การศึกษาพยาธิสภาพต่อมย่อยอาหารของหอยเชอรี่ (*Pomacea canaliculata*) จากพื้นที่อาศัยซึ่งได้รับผลกระทบของมนุษย์ที่ต่างกันในจังหวัดชลบุรี

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บทคัดย่อ

จังหวัดชลบุรีตั้งอยู่ในพื้นที่ภาคตะวันออกของประเทศไทยได้รับการกำหนดให้เป็นส่วนหนึ่งของพื้นที่เขตเศรษฐกิจพิเศษภาคตะวันออก ซึ่งการพัฒนานี้อาจส่งผลต่อคุณภาพน้ำจืดในจังหวัดชลบุรี การเปลี่ยนแปลงทางพยาธิสภาพเป็นตัวบ่งชี้ที่ดีของผลของมลพิษต่อสิ่งมีชีวิตที่ได้รับสารพิษและสามารถใช้เป็นเครื่องมือในการบ่งชี้เพื่อแสดงถึงความเป็นพิษในเบื้องต้นได้ ต่อมย่อยอาหารของหอยเป็นบริเวณที่สำคัญในการสะสมและการกำจัดสารพิษ ด้วยเหตุนี้การเปลี่ยนแปลงของต่อมย่อยอาหารของหอยจึงสามารถใช้เป็นตัวชี้วัดทางชีวภาพสำหรับการติดตามมลพิษทางสิ่งแวดล้อมได้ งานวิจัยนี้จึงศึกษาพยาธิสภาพต่อมย่อยอาหารของหอยเชอรี่ (*Pomacea canaliculata*) จากพื้นที่อาศัยในแหล่งน้ำจืดที่ต่างกันในจังหวัดชลบุรี ได้แก่ พื้นที่อ้างอิง (เป็นพื้นที่นอกเขตจังหวัดชลบุรี มีค่า DO อยู่ในช่วง 3.13–4.93 mg/L ซึ่งมีสภาพแวดล้อมของน้ำจืดค่อนข้างดี) พื้นที่ป่า พื้นที่เกษตรกรรม และพื้นที่ชุมชน ตลอดจนตรวจสอบคุณสมบัติทางพยาธิสภาพของหอยจากสิ่งแวดล้อมเหล่านี้ รวมทั้งคณะผู้วิจัยได้วัดตัวบ่งชี้ทางกายภาพพื้นฐานของคุณภาพน้ำด้วย จากการศึกษาทางเนื้อเยื่อวิทยาบริเวณต่อมย่อยอาหารของหอยเชอรี่จากพื้นที่อ้างอิง พบว่า ต่อมย่อยอาหารมีลักษณะเป็นท่อ ประกอบด้วยเซลล์ย่อยอาหารและเซลล์เบโซฟิลิก นอกจากนี้ยังพบแกรนูลสีเข้มกระจายอยู่ทั่วบริเวณต่อมย่อยอาหารด้วย โดยการเปลี่ยนแปลงทางพยาธิสภาพของต่อมย่อยอาหารที่เกิดจากสิ่งแวดล้อม ได้แก่ การเสียสภาพของเนื้อเยื่อบุผิว การลดลงของขนาดช่องว่างภายในท่อต่อมย่อยอาหารและพบการเพิ่มจำนวนของเซลล์เบโซฟิลิกและแกรนูลสีเข้ม จากผลการศึกษาพบว่าต่อมย่อยอาหารของหอยเชอรี่จากพื้นที่เกษตรกรรมมีการเสียสภาพของเนื้อเยื่อบุผิวอย่างชัดเจนเมื่อเปรียบเทียบกับพื้นที่อ้างอิง เนื่องจากการศึกษาครั้งนี้เป็นการศึกษาเบื้องต้นโดยมีจำนวนตัวอย่างน้อยและผลการศึกษานี้

ยังไม่ชัดเจนว่าสามารถนำต่อมย่อยอาหารของหอยเชอรี่ไปใช้เป็นตัวชี้วัดทางชีวภาพสำหรับการติด-ตามคุณภาพน้ำในสิ่งแวดล้อมของจังหวัดชลบุรีได้ อย่างไรก็ตามคณะผู้วิจัยเสนอให้มีการศึกษาพยาธิสภาพต่อมย่อยอาหารของหอยชนิดนี้และหอยฝาเดียวน้ำจืดชนิดอื่นเพื่อใช้เป็นเครื่องมือสำหรับเป็นตัวชี้วัดคุณภาพน้ำในสภาพแวดล้อมน้ำจืดของพื้นที่จังหวัดชลบุรีและพื้นที่อื่นในประเทศไทย

คำสำคัญ: *Pomacea canaliculata* ต่อมย่อยอาหาร พยาธิสภาพ ตัวชี้วัดทางชีวภาพ การติดตาม

A Histopathological Study of the Digestive Gland of the Golden Apple Snail (*Pomacea canaliculata*) from Different Human–Impacted Habitats in Chonburi Province, Thailand

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Abstract

Chonburi Province in eastern Thailand has been designated part of the Eastern Economic Corridor (EEC). This development might affect freshwater quality in Chonburi Province. Histopathological alteration is a good indicator of the effects of pollution on exposed organisms and provides an early indication of toxicity. The digestive gland of molluscs is the major site of pollutant accumulation and detoxification. Therefore, digestive gland alteration of molluscs can be used as a biomarker for monitoring environmental pollution. The present study investigated the histopathology of the digestive gland of the golden apple snail (*Pomacea canaliculata*) from different freshwater habitats in Chonburi Province. This included: a reference site (occurring outwith Chonburi Province and with a DO range of 3.13–4.93 mg/L, i.e., a putatively 'good' freshwater environment), a forest site, an agricultural site, and an urban site. To examine histopathological properties of snails from these environments, we also measured some basic abiotic indicators of water quality. The histological study revealed that the digestive gland of the snail from the reference site was characterized by tubules composed of digestive and basophilic cells. In addition, we found dark granules scattered throughout the digestive glands. Environmentally–induced histopathological alterations to the digestive glands were indicated by degeneration of epithelium, a decreased lumen space in the digestive tubules and an increase in the number of basophilic cells and dark granules. The result revealed that the digestive glands of *P. canaliculata* from the agricultural site showed particular degeneration of the epithelium when compared with the reference site. Due to low sample sizes, the present study is preliminary and it is not absolutely clear whether the digestive gland of *P. canaliculata* can be used as a biomarker for monitoring water quality in the aquatic environment. However, we encourage histopathological examination of the digestive gland of this and other freshwater gastropods as a promising tool to monitor water quality in the aquatic environment of Chonburi Province and elsewhere in Thailand.

Keywords: *Pomacea canaliculata*, Digestive gland, Histopathology, Biomarker, Monitoring

Introduction

The influence of land use on environmental pollution is most pronounced when there are accelerated environmental changes such as deforestation for agricultural or industrial purposes and growth of urban areas. Chonburi Province in Thailand is one of three eastern provinces in the Eastern Economic Corridor (EEC). The EEC Development Plan, initiated in 2017, falls under the umbrella of the Thailand 4.0 scheme, aiming to revitalize and enhance the well-known Eastern Seaboard Development Program that has encouraged industrial and economic development in Thailand. The EEC Development Plan will lead to significant economic development and a transformation of Thailand‘s investment in physical and social infrastructure in the area (Eastern Economic Corridor Office, 2017). On a more negative note, however, the development of Chonburi Province in the EEC might affect the natural environment, especially the aquatic environment and freshwater quality. Due to expansion of industry, transportation and urban areas, there is an increased wastewater discharge into the freshwater canal and pond environment. Hence, the water quality of the freshwater aquatic environment in Chonburi Province may be deteriorating.

Several tools or biomarkers may be used to assess pollutant contamination of organisms. These include: toxicity assessment, histopathological evaluation, and biochemical assessment. Histopathological techniques provide a rapid, sensitive, reliable, and inexpensive tool for assessment of stress responses to xenobiotics (Sharaf and Shehata, 2015). Several authors have reported the importance of histopathological alterations as a good tool for revealing the effects of pollutant contamination on tissues of exposed organisms, and this technique can provide an early indication of toxicity (Auffret, 1988; Sreeram and Menon, 2005; Dummee et al., 2015; Sharaf and Shehata; 2015).

The digestive gland or hepatopancreas of molluscs is the major site of pollutant accumulation and detoxification of substances such as heavy metals (Dummee et al., 2012; Vasanthi et al., 2012) and petroleum hydrocarbons (Sharaf and Shehata, 2015). Digestive gland alterations are a reflection of disturbances at a molecular level. Identification of these disturbances can aid the understanding of the impact of pollutants and other stress factors on the whole animal (Vasanthi et al., 2012). Molluscs collected from polluted sites have structural alterations to the epithelium of the digestive gland. This is likely due to an increase in lipid accumulation and granule formation in digestive tubules, a process that has been observed in mussels (*Mytilus edulis*) (Lowe, 1988; Wedderburn et al., 2000). Sharaf and Shehata (2015) studied histopathological alterations of the digestive gland of *Cyclope neritea* (gastropoda) that were collected from a polluted site containing heavy metals and petroleum hydrocarbons. In this study the digestive gland showed increased secretion from the digestive cells, causing the nuclei to start unusual divisions, and a great increase in numbers of dark granules. The digestive cells degenerated and began lysing, while most of the tubules were damaged. Similar results have been reported in *Lymnaea luteola* (gastropoda) and *Anodonta cygnea* (bivalvia) that were exposed to paraquat and zinc, respectively (Kanapala and Arasada, 2013; Moëzzi et al., 2013). In addition, the digestive gland of the golden apple snail (*Pomacea canaliculata*) and the snail, *Helix aspersa,* can be used as an oxidative stress biomarker for monitoring water quality (Arrighetti et al., 2018; Abdel-Halim et al., 2013). In summary, the digestive gland of molluscs can be used as a reliable biomarker for monitoring pollutant contamination in an aquatic environment.

P. canaliculata is native to South America and was introduced into Thailand in the 1990s from Japan, Taiwan, and the Philippines (Mochida, 1991; Halwart, 1994; Naylor, 1996). *P. canaliculata* helps control the growth of benthic algae and eliminates leftover food in aquariums and farming (Thaewnon–Ngiw et al., 2004). This snail is well known as a serious rice pest in most Southeast Asian countries because it damages the rice, feeding voraciously on young rice seedlings (Naylor, 1996). In Thailand, histopathological alteration of the digestive gland of *P. canaliculata* is reported to be of use as a biomarker for monitoring several pollutant contaminations. Krautrachue et al. (2011) studied *P. canaliculata* in Mae Klong River, Samut Songkhran Province, Thailand and Dummee et al. (2012) studied *P. canaliculata* in the Beung Boraphet reservoir, Nakhon Sawan Province, Thailand. In addition, the study by Dummee et al. (2015) in the laboratory indicated that snails can serve as bioindicators of water quality for the following reasons: (1) they are ubiquitous in aquatic habitats and an important component of freshwater food webs, and (2) they are easy to harvest. The objective of this study was to investigate the use of the digestive gland of *P. canaliculata* as a biomarker for monitoring water quality in different Thai habitats including: a reference site (occurring outwith Chonburi Province and with a DO range of 3.13–4.93 mgL, i.e., a putatively 'good' freshwater environment), a forest site, an agricultural site, and an urban site. This study represents a first step towards a reliable thesis that *P. canaliculata* histological examination can be used to monitor water quality problems in Chonburi Province, Thailand, and elsewhere.

Research Methodology

Field sampling

Twenty *Pomacea canaliculata* snail samples were hand–collected from four different habitats in Thailand: (1) reference site: Ban Pho District, Chachoengsao Province (13º 39.376' N, 100º 58.761' E) – this site is a rice paddy field. It has the highest dissolved oxygen range is 3.13–4.93 mg/ L of all sites considered in the present study, it is not in Chonburi Province, and snails from this reference site show a normal histopathology. Consequently, this site has been chosen as a reference site in the present study; (2) forest site: Bothong District, Chonburi Province (13º 09.072' N, 101º 35.837' E) – this site is characterized by sparse forest. It is close to a mountain and a rubber plantation. In addition, this site is far from a city; (3) agricultural site: Phanat Nikhom District, Chonburi Province (13º 31.208' N, 101º 12.017 E) – this site is a watercourse of freshwater and the cement canals are used as a water supply for rice paddy fields. This site is near residential households; and (4) urban site: Muang District, Chonburi Province (13º 16.898' N, 100º 56.706' E) – this site is a pond built by humans and this pond has been use for fishing. The site is close to a buildings and streets in a Saen Suk Sub–district. These locations represent a variety of habitats in relation to land use (Palmeirim et al., 2014). Three replicates were obtained in each of these four habitats and water quality parameters were quantified in the field during January 2017. These parameters were: water temperature (ºC), pH, dissolved oxygen (DO; mg/L), conductivity (µs/cm) and salinity (ppt). They were measured by using an YSI–85 multi–meter.

Histological study

Three snail samples were taken from each site (shell height 32.2–49.2 mm). The shell was then cracked and the soft tissue removed from the shell. Our sample size (n = 3) is consistent with the methods of Radwan et al. (2010), but ultimately is lower than desired and was constrained by logistic factors among others. Next, the digestive gland was dissected and fixed in Davidson's fixative for 24 hours. After that, the digestive glands were washed with 70% ethanol three times. Then they were dehydrated through an ethanol series (80%, 90%, and 100%), cleared in dioxane, and embedded in paraffin wax (Bancroft and Gamble, 2008).

Sections (6 µm) were cut using a rotary microtome (HM 360, Kinetics Corporation). The sections were stained with Hematoxylin and Eosin Y. The histopathological alterations in the digestive glands were examined under a compound light microscope (Olympus; BX51). Digital images were captured with an Olympus (series DP22) digital camera. Three images in each habitat were randomly taken at 400X magnification and analyzed (the number of basophilic cells and dark granules) using ImageJ (Papadopulos et al., 2007). The image size was approximately 0.095 mm^2. We measured the average number of basophilic cells ($N_{ba} = N_b/A_e$), and the average number of dark granules ($N_{da} = N_d/A_e$), where N_b = number of basophilic cells in the epithelium, N_d = number of dark granules in the epithelium and A_e = area of epithelium (modified from Klobucar et al., 2001). Histopathological changes in the digestive gland were described and classified according to the frequency of appearance of such alterations with the following criteria: the qualitative measurement of degeneration of epithelia and decrease space of lumen were scored values: 0 = not observed, + = mild (visually noticeable alteration relative to the reference present in one of the slides analyzed), + + = moderate (visually noticeable alteration relative to the reference present in two of the slides analyzed), + + + = severe (visually noticeable alteration relative to the reference present in all three of the slides analyzed) and score values for number of basophilic cells and number of dark granules were quantitative measurements: - = mild (average number of basophilic cells and dark granules were less than the reference site below 10%), - - = moderate (average number of basophilic cells and dark granules were less than the reference site between 10-15%), - - - = severe (average number of basophilic cells and dark granules were less than the reference site above 15%), + = mild (average number of basophilic cells and dark granules were more than the reference site below 10%), + + = moderate (average number of basophilic cells and dark granules were more than the reference site between 10-15%), + + + = severe (average number of basophilic cells and dark granules were more than the reference site above 15%).

Results

Field sampling of abiotic indicators of water quality

Water quality data are presented as ranges and shown in Table 1. Water temperature was highest (29.8–31.2°C) in the forest site and lower in the reference site, agricultural site, and urban site (28.4–29.1, 26.3–27.1, and 27.4–28.0°C, respectively). The highest pH value was found in the urban site and was 7.43–7.80. The pH at the reference site, forest site, and agricultural site (7.09–7.13, 6.30–7.04, and 6.72–6.86, respectively) was lower. Conductivity was highest (1,916–2,512 and 593–

1,828 µs/cm) in the reference site and agricultural site, respectively. In addition, salinity was highest 0.9–1.2 ppt. in the reference site and was lower in the forest site, agricultural site, and urban site (0.1, 0.3–0.9, and 0.4 ppt., respectively). The highest value of dissolved oxygen was in the reference site and forest site. This was 3.13–4.39 and 3.08–3.44 mg/L, respectively. On the other hand, there was a decrease relative level of dissolved oxygen in both the agricultural site and the urban site (0.11–1.23 and 2.18–3.68 mg/L, respectively).

Histological study

The digestive glands are composed of numerous tubules, which consisted of digestive and basophilic cells. The digestive cells were of a columnar epithelial type and these cells contained numerous vacuoles. The nucleus is basal in the digestive cell. The basophilic cells are scarce in the digestive gland and the basophilic cells are pyramid–like in shape and these cells occur between digestive cells. Moreover, dark granules were found in the basement membrane of the digestive gland. The digestive gland of *Pomacea canaliculata* from the reference site was composed of abundant digestive cells and scarce basophilic cells. The digestive cells were of normal columnar epithelium and the digestive tubules were of wide lumen. In addition, few dark granules were found along the digestive gland epithelium (Figure 1a). The digestive glands of *P. canaliculata* from the forest site showed degeneration of epithelium and the lumens decreased in space. The number of basophilic cells highly increased, and these were between digestive cells. In addition, the digestive gland of snails from forest site had a higher number of dark granules than the reference site. (Figure 1b). Parts of the digestive gland of *P. canaliculata* from the agricultural site showed degeneration of the epithelium resulting in vacuolization. The lumens of the digestive tubules were a highly decreased in space. The number of basophilic cells of snails from the agricultural site was highly increases. The number of dark granules were decreases compared to the reference site (Figure 1c). The digestive glands of *P. canaliculata* from the urban site showed the normal of epithelium and some digestive tubules of snails showed a decrease in the space of the lumen. Snails from the urban site showed numerous basophilic cells. The number of dark granules were highly decreased compared to the reference site (Figure 1d). The histopathological alterations are shown in Table 2.

Discussion

Water quality in the sites considered here was considered ‘medium clean’ with water temperature, pH, dissolved oxygen, conductivity, and salinity generally well below accepted limits set by the Thai Pollution Control Department (2010), except dissolved oxygen which was very low at the agricultural site (0.66 mg/L). The acceptable standard of dissolved oxygen at an agricultural site as defined by the Thai

Table 1 Data on water quality from habitats of *P. canaliculata*

Parameters	Site			
	Reference	Forest	Agriculture	Urban
Water temperature (^{O}C)	28.4–29.1	29.8–31.2	26.3–27.1	27.4–28.0
pH	7.09–7.13	6.30–7.04	6.72–6.86	7.43–7.80
Conductivity (µs/cm)	1916–2512	211.6–213	593–1828	945–960
Salinity (ppt)	0.9–1.2	0.1	0.3–0.9	0.4
Dissolved oxygen (DO; mg/L)	3.13–4.39	3.08–3.44	0.11–1.23	2.18–3.68

Table 2 Histopathological alterations of the digestive gland of *P. canaliculata*

Histopathological alterations	Site			
	Reference	Forest	Agriculture	Urban
Degeneration of the epithelia	NA	+	+ +	0
Decrease space of lumen	NA	+	+ + +	+
Number of basophilic cell	NA	+ + +	+ + +	+ + +
Number of dark granule	NA	+ +	- -	- - -

NA = not applicable, n = 3 sample sizes. Score value for degeneration of epithelia and decrease space of lumen: 0, not observed; +, mild; + +, moderate; + + +, severe. Score value for number of basophilic cell and number of dark granule: -, mild; - -, moderate; - - -, severe; (average number of basophilic cells and dark granules were less than the reference site below 10%, between 10–15% and above 15% respectively) +, mild; + +, moderate; + + +, severe (average number of basophilic cells and dark granules were more than the reference site below 10%, between 10–15% and above 15% respectively).

Pollution Control Department is 4.0 mg/L. Low dissolved oxygen was reported by Wilcock et al. (1995) in an agricultural site (dairy farming), Whangamaire Stream catchment, North Island, New Zealand, hence, the histopathological alterations of the digestive gland of the snail in the present study may be due to other environmental factors such as insecticide (Hamlet et al., 2012; Radwan et al., 2008; Sharaf et al., 2015), causing degeneration of the digestive tubules.

These studies showed that degeneration of epithelium were rarely observed in the digestive cells of snails from the forest site and observed more in the digestive cells of snails from the agricultural site. However, snails from the urban site showed normal epithelium in the digestive cells. Damage to the epithelium was observed in the digestive tubules in a phenomenon known as vacuolization. Similar pathological changes were observed by Arrighetti et al. (2018) after exposure of *P. canaliculata* to cypermethrin. Moreover, in the digestive gland of the bivalve mollusc *Crenomytilus grayanus* from Southwestern Pethe,

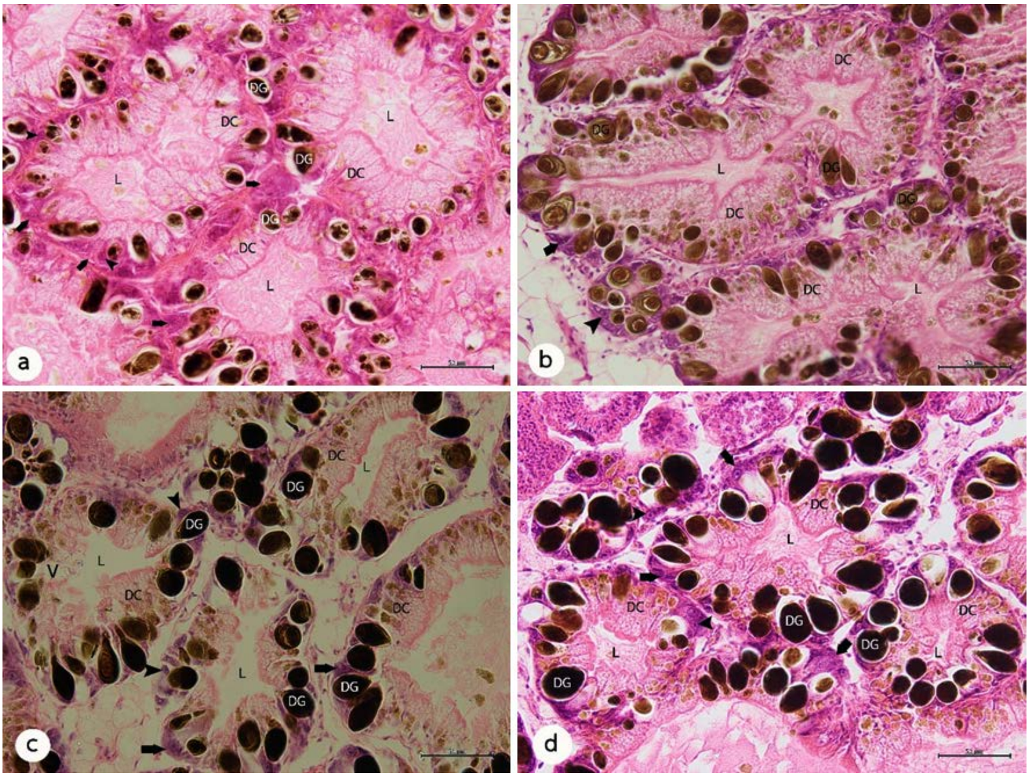

Figure 1 Digestive gland preparations from *P. canaliculata*: (a) Digestive gland of the snail from the reference site showing numerous tubules that were composed of digestive cells (DC) and basophilic cells (➡) surrounding the lumen (L). Moreover, few dark granules (DG) and nuclei (➤) formed the basement membrane of the digestive gland. (b) Digestive gland of the snail from the forest site showing that the numerous dark granules. (c) Digestive gland of the snail from the agricultural site showing the epithelium vacuolization (V). (d) Digestive gland of the snail from the urban site showing numerous basophilic cells.

Great Bay, Sea of Japan, there was an increase in vacuolization of digestive cells and this phenomena is recorded in bivalves as a consequence of exposure to xenobiotics of both organic and inorganic nature (Usheva et al., 2006). In the snail *M. cornuarietis* this phenomenon appears to occur due to exposure to $PtCl_2$ (Osterauer et al., 2010). Damage to the epithelium of the digestive tubules is an initial degenerative process that ends with epithelia necrosis, indicating systemic damage and resulting in vacuolization. This damage may be caused by oxidative stress, which damages the macromolecules in the cell, including DNA, proteins, and lipids (Bhagat et al., 2016). These histological alterations may affect biochemical pathways, leading to failure of digestive gland function. Desquamation (cell peeling) of digestive cells in molluscs under the impact of various pollutants is often recorded in pathology, both under anthropogenic pollution in the field and in the laboratory conditions (Auffret 1988; Usheva et al., 2006; Osterauer et al., 2010). The agricultural site considered in our study is a watercourse of freshwater, with the canal used as a water supply for rice paddy fields. This site is near residential households. Discharges from the surrounding rice paddy fields and wastes from domestic households may affect to water quality. This could explain the high tolerance of *P. canaliculata* to different environmental contaminants. Snails can survive in an agricultural sites while dissolved oxygen range in water is low, pH value range is weak acid, and water is turbid. Similar results were observed by Chaichana and Sumpan (2015).

The second histopathological alteration of the digestive gland we observed was decreasing lumen space in the digestive tubules. In the snails from the agricultural site were observed a very narrow tubule lumen when compared to the snails from the reference site. In the snails from the forest site and the urban site, there was observed a slightly decreased lumen space in the digestive tubules. Similar pathological changes were observed by Dummee et al. (2012, 2015) after exposure of snails to heavy metals. The authors reported that some digestive cells and their nuclei became highly dilated resulting in a very narrow tubular lumen.

In general, digestive cells are always more abundant than basophilic cells, except under stress conditions, when an increase in the number of basophilic cells occurs; a process called “cell–type replacement” (Arrighetti et al., 2018). Snails from the forest site, the agricultural site and the urban site had an increase in number of basophilic cells when compared to the reference site. The phenomenon is observed in the digestive gland of *M. cornuarietis* exposed to $PtCl_2$ (Osterauer et al., 2010), in *M. cornuarietis* exposed to Cu and Li (Sawasdee et al., 2011), and in *P. canaliculata* from an agricultural site (Dummee et al., 2012). Histopathological alterations similar to those reported in the present study, such as the basophilic cells increasing in number, have also been shown to occur in the digestive gland of *P. canaliculata* when exposed to cypermethrin (Arrighetti et al., 2018).

The last histopathological alteration of the digestive gland was an increase in number of dark granules. The digestive glands of *P. canaliculata* from the forest site had a higher number of dark granules than the reference site. The increase in number of dark granules has also been shown to occur in the digestive gland of *M. cornuarietis* when exposed to Cu and Li (Sawasdee et al., 2011) and in *P. canaliculata* when exposed to Cu (Dummee et al., 2015). The same occurred to *P. canaliculata* (Arrighetti et al., 2018) exposed to insecticide. The increase in number of dark granules may represent the activation detoxification mechanisms in the digestive gland as reported by Mason and Simkiss (1982). Within gastropods, the digestive glands are particularly productive of non–mineralized granules which take the form of condensed sulfur–containing residual body in the lysosomal system (Dummee et al., 2012). On the other hand, the digestive gland of snails from the agricultural site and the urban site had a lower number of dark granules than the reference site. However, the dark granules in digestive gland of snails from the agricultural site and the urban site showed larger size than glands taken from the reference site, showed in the Figure 1c and 1d. This study did not measure the average epithelial area filled with dark granules. For a better result than the present study, a the future study might measure average epithelial area filled with dark granules, following a study of the freshwater snail, *Planorbarius corneus* (gastropod), by Klobucar et al. (2001).

In gastropods, the digestive gland or hepatopancreas plays a major role in contaminant uptake, accumulation, intracellular food digestion, and the metabolism of inorganic and organic chemicals (Marigómez et al., 2002; Usheva et al., 2006). Damage to the digestive gland may lower the ability of the snail to digest food (Osterauer et al., 2010). Vasanthi et al. (2012) reported that the digestive gland of a mussel, *Perna viridis,* collected from Ennore estuary, India (a site polluted by heavy metals), showed necrosis of the cells in the lumen. Digestive cells were compressed and there was a loss of digestive tubules, which was also observed in oil–exposed clams, *Macoma calcarea* (Neff et al., 1987) and mussels, *Mytilus edulis,* exposed to heavy metals (Wedderburn et al., 2000). Severe reactions to environmental contamination become evident in the digestive gland because it is the main metabolic organ in snails and is also involved in accumulation and detoxification of pollutants (Abdel–Halim et al., 2013; Arrighetti et al., 2018; Mleiki et al., 2018).

In the present study, histopathological alterations of digestive glands of the golden apple snail, *P. canaliculata* in different human-impacted environments included damage to the epithelium of the digestive tubules and the narrow tubular lumen observed in *P. canaliculata* in our study could serve as a biomarker for monitoring pollutant contamination in the aquatic environment (Dummee et al., 2015). The increased number of basophilic cells seen in our study is similar to the effects seem with *P. canaliculata* from Beung Boraphet reservoir, Nakhon Sawan Province, Thailand (Dummee et al., 2012). In addition, there was an increased number of dark granules. These symptoms were also observed in the digestive glands of *P. canaliculata* from Mae Klong River, Samut Songkhram Province in Thailand (Kruatrachue et al., 2011). However, *P. canaliculata* is able to tolerate relatively severe environment contamination. For that reason, the digestive gland of these snails may be less suitable as a biomarker of water quality in the aquatic environment of Chonburi Province, Thailand, and elsewhere.

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