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Artikel Asli/*Original Articles*

Morphometric and Gravimetric Indices of Two Populations of Rice Frog (*Fejervarya limnocharis*) Naturally Exposed to Different Environmental Cadmium Levels

(Indeks Morfometrik dan Gravimetrik Dua Populasi Katak Sawah (*Fejervarya limnocharis*) yang terdedah Secara Semulajadi pada Aras Kadmium Persekitaran yang Berbeza)

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ABSTRACT

In this study, morphometric and gravimetric indices were used to determine the impact of cadmium exposure on a sentinel species. Scaling coefficient, condition factor (CF), hepatosomatic index (HSI), renosomatic index (RSI) and gonadosomatic index (GSI) were compared between rice frogs (Fejervarya limnocharis) exposed to different environmental cadmium levels. The result showed that frogs caught from the contaminated site had significantly higher CF (10.296), RSI (0.413) and female GSI (7.594) than frogs from the contaminated site (7.594, 0.380 and 1.594, respectively). For Scaling Coefficient and HSI, albeit being statistically insignificant, these indices showed a similar trend. On the other hand, the male GSI showed a reverse trend where frogs from contaminated site showed higher values than their counterpart from the reference site. However, the differences were not statistically significant. This research concluded that there is a relationship between cadmium contamination with morphometric and gravimetric indices. Therefore, it is suggested that the use of these data could give an idea on the effect of cadmium exposure on the rice frog.

Keywords: Fejervarya limnocharis; morphometric indices; gravimetric indices; cadmium; Tak Province

ABSTRAK

Dalam kajian ini, indeks-indeks morfometrik dan gravimetrik telah digunakan untuk menentukan impak dedahan kadmium terhadap spesis sentinel. Scaling coefficient, condition factor (CF), indeks hepatosomatik (HSI), indeks renosomatik (RSI) dan indeks gonadosomatik (GSI) telah dibandingkan antara katak sawah (Fejervarya limnocharis) yang terdedah kepada aras cadmium sekitaran yang berbeza. Hasil menunjukkan bahawa katak yang ditangkap dari kawasan rujukan secara signifikannya mempunyai nilai CF (10.296), RSI (0.413) dan GSI betina (7.594) yang lebih tinggi berbanding katak dari kawasan tercemar (7.594, 0.380, 1.597). Untuk Scaling coefficient dan HSI, walaupun tidak berbeza secara signifikan, indeks-indeks ini menunjukkan trend yang serupa. Sebaliknya, GSI jantan menunjukkan trend yang berlawanan di mana katak dari kawasan tercemar mempunyai nilai yang tinggi berbanding katak dari kawasan rujukan. Walau bagaimanapun, perbezaan ini adalah tidak signifikan. Kajian ini menyimpulkan bahawa wujud hubung kait antara pencemaran cadmium dengan indeks-indeks morfometrik dan gravimetrik. Oleh yang demikian, adalah dicadangkan bahawa penggunaan data-data ini boleh member gambaran mengenai kesan dedahan kadmiumter hadap katak sawah.

Kata kunci: Fejervarya limnocharis; indeks morfometrik; indeks gravimetrik; kadmium; Wilayah Tak

INTRODUCTION

Morphometric and gravimetric data has always been a good indicator of the effect of pollution on an organism. Often, pollutant has a direct effect on growth of an organism. According to Monserrat et al. (2007), morphological and physiological alterations have been reported in individuals exposed either naturally or experimentally to different pollutants. Kitana et al. (2007) reported that morphometric analysis was used to compare site-related differences in body size. For instance, in a study by Norris et al. (2000), it was found that the hepatosomatic index of trout living in uncontaminated site was greater than those living in sites contaminated by cadmium and zinc. The same study also reported that there was an association between high renal cadmium with low hepatosomatic index in many fish species. Maes et al. (2005) reported that recent studies have established relationships between metal toxicity with changes in gravimetric indices of European eel, especially hepatosomatic index. Therefore, the significance of assessing biometric response (weight, condition, growth) has been suggested as an important measure of pollution impact on an organism (Van Straalen & Timmermans 2002).

Over the years, weight-length relationship and condition factor has been used to assess the well-being of fish species. The weight-length relationship can be used to reflect the impact of the environment on the growth of an organism. From this relationship, the value of Scaling coefficient is able to be determined. Eastwood and Couture (2002) stated that the Scaling coefficient is a descriptor of growth pattern of a specific population or organisms. The Scaling Coefficient has been proposed to be used as a bioindicator for long term stress in populations subjected to environmental pollution. As for condition factor (CF), Bervoets and Blust (2003) stated that it is used to express the overall wellbeing of an individual. Hence, CF is a useful tool to assess the effect of pollution on individuals. In addition, Urena et al. (2007) mentioned that condition factors are indicative of overall health and therefore, are good candidate to be considered when studying the effect of metal exposure. A study by Hansen et al. (2006) revealed that the condition factors are lower in metal-exposed population as compared to reference population which is an indicative of the effect of metal exposure on fitness of fish. Maes et al. (2005) also stated that there is a clear relationship between increased heavy metal content with lower condition factor. The use of these parameters in *F. limnocharis* is novel because it has never been used in amphibians before

In this study, *F. limnocharis* has been chosen to be a sentinel species for cadmium contamination based on a few criteria, including 1) not threatened by extinction 2) ubiquitous 3) of suitable size 4) has large distribution area and 5) has a stable population (IUCN, Conservation International and Nature Serve 2006). This study aimed to compare the morphometric and gravimetric indices of two populations of rice frogs exposed to different environmental cadmium levels.

MATERIALS AND METHOD

Frogs were caught live at night time during visual encounter survey (Crump & Scott 1994) and were collected on a monthly basis during November 2007 and October 2008 from several rice fields in Mae Tao and Mae Pa in Mae Sot District, Tak Province (Figure 1). The contaminated site, Mae Tao, was located at 16o45'13"N; 98o35'25"E. This area is irrigated by the Mae Tao Creek. Simmons et al. (2005) reported that there were elevated cadmium levels in the paddy soils and rice grain in vicinity of Mae Tao Creek downstream of a zinc mining area. The reference site, Mae Pa, was located 8.4 km north of the contaminated site at 16o40'43"N; 98o35'36"E. The area is irrigated by Huay Luek Creek and not on the path of the cadmium contamination plume. Preliminary analysis showed that the cadmium concentrations were 0.0007 mg/L (water) and 0.0988 mg/kg (sediment) at the reference site. The concentration at the contaminated site was 0.0015 mg/L (water) and 1.0110 mg/kg (sediment).

Even though samples were collected on monthly basis, all data were analysed on a tri-monthly basis. Data from all twelve months were pooled into four groups based on the average total rainfall of each groups as follows:

Early dry season:	November 2007 – January 2008
Late dry season:	February 2008 – April 2008
Early rainy season:	May 2008 – July 2008
Late rainy season:	August 2008 – October 2008

The seasonal average rainfall was obtained from World Meteorological Association (2007). Seasons of rain and dry were chosen because while *F. limnocharis* in this area are essentially continuous breeders, they more optimized for cyclic reproduction mode with two breeding cycles during the rainy season (Othman et al. 2011). Therefore, the rainy season would also determine their energy budget and food consumption.

The frogs were transported live to the lab where they individually subjected to cold anaesthesia procedure before sacrificed by double-pith at the brain and spinal cord (Tharp & Woodman 2002). Then the frogs were weighed and their snout-vent lengths (SVL) were weighed. Each frog was then biopsied and their livers (n = 206), kidneys (n = 206), ovaries (n = 94) and testes (n = 111) were removed and weighed. The organs were placed in plastic bags and then frozen until further analysis. The weight and the snout-vent length data (n = 647) were used to determine Scaling Coefficient and condition factor. Scaling Coefficient was calculated from the extrapolation of the logarithmic transformed weight-length relationship by linear regression. The logarithmic-transformed weight-length relationship was also used to determine condition factor of each population. Hepatosomatic index (Loumbourdis & Vogiatzis 2002), renosomatic index and both male and female gonadosomatic indices (Goodwin et al. 1992; Tilton et al. 2003; Maitra et al. 2007) were calculated for each frog. These indices were based on the weight ratio of each tissue (liver, kidney, testis and ovary) to the body weight.

All data were statistically analysed with two-way ANOVA and Student-Newman Keuls test using the SigmaStat 2.0 program.

RESULTS AND DISCUSSION

We have previously reported that the cadmium concentrations in water and soil samples from Mae Tao were significantly higher than samples from Mae Pa (Othman et al. 2009). In addition, we also found that the result also showed that frogs from contaminated site had significantly higher hepatic, renal and gonadal cadmium levels than frogs from reference site.

Figure 2 shows that the quarterly average Scaling Coefficients of *F. limnocharis* caught from the sampling sites in Mae Pa range from 2.783 to 3.223. For Mae Tao, the range extends from 2.762 to 2.978. However, the results are not statistically significant when compared between

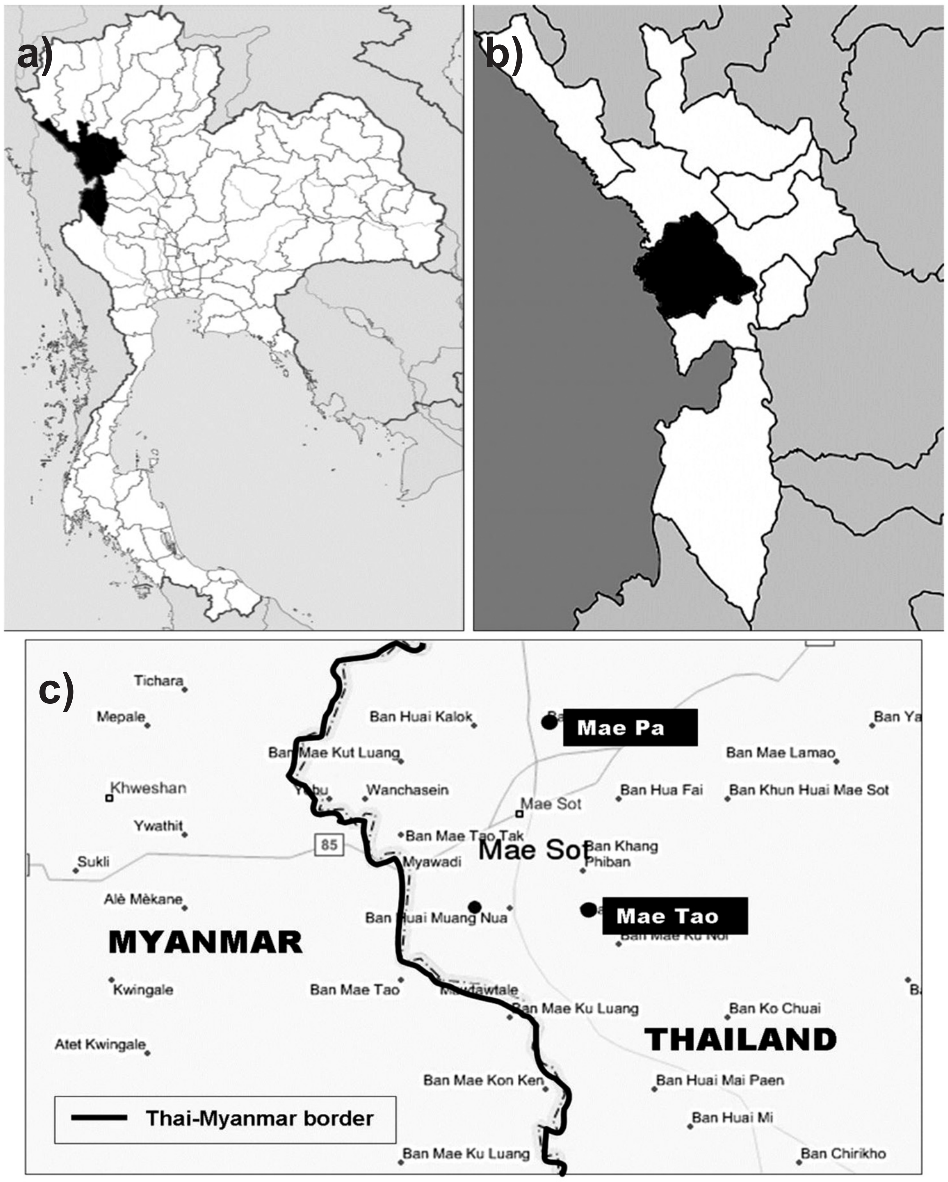

FIGURE 1. A Thailand map showing the geographic location of a) Tak Province; b) Mae Sot district; and c) sampling sites of Mae Pa (reference site) and Mae Tao (contaminated site)

seasons ($p = 0.053$) dan between stations ($p = 0.082$) The result also shows that in both sites, the highest Scaling Coefficients are recorded from frogs caught during the late rainy season. The lowest is found in frogs caught during the late dry season. The figure also shows that both Mae Pa and Mae Tao exhibit similar trend of Scaling coefficient seasonal fluctuation. The overall average Scaling Coefficient for frogs caught from Mae Pa is 2.975, while for Mae Tao, the overall average value is 2.856.

In Figure 3, the result shows that condition factors of *F. limnocharis* caught from Mae Pa range from 9.781 to 11.093 while for those caught from Mae Tao, the range is from 9.511 to 9.931. All mean differences between seasons and stations are statistically significant ($p < 0.001$). In Mae Pa, the frogs have the highest condition factor during the late rainy season and the lowest condition factor during the early rainy season. However in Mae Tao, the highest

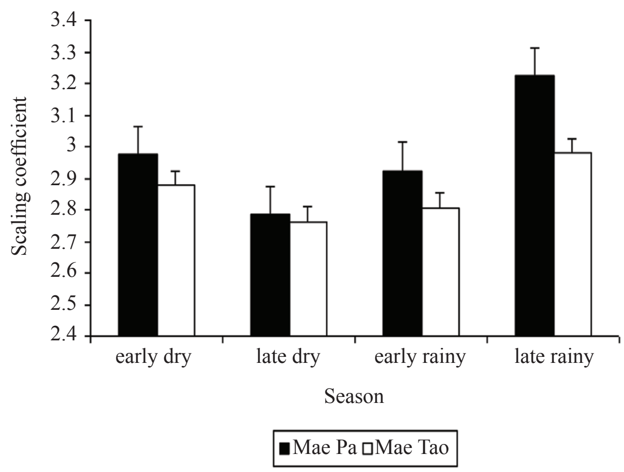

FIGURE 2. Quarterly average Scaling Coefficient of *F. limnocharis* caught from Mae Sot, Tak

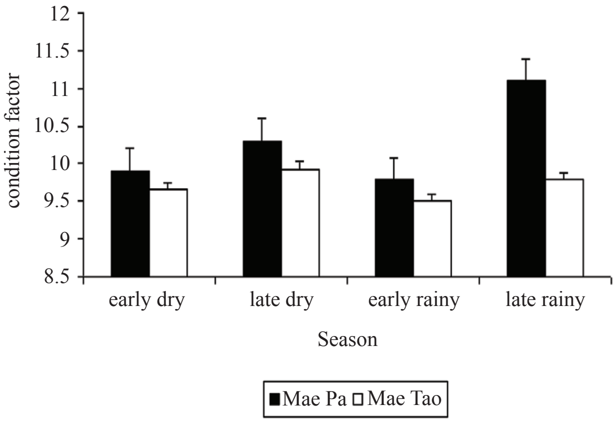

FIGURE 3. Quarterly average condition factor of *F. limnocharis* caught from Mae Sot, Tak

condition factor is recorded during the late dry season while the lowest is recorded during the early rainy season. The overall average condition factor for frogs caught from Mae Pa is 10.296 while the value is 9.720 for frogs caught from Mae Tao.

The differences between the hepatosomatic indices (HSI) of *F. limnocharis* caught from Mae Pa and Mae Tao is illustrated in Figure 4. The mean differences between seasons are statistically significant ($p < 0.001$) while the differences between station are not statistically significant ($p = 0.359$). The graph shows that HSIs of the rice frog caught from Mae Pa range from 1.578 to 2.074. Rice frogs caught from Mae Tao have HSIs that ranged from 1.529 and 2.025. The figure also shows that in both sites, the highest HSI is recorded during the early rainy season, while the lowest during late dry season. It is also observable that both sites show similar trend in the seasonal fluctuation of HSIs. The overall average HSI for rice frogs caught from Mae Pa is 1.913 while the value is 1.845 for rice frogs caught from Mae Tao.

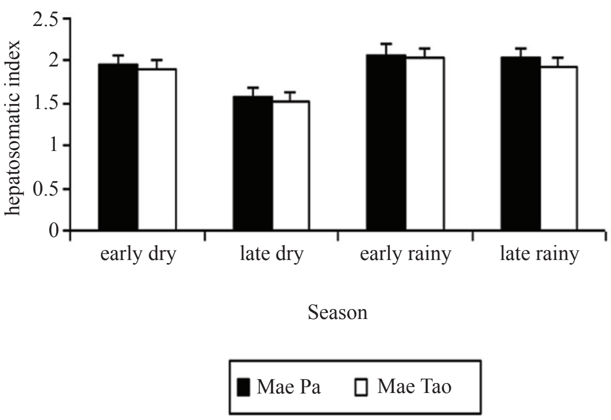

FIGURE 4. Quarterly average hepatosomatic index of *F. limnocharis* caught from Mae Sot, Tak

For renosomatic indices (RSIs), the quarterly average is shown in Figure 5. All mean differences between seasons and stations are statistically significant ($p < 0.001$) Average RSIs of *F. limnocharis* caught from Mae Pa range from 0.372 to 0.448. The highest RSI of these frogs is recorded in during the early rainy season while the lowest is recorded during the late rainy season. The overall average RSI of frogs caught from Mae Pa is 0.413. For Mae Tao frogs, the RSIs range from 0.354 to 0.411. In both sites, the highest RSIs are recorded during the early rainy season. The lowest RSI is recorded during the early rainy season. The overall average of RSI for *F. limnocharis* caught from Mae Tao is 0.380.

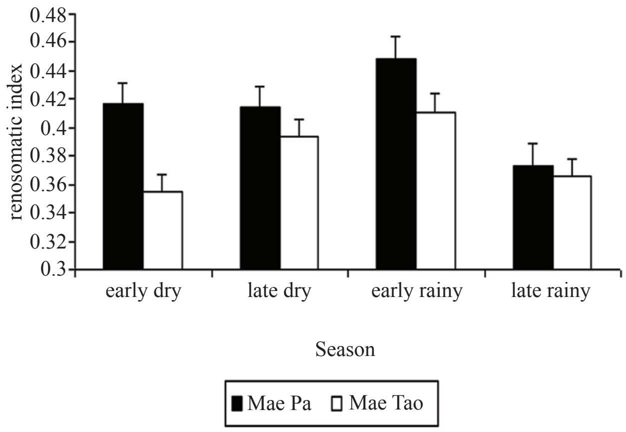

FIGURE 5. Quarterly average renosomatic index of *F. limnocharis* caught from Mae Sot, Tak

Figure 6 exhibits the quarterly average female gonadosomatic indices (GSI) of *F. limnocharis* caught from Mae Sot, Tak. All mean differences between seasons and stations are statistically significant ($p \leq 0.001$). The graph shows that female GSI of frogs from Mae Pa ranges from 3.591 to 12.082. On the other hand, female GSI of *F. limnocharis*caught from Mae Tao is recorded to be in the range of between 2.287 to 7.702. The overall average female GSI for frogs caught from Mae Pa is 7.594 while for the frogs caught from Mae Tao, the average female GSI is 4.919. The highest average female GSI is recorded in frogs caught during the late rainy season (Mae Pa) and late dry season (Mae Tao). For both stations, the lowest average female GSI is recorded in frogs caught during the early dry season. For male GSIS, *F. limnocharis* caught from Mae Pa range from 0.109 to 0.249, while for those caught from Mae Tao, the range was recorded to in the range of 0.113 to 0.290 (Figure 7). The mean differences between seasons are statistically significant ($p < 0.001$) while the differences between station are not statistically significant ($p = 0.767$). The highest male GSI in Mae Tao frogs occurs in those caught during the early rainy season (Mae Pa) and late dry season (Mae Tao). The lowest is recorded from those caught during the early dry season. The overall average male GSI for frogs caught from Mae Pa is 0.192 while the value is 0.196 for frogs caught from Mae Tao.

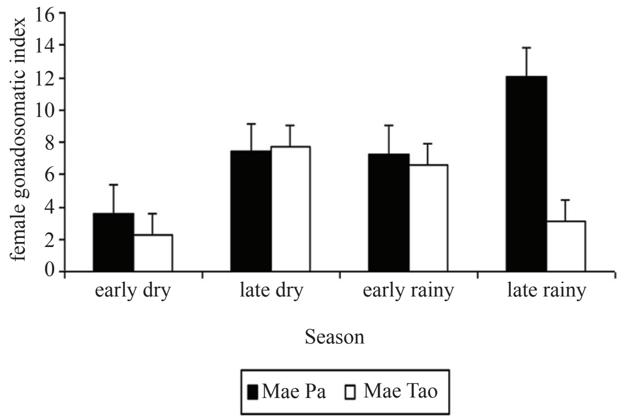

FIGURE 6. Quarterly average female gonadosomatic index of *F. limnocharis* caught from Mae Sot, Tak

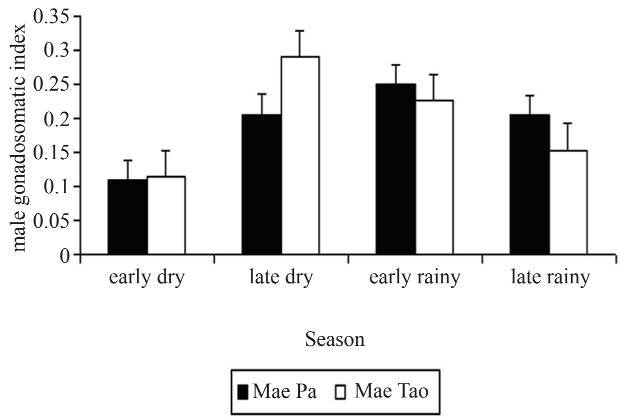

FIGURE 7. Quarterly average male gonadosomatic index of *F. limnocharis* caught from Mae Sot, Tak

In all the seasons, the differences in the mean values of Scaling coefficient of frogs caught from both Mae Pa and Mae Tao are not statistically significant. Scaling Coefficient reflects the ratio between logarithmic modes of weight and length. This means that for a certain snout-vent length, the difference in body weight of the rice frog may indicate whether the frog is in better condition or not. Therefore, it can be inferred that at this instance, cadmium accumulation is not severe enough to affect the ratio between weight and length of *F. limnocharis* caught from both reference and contaminated sites. Thus the Scaling Coefficient is not significantly affected by the stress from living in a cadmium contaminated site. However, even though the differences are not statistically significant, there is an observable trend that shows frogs from reference site had higher Scaling Coefficient than *F. limnocharis* caught from the contaminated site. Eastwood and Couture (2002) suggested that long term stress in population subjected to environmental pollution may lead to reduced weight to length ratio, hence reduced Scaling coefficient. Therefore, even though not statistically significant, the results showed there is a tendency that stress from cadmium accumulation in *F. limnocharis* may affect growth and development. This may eventually lead to reduced weight to length ratio. Perhaps, a longer duration of exposure may yield significant differences because Rosa et al. (2008) stated that age and exposure is an important factor in wildlife toxicology.

While Scaling coefficient values do not show statistically significant differences between both Mae's Pa and Mae Tao's frogs, condition factor (CF) shows a clearer picture. There are two important premises that can be derived from the result of the research. Firstly, it is shown that the difference in season plays a statistically significant role in the change in the CFs of the frogs. This means that in different seasons, the frogs live in different conditions which eventually affect their overall wellbeing. This is because condition factor is a provider of information on the health status (Linde-Arias et al. 2008), overall health (Urena et al. 2007) and general well-being (Bervoets & Blust 2003) of an organism. However, a more important point is revealed in the second premise derived from the result of this research. The result shows that there is a statistically significant difference in the CFs between *F. limnocharis* caught from the reference site than those caught from the contaminated site. Mae Pa's frogs had higher CF than Mae Tao's frogs. This is in line with the results obtained by other researchers where metal-exposed Salmo trutta was found to have lower CF as compared to reference populations (Hansen et al. 2006; Norris et al. 2000). Therefore, exposure to heavy metal especially cadmium leads to diminished quality of living condition which results in diminished CF.

Figure 3 shows the quarterly average hepatosomatic indices of *F. limnocharis* caught from both reference and contaminated sites. The differences in the average HSIs between the frogs caught from the contaminated site and those caught from reference site is not statistically significant. However, there is a common trend that can be observed despite the differences being statistically not significant. The data shows that in all the seasons, the frogs from Mae Pa have higher HSIs than frogs from Mae Tao. According to Hansen et al. (2006), cadmium has the ability to induce the formation of reactive oxygen species. This will then result in the generation of oxidative stress (Loumbourdis et al. 2007) which will then lead to an increase in lipid peroxidation (Mouchet et al. 2006; Alvarez et al. 2004; Isani et al. 2008). Increased lipid peroxidation will eventually result in cell membrane damage and cell death, either via apoptosis or necrosis (Norris et al. 2000). This is then followed by the reduction of liver weight and consequently the reduction in HSI. This may be a plausible explanation on why *F. limnocharis* caught from contaminated site tend to have lower HSIs than those caught from the reference site.

For renosomatic index, the difference in average values between rice frogs caught from Mae Pa and those caught from Mae Tao is statistically significant. The result shows that *F. limnocharis* living in cadmium contaminated site has lower RSI than frogs from the reference site. There has been very limited explanation and information on how cadmium causes the reduction in kidney size, hence the reduction in RSI of an organism. One possible cause is that the reduction could be attributed to the same mechanism that causes the reduction in liver weight. Exposure to cadmium leads to production of reactive oxygen species which imposes oxidative stress to the kidney cells. Oxidative stress causes lipid peroxidation which results in cell membrane destruction and cell death. However, more detailed and extensive studies need to be done in order to explain the mechanism how cadmium accumulation could lead to reduced kidney weight.

Differences in the female gonadosomatic index between *F. limnocharis* caught from cadmium contaminated site with those from reference site are statistically significant. The connection between cadmium accumulation with reduced ovarian weight could be attributed to the metal's effect on vitellogenesis. In a study on Oncorhyncus mykiss, Bon et al. (1997) found out that during the period of endogenous vitellogenesis and exogenous vitellogenesis, there is a great correlation between vitellogenin levels with female GSIs. In another study, the presence of cadmium leads to the inhibition of vitellogenin production (vitellogenesis) in *Platichthysflesus* (Povlsen 1990). This is because cadmium treatment and the resulting metallothionein synthesis compete with vitellogenesis in the liver cells. Apart from that, sub-lethal exposure to cadmium is often demonstrated by a marked hypocalcemic response (decrease in total blood plasma calcium) which may impair vitellogenesis (Haux et al. 1998). Therefore the disruption of vitellogenesis and the decline in vitellogenin level may be linked, although not exclusively, to cadmium exposure. Therefore, if cadmium can impair vitellogenesis and there is a great correlation between vitellogenin levels with female GSI, it can be inferred that cadmium exposure may be able to result in the reduction of female gonadosomatic indices. This could explain why *F. limnocharis* caught from the contaminated site has lower female GSI than those caught from the reference site.

However, for male gonadosomatic index, the result offers no clear explanation. The result shows that there are no significant differences in male GSIs of rice frog caught from both reference and contaminated site ($p = 0.767$). A mixture of contradicting trend is also apparent in the result. During both early and late dry season, the quarterly average male GSI for frogs from the contaminated site is higher than those from the reference site. However, during both early and late rainy season, the male GSI showed a completely reverse trend where the quarterly average for frogs from the contaminated site is lower than those from the reference site. Most probably, longer period of study that involves two or more annual cycle is required to fully understand the fluctuation and changes in male GSIs.

CONCLUSION

This research found that there is a connection between cadmium contamination with morphometric and gravimetric indices of *F. limnocharis* living in contaminated and reference sites in Mae Sot, Tak. Albeit being statistically not significant, there is a trend that frogs from contaminated site had lower Scaling Coefficient and hepatosomatic index than frogs from reference site. This trend is even more apparent in the differences in condition factor, renosomatic index and female gonadosomatic index where the differences are statistically significant. For these parameters, *F. limnocharis* caught from Mae Pa had higher condition factor, renosomatic index and female gonadosomatic index than those caught from Mae Tao. On the other hand, result for male gonadosomatic index is rather mixed with contradicting trend according to season. However, these differences are not statistically significant. In conclusion, the use of morphometric and gravimetric indices could give an idea on what would be the effect of cadmium contamination on the rice frog. Therefore, it is suggested that the use of morphometric and gravimetric can be used to determine whether *F. limnocharis* is a suitable candidate for sentinel species for cadmium contamination. However, it needs to be emphasized that morphometric and gravimetric data cannot be used alone in making the decision. These data need to be used in complementary with other parameters, especially contaminant analysis data.

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