
Efficacy of Four Different Feeds for Producing Hatchery Raised Red Claw Crayfish (*Cherax quadricarinatus*)

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Kiriyakit, A. and Suwannagate, K. (2018). Efficacy of four different feeds for producing hatchery raised red claw crayfish (*Cherax quadricarinatus*). International Journal of Agricultural Technology 14(5):693-704.

Abstract This research was initiated in order to determine effectiveness of locally available Thailand feeds and homemade (domestically manufactured) diets for best production of hatchery raised Red Claw Crayfish (*Cherax quadricarinatus*). A higher cost commercially manufactured shrimp feed was also examined. Brooding red claw crayfish were individually reared and fattened with four different feed treatments; earthworm segments, oyster meat, homemade mixed feed and commercial shrimp pellet feed. Feeding occurred for 30 days, prior to transfer of all red claw crayfish to a pool mating tank. Gravid females for each feed treatment were collected and recorded at 15 day intervals, until day 45 of the breeding period. Total hatchery production for incubation times, number and size of free living juveniles, as well as survival and growth of 1 month old crayfish were determined, comparing performance of all brood crayfish. Results showed that brood crayfish fattened with oyster meat and homemade mixed feed, spawned faster and with higher fecundity, than brood crayfish fattened with earthworm segments and commercial shrimp pellet feed. Brood crayfish fattened with oyster meat spent longest incubation times. However, they gave similarly free living juvenile recruitment numbers to those brood fattened with homemade mixed feed and shrimp pellet feed. Even though the brood crayfish fed homemade mixed feed produced largest sized free living juveniles, juveniles fed with oyster meat showed better overall growth. Brood crayfish fattened with earthworm segments required shortest incubation times and produced highest numbers of free living juveniles. However, their free living juveniles were of the smallest size. Therefore, homemade feeds, or oyster meat feeds, are suggested to be the best choice for a maturation feed for red claw crayfish, for breeding. A better production performance, lower cost and simple preparation were observed factors.

Keywords: Hatchery production, Red Claw Crayfish, *Cherax quadricarinatus*

Introduction

Red claw crayfish, *Cherax quadricarinatus* are tropical freshwater crayfish, originating from Australasia. They were introduced for aquaculture into several tropical and subtropical countries (Ahyong and Yeo 2007; Lodge *et*

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al., 2012). Crayfish are often called “freshwater lobster” for their physical resemblance to a lobster and its large size. They are considered high potential for aquaculture farming in Southeast Asia, because of their rapid growth rate, ease to feed, breed, and harvest, as well as a high tolerant to handling and strong marketability (New, 2003; Edgerton, 2005). Interest for aquaculture and ornamental pet trade has resulted in wide translocations of the red claw crayfish species within Australia, Southeast Asia, and Central/South America (Christina *et al.*, 2011).

Red claw crayfish, *C. quadricarinatus*, were imported into Thailand for Royal Project Research, since 2005. Results indicated that *C. quadricarinatus* can perform at levels having aquaculture potential in Thailand (Apirakpongsa, 2013). Hopefully *C. quadricarinatus* can become a significant aquaculture species, and a possibly rival of the giant freshwater prawn (*Macrobrachium rosenbergii*). However, the current constraint of *C. quadricarinatus* growth in Thailand is insufficient seeded to supply from hatcheries, a major limiting factor in commercial viability. At present, low production capabilities and high costs do not allow for juvenile sales to any other market than for ornamental aquarium trade. The need for proven seed production, to optimize quantity and quality of juveniles and to lower seed stock price, is a must, if industry wants to maintain its present growth rate. To achieve this understanding, better egg production and hatchery management of *C. quadricarinatus*, under culture conditions, is imperative.

Exogenous or external factors provide broodstock with the essential nutrients required for gonadal development and performance of seedstock (El-Sayed and Kawanna, 2008). Red claw crayfish, generally grow well when fed diets developed for other crustaceans (Saound, *et al.*, 2012), but specific diet requirements, allowing for maximum broodstock potential have not been suggested. For *C. quadricarinatus* culture to further develop, juvenile red claw numbers must increase dramatically. In the present study, we evaluated four different maturation feeds on production efficiency of red claw crayfish (*C. quadricarinatus*), under hatchery conditions.

Materials and methods

Experimentals Design

Effects of maturation feeds on fecundity, spawning success, egg incubation period, hatching success, and juvenile growth and survival of *C. quadricarinatus* were studied using a Completely Randomized Design. Experimental feeds included in the evaluation were: 1) earthworm segments, 2)

oyster meat, 3) homemade feed diet, and 4) commercial shrimp pellets feed, with 5 replications. The experiment was performed at the Rajamangala University of Technology Tawan-ok Chanthaburi Campus, Thailand.

Experimental Animals

One hundred and twenty Red claw crayfish, *C. quadricarinatus* (13.97±0.51 cm TL) of 1 year age were selected as experimental broodstock. They were purchased locally, from a private Sa Kaeo farmer. Before the experiment began, all crayfish were held for a 14-days broodstock acclimation period.

Experimental tanks

Experiment tanks were divided into 4 categories; broodstock rearing tanks, breeding tanks, gravid females rearing tanks and nursing tanks. Sixty black plastic boxes (20cm x30cm x 15cm) connected to a recirculating water system (water circulated through each box at a rate of 10 liters/minute) were used. Each rearing tank contained one individual red claw crayfish broodstock. Four concrete tanks (2x3 meter width and 0.6 meter depth) were used for crayfish breeding-mating tanks. Three air stones provided aeration for the tanks. Ten PVC pipe shelters (5-cm diameter x 12-cm length, 1 pipe/crayfish) and one sheet shelter net (200×300 cm.) were placed in each of these tanks. Twenty 60×37.5×37.5 cm. aquariums were used for housing gravid females; also known as nursing tanks. One air stone and a piece of PVC pipe were placed in each aquarium, per gravid female. However, when these aquariums were used for nursing crayfish juveniles, two air stones and one sheet shelter net (50×90 cm.) were added to each aquarium.

Experimental Feed preparation

Four types of feed (Fig.1b) were tested, as treatments:

- (1) Earthworms, African night crawlers of *Eudrilus eugene* species were representative of the live feed tested. Live worms were purchased in Chanthaburi, Thailand, from a local private farmer. After purchase, they were acclimated for 7 days in culture boxes (50-cm diameter x 15-cm high), containing soil substrate, and prior to use. Two hundred and seventy gram African night crawlers were stocked in each culture box and fed 10% of their body weight, three times a week, with fermented cow dung. This feed was spread on top of the soil substrate and water was sprinkled on it. The

- feed worms were captured, cleaned and cut into small segments (1 centimeter length) before being fed to experimental crayfish.
- (2) Oyster (*Saccostrea commercialis*) is representative of fresh feed. The oyster meat was purchased from the local Chanthaburi seafood market. It was cut into small pieces before being fed to experimental crayfish.
 - (3) Homemade feed diet was made from squid, shrimp and pumpkin, in equal ratios (20 g.). All ingredients were mixed and ground, using a fruit blender (EBR7804S Electrolux, Thailand). Ten grams of gelatin powder were diluted in a liter of water, and then boiled. The boiled gelatin was then added to the mixed ingredients. After the gelatin solidified, it was sliced into small pieces (1 centimeter length), before being fed to experimental crayfish
 - (4) A commercial pellet feed of 35% protein was tested (commonly used for Giant Tiger Shrimp). This feed is locally available in Chanthaburi area, and is representative of a high priced feed option. These feed pellets were 0.4 centimeters in length.

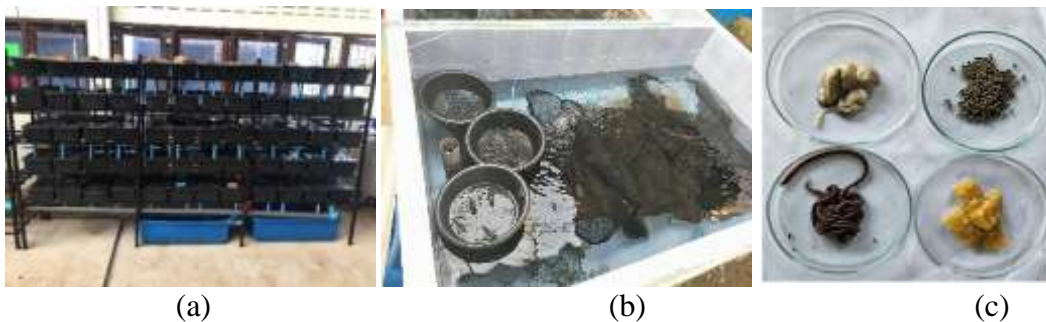


Figure 1. Experimental facility; broodstock rearing tanks (a); breeding-mating tanks (b); feed treatments (c)

Experimental procedure and data collection

Eighty female and forty male red claw crayfish were individually rearing in the brood rearing boxes (Fig.1, a) for 30 days before pool mating. Each sex was divided into 4 groups. Each group were fed two times daily (08.00 am and 07.00 pm) by four feed treatment. The total amount fed to each box was 3% body weight per day. Uneaten feed and debris were siphoned from each box every day. Biofilters were back-washed once a week. Approximately 5% of the 500-liter system volume was replaced bi-weekly. Water was continuously aerated and temperature was maintained at 28 ± 3 °C.

After 30 days of fattening, all crayfish were transferred to 4 mating tanks (Fig.1, b), stocking at a female to male ratio of 2:1. Therefore, each mating tank comprised of 20 females and 10 males. Prior to stocking, female crayfish were weighed and measured in total length. Feeding in each mating tank continuously followed the treatment. Spawned female were monitoring and randomly checked every 15 days. Gravid females were counted, and the egg stage of their spawn was noted (Medley, 1994). Gravid females of each treatment (5 replications) were then carefully removed and introduced separately into the 60×37.5×37.5 cm. aquariums "hatching" tanks. Each aquarium was observed for the presence of free living juveniles. Once free-living juveniles were noticed, the female was removed, the remaining juveniles were counted and randomly (30%) measured weight and length, and the release date recorded.

One hundred juvenile red claw crayfish were randomly selected from each treatment and grown in 60×37.5×37.5 cm. aquariums, to evaluate growth and survival. They were fed twice daily (8:00 am and 7:00 pm) ad libitum with egg tofu (40% protein). Uneaten feed and debris were siphoned from each aquarium and water exchanged at 10% every day. Juvenile red claw were reared for 30 days, at which time they were weighed and counted for harvest.

Water quality was routinely monitored in all four types of experimental tanks. Temperature, dissolved oxygen and pH were checked daily using a multi-parameter analyser (C932 Consort, Belgium). Total alkalinity was checked weekly using Alkalinity Test Kit.

Statistical Analysis

Number of gravid females, achieved within 45 days of breeding was analysed for spawning success and percentage of trial group. Data recorded included: incubation times (days), number and size of free living juveniles at the time of release, growth and survival of juvenile at 30 days of raising, were analysed using a one-way analysis of variance (ANOVA), and significant different among treatment means were evaluated with Duncan's new multiple Range Test. Significant levels were considered at $P < 0.05$.

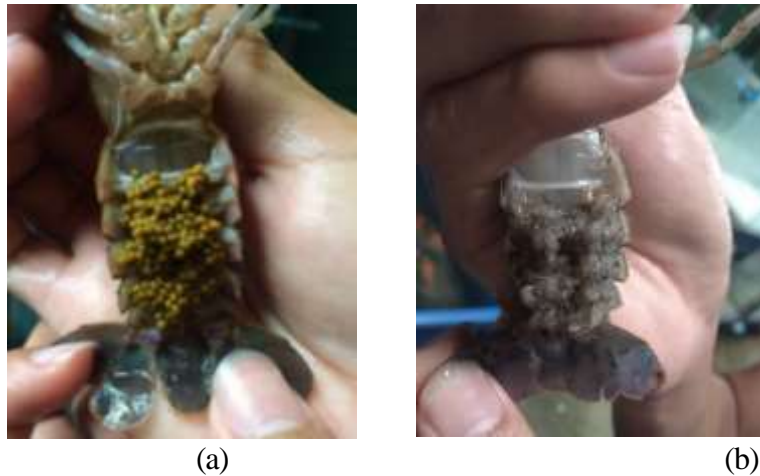


Figure 2 Gravid female with olive or khaki egg color (a) and juveniles were fully formed and released from the female (b)

Results

Effectiveness of maturation feed on spawning of Red claw crayfish

Brooding red claw crayfish were fattened with four different feeds for a month, prior to mating. It was found that at first 15 days of breeding, maximum number gravid females were achieved from treatments fed with oyster meat, following by treatments fed with homemade feed diet, and then commercial shrimp pellet feed and lastly earthworm segments. However at 45 days of breeding, brood crayfish, that were fed oyster meat and homemade feed diet, produced highest total of gravid females. Brood crayfish fed earthworm segments had lowest total of gravid females, throughout the 45 days period (Table 1). All gravid female collected at every 15 days interval of breeding were in similarly stage, olive or khaki egg color (Fig. 2a).

Effectiveness of maturation feed on total hatchery production

This trial found that fattening with four different feeds, resulted in significant differences in incubation time and numbers of free living juveniles ($P < 0.05$). Brood females, fattened with oyster meat, resulted in longer incubation times (42.60 ± 1.47 days) compared to brood females fattened with earthworm segments (37.8 ± 0.49 days) and homemade feed diet (38.20 ± 0.49 days). However, their incubation time was not different from brood females fattened with commercial shrimp pellet feed (39.40 ± 1.47 days) (Fig.3)

At the time of release, brood crayfish, fattened with earthworm segments, gave highest number of free living juvenile, significant higher ($P < 0.05$) than brood crayfish fattened with all other tested feeds. There was no different among the oyster meat, homemade feed diet and commercial shrimp pellet feed, in regards to numbers of free living juveniles. Brood females fattened with earthworm segments, also showed higher number of free living juveniles per gram of female body weight ($P < 0.05$), than brood females fattened with other feed (Table 2).

Table 1 Number of gravid females, collected at 15 day breeding intervals and percent spawning success, within 45 days of breeding.

Experimental feed	Number of females	Number of gravid females			Total	Spawning percentage
		15 day	30 day	45 day		
Earthworm segments	20	2	3	2	7	35%
Oyster meat	20	7	2	1	10	50%
Homemade feed diet	20	6	2	2	10	50%
Commercial Shrimp pellet feed	20	5	3	1	9	45%

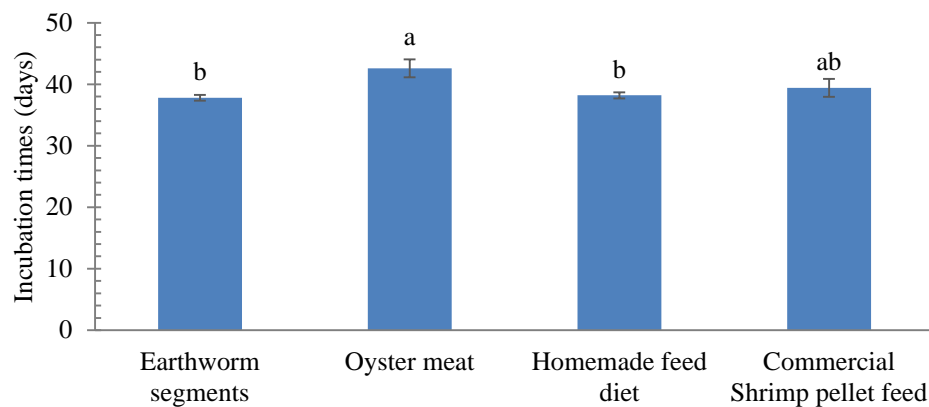


Figure 3. Mean (SE) days required for free living juveniles to be released from gravid females, fattened with four different feed, at 28 ± 3 °C. Significant differences are indicated by different letters above the error bars within each variable ($P < 0.05$; $n=5$)

Table 2 Number of gravid females, collected at 15 days interval of breeding and percent spawning success, within 45 days of breeding.

Experimental feed	Average size of gravid female		Number of free living juvenile	Free living juvenile per female body weight (juvenile/g.)
	Weight (g.)	Length (cm.)		
Earthworm segments	57.15±3.29	13.98±0.12	616.40±23.01 ^a	10.87±0.47 ^a
Oyster meat	57.38±3.65	13.98±0.11	467.60±49.36 ^b	8.27±0.95 ^b
Homemade feed diet	47.83±8.96	13.98±0.12	356.20±24.83 ^b	9.25±2.50 ^b
Commercial Shrimp pellet feed	57.18±4.24	13.98±0.11	421.00±64.82 ^b	7.60±1.29 ^b

*Mean values (mean ± SE) in column with different superscript are significantly different (P<0.05).

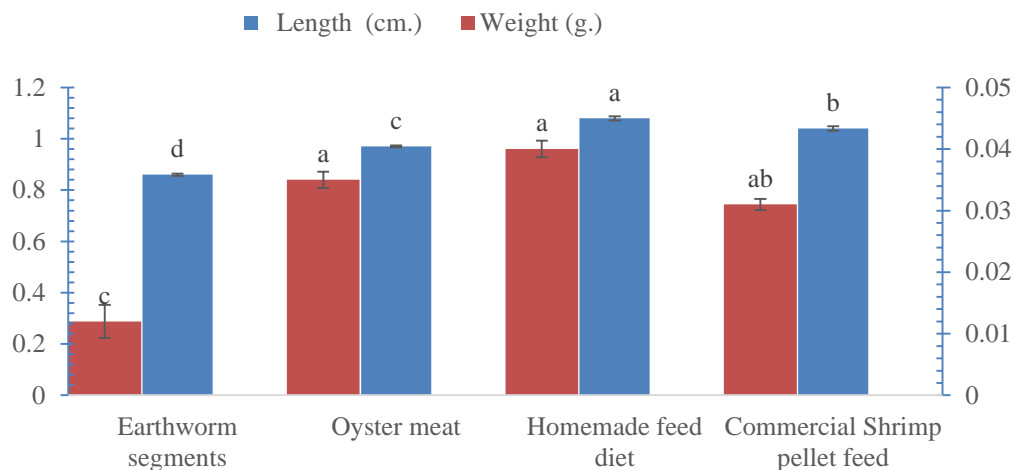


Figure 4. Mean (SE) length and weight of free living juveniles, at time of release, from gravid female, fattened with four different feed at 28±3 °C. Significant differences are indicated by different letters above the error bars within each variable (P <0.05; n=5)

At the time of release, brood females, fattened with homemade feed diet gave larger free living juveniles size, than those brood female fattened with all other tested feeds (P<0.05). Brood females, fattened with earthworm segments gave smallest free living juveniles size (P<0.05) (Fig.4).

Free living juveniles from each treatment (N=100) continued to be raised for 30 additional days, under experimental condition, to compare seed performance from each brood treatment. Results showed that red claw crayfish seed from brood crayfish, fattened with oyster meat obtained the greatest growth; in contrast, seed from brood crayfish, fattened with earthworm segments showed lowest growth (Table 3). There was no different among brood crayfish, fattened with earthworm segments, oyster meat, homemade feed diet and commercial shrimp pellet feed, in regards to crayfish survival ($P>0.05$). At the end of this experiment, brood crayfish survival, fattened with earthworm segments, oyster meat, homemade feed diet and commercial shrimp pellet feed showed 47.20 ± 2.11 , 46.40 ± 3.33 , 52.40 ± 2.29 and 48.40 ± 3.91 percent, respectively.

Table 3 Mean (SE) size and growth rate of crayfish seed, reared from broodstock, fattened with four different feed within 30 days of release

Experimental feed	Average size at the end of experiment		Average growth rate	
	Weight (g.)	Length (cm.)	Weight gain (mg./day)	Length gain (mm./day)
Earthworm segments	0.20 ± 0.01^b	2.04 ± 0.05^c	6.40 ± 0.20^c	0.38 ± 0.01^b
Oyster meat	0.34 ± 0.03^a	2.26 ± 0.10^a	10.29 ± 0.46^a	0.43 ± 0.01^a
Home-made feed diet	0.27 ± 0.03^a	2.31 ± 0.08^b	7.86 ± 0.47^b	0.41 ± 0.01^a
Commercial Shrimp pellets feed	0.30 ± 0.05^a	2.34 ± 0.09^b	8.85 ± 0.66^b	0.42 ± 0.01^a

*Mean values (mean \pm SE) in column with different superscript are significantly different ($P<0.05$).

All water quality parameters remained in acceptable standards for culture throughout the experiment, with temperature 28 ± 3 °C, pH 7.5-8.1, un-ionized ammonia (NH_3) 0.01-0.03 mg/L, nitrite (NO_2) 0.01-0.02 mg/L., dissolved oxygen levels 5-7 mg/L and total alkalinity (as CaCO_3) averaged 125 mg/L.

Discussion

Presently there are no commercial pellet feeds in Thailand, made specific for red claw crayfish, especially feed for maturation and broodstock development. Crayfish farmers in Thailand most commonly will use marine shrimp or freshwater prawn feeds for all crayfish stages. , Commercially

available shrimp or prawn feed are generally 30 - 43% protein, based on size. As well, the larger the feed pellet size, the more the protein content decreases. Commercial shrimp or prawn feed pellets, having a size of 0.3-0.4 mm length, contain 25 - 32 % protein. This is the main feed used for red claw crayfish that have attained a size of more than 10 grams body weight, and includes brood red claw crayfish. However, protein requirements during crayfish maturation and reproduction are much higher, comparing to non- reproductive stages (Harrison, 1990). A range of 40-45 percent crude protein and 14.65 - 16.72 kJ g⁻¹ energy content supports higher eggs production and hatchability, in red claw crayfish broodstock (Ladan, 2004). For this reason, local feed ingredients containing required nutrition to support higher juvenile red claw crayfish production were investigated in this study. Such feeds would also need to be cost effective or more reasonable than commercially available feeds. After brood crayfish were fattened with 4 different feeds types for 30 days before released to continuously mating for 45 days, it was found that the brood fed with oyster meat (37 % protein content) and homemade feed diet (40 % protein content) spawned better and faster than the brood fed with earth worm segments and commercial shrimp pellet feed (Table 1). This is likely due to oyster meat and homemade feed diets being rich in protein and cholesterol, also contain sexual steroids that trigger vitellogenesis. Raw oysters contain high amounts of cholesterol, 231 mg/100 g. (Bureau of Nutrition, 2014), including micronutrients such zinc. Squid also contain high amounts of cholesterol, 251 mg/100 g. (Bureau of Nutrition, 2014). Zinc, contained in oyster (25 mg/100 g., Bureau of Nutrition, 2014) and pumpkin (10 mg/100 g., Bureau of Nutrition, 2014), also can have effects on growth performance, antioxidant responses and reproductive performance in different fish species (Egwurugwu, *et al.*, 2013; Jiang *et al.*, 2016). Therefore, brood crayfish fed oyster meat or homemade feed diet for several consecutive days exhibited higher gonadal maturation resulted in enhanced spawning performances. It is suggesting that oyster meat and homemade feed diet are useful in manipulating gonadal activity. There is no report on cholesterol amount containing in earth worm segments or commercial pelleted feed, only records of lipid amounts (7-10 % in earth worm (Edwards, 1985) and 4-5% in commercial pellets feed). It is assumed that low lipid counts mean low cholesterol contained in the feed. Number and size of free living juveniles, were considered critical factors for assessing total hatchery production. The nutritional analysis indicated that earthworm (*E. euginae*) segments served as a rich protein source (60-70%) but also as a source of essential amino acids (Anitha and Jayraaj, 2012); meaning that earthworm proteins were of high quality. Proteins act as source of amino acids, essential for the early stages of embryogenesis (Wilson, 2002). Earthworms might

provide balance of essential amino acids in the eggs, results to successfully embryonic development. Results found that shortest incubation times and highest number free living juvenile were obtained from the brood fattened with earthworm segments (Fig.2; Table 2). However, their size was smallest; and also having lowest growth (Table 3). This is likely as a result of females with greater amounts of resources available for reproduction, are expected to produce more offspring (Goncalves, *et al.*, 2011). Thus, more offspring competing for the same amount of resources can only lead to smaller size free living juveniles. Brood female crayfish fed with earthworm segments produce more offspring; but, their offspring shown lower growth performance. A higher growth performing crayfish juvenile is more preferred; when factoring in production and variable marketing costs, necessary for business planning, cash flow and break even.

Acknowledgment

This work was supported by grants from the Rajamangala University of Technology Tawan-ok, Thailand. The author would like to offer particular thanks to Mr. Suttaya Pong-Rad, responsible for experimental facilities support. The authors also would like to extend their warmest gratitude to Mr. Eric Hutching, for his review.

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(Received: 29 July 2018, accepted: 30 August 2018)