom sediments during the draining-down of these ponds. When the ponds were emptied a red coloured silt was observed at the bottom, indicating high Fe content in the sediment. In aquatic environments, leaching of bedrock is one of the main reasons for release of trace elements into the water (Ahmed et al., 2011; Khatri & Tyagi, 2015). Al average concentration was higher during the spring than the safe concentration for aquatic life or aquaculture (Table 1 & 4). But the concentration was not toxic to pond biota as Al is more soluble and potentially toxic to freshwater biota if the pH decreases below 6.0 (Gensemer & Playle, 1999), and the pH of marron pond water was higher than 7.8 throughout the year. All the physical and chemical water parameters showed seasonal variations (Table 6) and were within the optimum range for marron (Cole et al., 2019).

The information regarding the effects of Mn concentration on primary productivity and it's relationships with plankton in aquaculture ponds is scarce. Mn naturally occurs in rocks, soil, and sediment, and is weathered into ground and surface water; resulting in various levels of Mn in natural waters (Reimer, 1999). It was positively correlated with the phytoplankton abundance, wet weight, dry weight and zooplankton wet weight. Mn form of superoxide dismutase is common in diatoms

(Wolfe-Simon et al., 2005). The growth of diatoms is dependent on Si, to grow the siliceous cell walls (Boyd, 2014). Si was positively correlated to phytoplankton species diversity and zooplankton abundance. Also, the Mn and Si concentrations were higher in old ponds (Table 3) Abu Hena et al., (2012) observed a higher Mn concentration in old ponds (*P. monodon*, Malaysia), author suggested the cause was age of ponds, liming activities and sediment leaching. In case of marron ponds the limestone was added only into the new ponds at the start of ponds utilization.

Ca and Mg, vital for aquatic plants and animals, showed negative correlations with the plankton species diversity and zooplankton wet weight. Our output of these negative correlations can be supported by the studies of Nasser & Sureshkumar, (2013) on interaction between microalgal species richness and environmental variables, where Chlorophyceae showed a negative correlation with Mg and Ca. Hulyal & Kaliwal, (2009) the abundance of Cyanophytes was negatively correlated with Ca and Mg. In a study by, Castillo-Soriano et al., (2010) chlorophyll *a* was negatively correlated with the Ca concentration in intensive culture of white leg shrimp (*Litopenaeus vannamei*). More research is needed to clarify the impacts of Ca on different aspects of primary production (Shi et al., 2013).

The seasonal variations of trace elements can significantly influence the algal growth (Fayissa, 2013; Talling, 1986). Our study suggests that the favourable season for plankton abundance and species diversity was autumn (March) (Table 2), which could be due in part to the lower volume of water present in the ponds due to the evaporation during the summer, causing higher dissolved concentrations of correlated trace elements. The phytoplankton abundance ranged from 24.1 x 10^4 to 290.8 x 10^4, showing the highest abundance after the warmer days, during autumn (Table 2 & 6). Affan et al., (2005) also observed that the period from February to March was the favourable time for phytoplankton in ponds (Bangladesh), because of the low volume of water, high solar radiation, rising temperature and suitable levels of nutrients favouring rapid cell multiplication. The plankton abundance, and phytoplankton species diversity were lower during the winter, which may be due to the draining of ponds in winter or the calm weather, less sunlight and lower temperatures. Phytoplankton often die with calm weather (Boyd, 1982) that reduces mixing with bottom water, which is an important nutrient source for phytoplankton growth in stratified systems (Margalef, 1978). The highest zooplankton abundance during the autumn shows that the phytoplankton abundance could have supported the zooplankton abundance. The phytoplankton abundance and chlorophyll *a* concentration are important factors for zooplankton abundance, zooplankton abundance is entirely dependent on phytoplankton abundance (Martinez-Cordova et al., 1998; Preston et al., 2003; O'Brien & de Noyelles, 1974).

The plankton productivity was significantly higher in old ponds (Figure 1), possibly due to the Mn and Si higher concentrations (Table 3) in old ponds, as both trace elements were positively correlated to plankton productivity. The phytoplankton abundance, species diversity and wet weight were significantly higher in old ponds. There was a significant interaction between season and pond age for zooplankton abundance ($p<0.05$). Where Zooplankton abundance and zooplankton dry weight was significantly higher in old pond during autumn and spring respectively. These results were similar to the findings of Cole et al., (2019) where author found the phytoplankton abundance, species diversity and zooplankton abundance were higher in old ponds. In contrast to our result, Abu Hena, (2005) reported that the new culture ponds had greater phytoplankton growth than old ponds, attributed to the higher Ca concentration in old ponds, leading to a higher rate of Ca- phosphate precipitation, in turn resulting into less availability of phosphate in the water to support plankton growth (Abu Hena et al., 2018).

CONCLUSIONS

The results demonstrated the effects of seasonal variations and pond age on trace elements and natural productivity, and the effects of seasonal variations of trace elements on the abundance of plankton in freshwater crayfish ponds. Dissolved concentrations of Co and Se were below the detectable limit, while Zn showed the most seasonal fluctuation among the trace elements. The plankton productivity was higher during the autumn and was also higher in old ponds than new. The plankton productivity of the ponds was mostly influenced by three trace elements, Mn, Si and P. These trace elements play a major role in the primary and secondary productivity of ponds as higher phytoplankton abundance can likely support higher numbers of zooplankton. The statistical analysis demonstrated that interaction between zooplankton and seasonality is strongly related to the pond age. Laboratory experiments with the trace elements addition should be performed to better understand their effect on plankton growth and species diversity.

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Conflict of interest

The described work is a part of PhD course of Miss. Smita Sadanand Tulsankar, with no conflicts of interest related to this article.

viii. REFERENCES

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ix. Table -1. Fluctuations of trace elements in all ponds during different seasons (mg L^{-1}).

Trace elements	Summer	Autumn	Winter	Spring
Ca	29.6±1.1^{b}	29.9±0.8^{b}	21.0±0.7^{a}	19.2±0.8^{a}
Mg	21.7±0.40^{c}	21.9±0.40^{c}	16.2±0.50^{b}	10.6±0.40^{a}
S	6.9±0.5^{b}	5.9±0.5^{ab}	5.8±0.3^{a}	5.1±0.3^{a}
Si	0.52±0.08^{a}	0.98±0.12^{b}	0.64±0.09^{a}	0.45±0.09^{a}
Al	0.05±0.01^{a}	0.08±0.01^{a}	0.20±0.05^{a}	0.40±0.07^{b}
Fe	0.03±0.03^{a}	0.05±0.05^{a}	0.08±0.02^{b}	0.1±0.02^{c}
P	0.01±0.001^{a}	0.05±0.001^{b}	0.01±0.001^{a}	0.01±0.005^{a}
Cu	0.008±0.0020^{b}	0.020±0.0020^{c}	0.001±0.0003^{a}	0.001±0.0002^{a}
Mn	0.003±0.001^{ab}	0.005±0.001^{b}	0.002±0.001^{a}	0.005±0.001^{b}
Zn	0.002±0.0003^{a}	0.02±0.0200^{b}	0.001±0.0001^{a}	0.005±0.0005^{b}
Se	ND	ND	ND	ND
Co	ND	ND	ND	ND

The values in the same row with different superscripts are significantly different ($p<0.05$) between the seasons. Data represented as (Mean ± SE): n=28. A P concentration in ponds of <0.02 mg L^{-1}, was recorded as 0.01mg L^{-1}; while a Cu concentration of <0.001mg L^{-1} was recorded as 0.0009 mg L^{-1}.

Table 2. Plankton abundance, species diversity, wet weight and dry weight in different seasons.

Plankton factors	Summer	Autumn	Winter	Spring
Phytoplankton abundance (10^{4} individuals/L)	24.1 ±7.85^{a}	290.8 ±41.3^{b}	90.8 ±21.2^{a}	136.9.3 ±14.7^{a}
Phytoplankton species diversity (Number of species/mL)	2.64 ±0.22^{a}	3.32 ±0.27^{b}	2.54 ±0.24^{a}	4.00 ±0.32^{b}
Phytoplankton wet weight (g/100mL)	0.04±0.01	0.05±0.01	0.02±0.01	0.06±0.01
Phytoplankton dry weight (mg/100mL)	2.0 ±0.4^{ab}	2.0 ±0.4^{a}	3.0 ±0.4^{b}	2.0 ±0.6^{a}
Zooplankton abundance (Ind/L)	88.7 ±16.5^{b}	277.9 ±27.3^{d}	192.8 ±26.0^{c}	26.5±4.4^{a}
Zooplankton species diversity (Species number/mL)	2.6 ±0.24^{b}	3.2 ±0.10^{c}	3.3 ±0.14^{c}	1.9 ±0.15^{a}
Zooplankton wet weight (g/100mL)	0.01 ±0.0004^{ab}	0.01 ±0.0004^{a}	0.01±0.0005^{b}	0.004 ±0.0007^{c}
Zooplankton dry weight (mg/100mL)	0.3 ±0.0^{bc}	0.4±0.1^{c}	0.2 ±0.0^{ab}	0.03 ±0.1^{a}

The values in the same row with different superscripts are significantly different ($p<0.05$). Data represented as (Mean ± SE): n=28.

Table 3. Trace elements concentrations in different aged pond water (mg L^{-1}).

Trace elements	Old ponds	New ponds
Ca	23.6±0.9^{a}	25.9±1.0^{a}
Mg	18.5±0.9^{a}	17.0±0.6^{a}
S	4.6±0.2^{a}	6.9±0.3^{b}
Si	0.86±0.1^{a}	0.49±0.1^{b}
Al	0.16±0.03^{a}	0.19±0.03^{a}
P	0.03±0.01^{a}	0.01±0.001^{a}
Fe	0.08±0.01^{a}	0.06±0.01^{a}
Cu	0.006±0.001^{a}	0.007±0.001^{a}
Mn	0.006±0.000^{a}	0.002±0.000^{b}
Zn	0.003±0.000^{a}	0.009±0.006^{a}
Se	ND	ND
Co	ND	ND

The values in the same row with different superscripts are significantly different ($p<0.05$) between pond age. Data represented as (Mean ± SE); (n=28).

Table 4. Means (mg L^{-1}) and standard error of total trace elements in water and their safe concentration for freshwater aquaculture species.

Trace elements	Average mean (mg L^{-1}) ± Std. error	For aquaculture mg L^{-1}
Ca	24.91±0.6	NF
Mg	17.62±0.5	<15[1]
Si	0.65±0.05	NF
Al	0.17±0.025	<0.03 (pH>6.5)[1]
P	0.02±0.006	<0.1[2]
Fe	0.07±0.007	<0.01[1]
Mn	0.004±0.001	<0.01[1,3]
Zn	0.007±0.004	<0.005[1]
Cu	0.007±0.001	<0.005 (varies with hardness)[2]
S	5.9±0.20	NF
Se	ND	<0.01[1]
Co	ND	NF

ND= Not detectable (Dissolved concentration were below the detectable level); NF= Not found in literature; 1: Meade, (1989); 2: DWAF (1996); 3: Zweig et al., (1999).

Table 5. Correlations between trace elements and plankton abundance, species diversity, wet weight and dry weight in marron ponds. This table shows only the seasons where significant correlations were observed.

Plankton parameters	Seasons	Pond age & Stocked life stage				
		Old	New	Juvenile	Grow-out	Brooder
Phytoplankton abundance	Summer	-	Mn & Fe ($^{+}$)	Mn ($^{+}$)	Mn, Fe, Al & ZA ($^{+}$)	Zn & Cu ($^{+}$)
	Autumn	-	-	-	-	S ($^{+}$)
	Winter	-	-	-	P, Zn & Cu ($^{+}$)	Mn (+)
Phytoplankton species diversity	Summer	-	-	Mg & Zn ($^{-}$)	-	-
	Autumn	-	-	P ($^{+}$)	-	-
	Winter	-	-	-	Si ($^{+}$)	-
Phytoplankton wet weight	Autumn	Cu ($^{-}$)	-	-	-	-
	Winter	-	-	-	Mn ($^{+}$)	P ($^{+}$)
Phytoplankton dry weight	Autumn	-	-	-	-	Al ($^{+}$)
	Winter	-	-	-	-	Mn ($^{+}$)
Zooplankton abundance	Summer	Zn ($^{-}$) & PA ($^{+}$)	-	S ($^{+}$)	Si ($^{+}$)	P ($^{+}$)
	Autumn	-	-	-	-	P ($^{+}$)
Zooplankton species diversity	Summer	-	-	PSD ($^{+}$)	-	PSD ($^{+}$)
	Autumn	P ($^{+}$)	-	-	-	-
	Winter	-	-	-	Mg ($^{-}$)	Ca ($^{-}$)
Zooplankton wet weight	Winter	-	-	Mg ($^{-}$)	Mn ($^{+}$)	-
Zooplankton dry weight	Autumn	P ($^{+}$)	-	-	P ($^{+}$)	Al & PDW ($^{+}$)
	Spring	-	-	-	-	P ($^{+}$)

The same row content shows positive ($^{+}$) and negative ($^{-}$) correlations in the different seasons ($p<0.05$ and $p<0.01$ level) and significant correlations ($r^2 \geq 0.72$), ($n=28$). Where, PA= Phytoplankton abundance, PSD= Phytoplankton species diversity, ZA= Zooplankton abundance, PDW= Phytoplankton dry weight.

Table 6. Physical and chemical parameters measured over the study period for all ponds.

Seasons / Parameters	Summer	Autumn	Winter	Spring
Temperature (ºC)	25.8 ± 0.2^{d}	13.4 ± 0.4^{b}	10.2 ± 0.2^{a}	17.8 ± 0.6^{c}
Dissolved oxygen (mg L^{-1})	11.2 ± 0.2^{b}	10.4 ± 0.2^{a}	11.3 ± 0.2^{b}	11.0 ± 0.1^{b}
pH	7.9 ± 0.07^{ab}	8.0 ± 0.03^{bc}	8.1 ± 0.06^{c}	7.8 ± 0.07^{a}
Turbidity (m)	0.76 ± 0.09^{a}	0.70 ± 0.07^{a}	0.89 ± 0.09^{a}	1.20 ± 0.08^{b}

The values in the same row with different superscripts are significantly different ($p<0.05$). Data represented as (Mean ± SE), ($n=28$).

x. Pond age and primary and secondary productivity of all ponds

Plankton producticity were compared on the basis of pond age. Plankton abundance, phytoplankton species diversity and wet weight were higher in old ponds than in new ponds (Fig. 1). There was a significant interaction between season and pond age for zooplankton abundance ($p<0.05$).

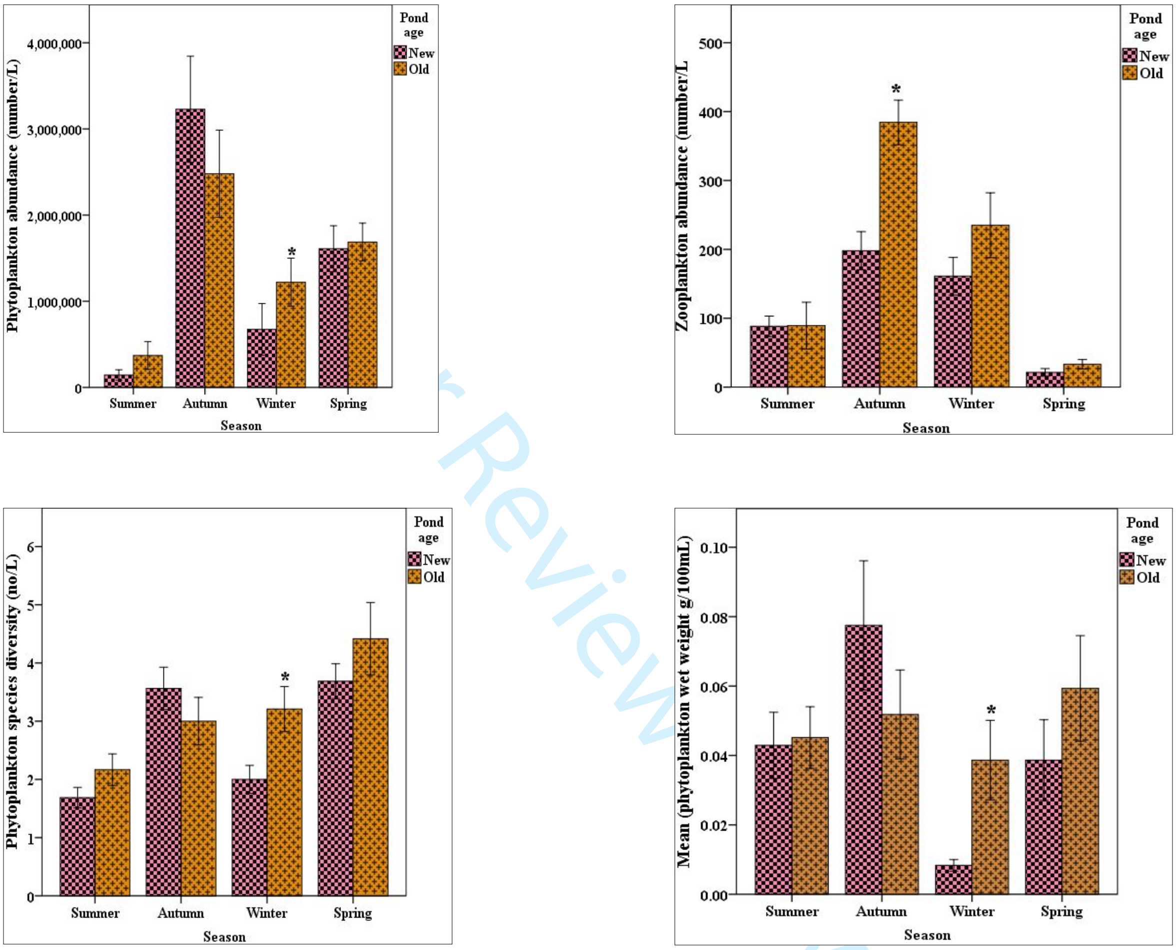

Fig 1. Plankton abundance, phytoplankton species diversity and wet in old and new ponds over the seasonal variation * shows the significant difference ($p<0.005$) between pond age. ($n=28$).