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REVIEW ARTICLE

Moult induction methods in soft-shell crab production

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Abstract

Soft-shell crabs are gaining attention internationally as a more lucrative option of selling commercially important portunid species due to their ease of consumption, high nutritional values, and unique and excellent taste. This product, however, is only attainable in captivity as crabs are harvested right after moulting when their exoskeletons are still soft. Among the most crucial factor in soft-shell crab production is the moult induction method. Shorter moult intervals imply more soft-shell crabs could be produced, increasing productivity and profit for farmers. This review describes the moulting event, soft-shell crab production process and production systems, and highlights the currently available and potential moult induction methods, including eyestalk ablation, limb autotomy, the use of ecdysteroid, phytoecdysteroid, biogenic amines and methyl farnesoate, the inhibition of moult-inhibiting hormone (MIH) and the regulation of physical parameters. This review further compares these moult induction methods and their benefits towards soft-shell crab production.

KEYWORDS
aquaculture production, crab aquaculture, moult induction method, moulting, portunid, soft-shell crab

1 | INTRODUCTION

Marine portunid crabs (Decapoda: Crustacea) are sought-after delicacies with high commercial value and of great aquaculture potential (Azra et al., 2020; Azra & Ikhwanuddin, 2015; Fazhan, Waiho, Darin Azri, et al., 2017; Zmora et al., 2005). Capture of marine portunid crabs support the livelihood of many local coastal communities (Stacey et al., 2019; Waiho et al., 2017) and with the increasing global catch trend of crabs, they are among the key players in the economic growth of many countries (FAO, 2018). Some portunid species, such as the mud crab of the genus *Scylla*, are being extensively cultured in the Asia-Pacific region due to their high market price (Fazhan et al., 2017b; Shi et al., 2019; Waiho et al., 2019). In addition to being sold in their hard-shell condition, marine portunid crabs are also being

In general, soft-shell crabs can be produced from any edible portunid crab species. Focus has been given, however, on species with high commercial value and abundant in their native geographical regions. For example, the Atlantic Blue Crab *Callinectes sapidus* is the primary species used to produce soft-shell crabs in the United States (Chaves & Eggleston, 2003); blue swimming crab *Portunus pelagicus* is used to produce soft-shell crabs in India (Maheswarudu et al., 2008); while mud crabs (*Scylla* spp.) are the preferred candidates for soft-shell crab production in many countries around Asia (Fujaya, 2011; Shelley & Lovatelli, 2011; Tobias-Quinitio et al., 2015). Eliminating the necessity of extracting meat from its hard exoskeleton, soft-shell crabs can be consumed whole and this is the primary factor for its higher market price. In addition, soft-shell crabs are also gaining popularity and perceived as a healthy food choice due

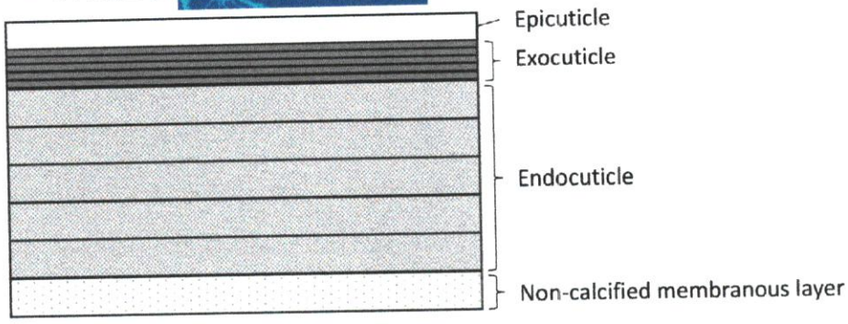

FIGURE 1 Primary layers of crab cuticle

nutritional value of the edible parts of soft- and hard-shell crabs, He et al., (2016) found that soft-shell crabs were of higher crude protein, moisture, ash and phosphorus contents than hard-shell crabs, whereas the total free amino acids were similar between them.

The production and commercialization of soft-shell crabs started about 150 years ago in the United States and is considered as among the first forms of aquaculture (Roberts, 1905), although it was reported that Native Americans were already consuming soft-shell crabs prior to the arrival of English settlers (Oesterling, 1988). In Asia, the rise in soft-shell crab production occurred during the early 1990s due to an increase in the demand for soft-shell crabs in both domestic and international markets (Tobias-Quinitio et al., 2015). Since then, various studies were conducted to increase the production of soft-shell crabs such as construction of efficient culture systems (Tavares et al., 2018), development of suitable diets (Lwin, 2018), and optimizing physical rearing parameters (Agus et al., 2014). Also, with the advent of technology, a greater understanding on the moulting process of crabs has been achieved by studying their transcriptomic gene expression (Shaymal et al., 2018; Tran et al., 2019). The global status of soft-shell crab production has been reviewed by Hungria et al., (2017). Although the exact production quantity of soft-shell crabs is unknown, most of the soft-shell crab exporting countries are main crab-producing countries, with the Asian continent as the main producer of soft-shell crabs (Hungria et al., 2017; Tobias-Quinitio et al., 2015) and the demand for soft-shell crabs exceed its supply.

In the production of soft-shell crabs, the main concern of farmers is how to induce moulting in crabs after stocking as shorter moult duration means shorter rearing time, lesser feed cost and other inputs, and therefore, greater profit (Rahman et al., 2020). This review describes the natural moulting process of crabs, the current knowledge on the mechanism behind it, moult induction methods currently used by soft-shell crab farmers and also other potential induction methods that could be used in large-scale culture in the future. Due to the narrative nature of this review, the methodology employed is briefly described (Baethge et al., 2019; Green et al., 2006). Two online databases, Web of Science and Scopus, were searched within the article title using the term "soft-shell crab", "molting" AND "crab", "eyestalk ablation", "limb autotomy", "limb removal", "molt induction" AND "crab", and "ecdysteroid" AND "crab", from 1975 to 2020. Only articles and reviews were included. In addition studies that may have not been indexed in either Web of Science or Scopus databases.

2 | MOULTING

2.1 | Moulting stages and the process

The main process that is involved in the production of soft-shell crabs is moulting. Moulting, also known as ecdysis, is a common phenomenon experienced by many invertebrates with exoskeletons, including crustaceans, to attain growth (Hyde et al., 2018). This process is risky because between moulting and the hardening of their new exoskeletons, crabs are incapacitated and are vulnerable to predators. At the same time, moulting is beneficial because it allows the regeneration of damaged tissues and missing appendages (Hopkins, 2001).

In portunid crabs, the moulting process can be divided into four main stages, that is intermoult, premoult, ecdysis and postmoult (Quinitio & Estepa, 2011; Roer & Dillaman, 2018). The cuticle layers involved during moulting are the epicuticle (outermost), exocuticle, endocuticle and non-calcified membranous layer (innermost) (Figure 1). The outermost epicuticle consists of an organic matrix rich in protein, lipid and calcium salts but without chitin whereas the organic matrix of the inner three cuticle layers is of protein and chitin microfibrils (Roer & Dillaman, 2018). The intermoult stage of portunid crabs is often the longest stage where cuticular formation is completed and the outer exoskeleton is hard – cuticle completely deposited and fully mineralized (Priester et al., 2005) – with minimal sign of epidermis separation from the exoskeleton (Freeman et al., 1987; Mangum, 1985). Most of the crabs stocked for soft-shell crab production are commonly in this moult stage.

The premoult stage can then be further classified into early premoult, mid premoult, and late premoult (Sugumar et al., 2013). Entering premoult stage, a new exoskeleton starts to form underneath and retracts from the cuticle of the old exoskeleton (early premoult stage). This separation is initiated by crustecdysone and caused by a moulting secretion of digestive, chitinolytic enzymes (Priester et al., 2005; Roer et al., 2001). This process is also known as apolysis. Eventually, the inner, non-calcified membranous layer and subsequent calcified layer of the old cuticle – the endocuticle – are

clear space between the new and old exoskeleton is visible, with no clear pigmentation. The subsequent mid premoult stage is characterized by the presence of pink pigmentation at the edge of the new exoskeleton as a result of carotenoid mobilization in the epidermis (Mangum, 1985). At the late premoult stage, its coloration changes from pink to dark red as maximum concentration of carotenoid is present (Mangum, 1985). It is also during this stage that the resorption rate of the digested old exoskeleton is at its highest (Priester et al., 2005).

At the beginning of moulting, ecdysial sutures at predictable sites such as underneath and at the rear of the carapace (posterior and lateral margins) are observed as the new exoskeleton expands from within. The dorsal carapace is still attached to the anterior margin of the old exoskeleton similar to a hinge. The new exoskeleton increases in size, along with the ecdysial sutures while the crab backs out from the old exoskeleton. Once completely detached from the old exoskeleton, the crab continues to take up more water in order to maximize the expansion of the new exoskeleton (Mangum, 1985; Waiho et al., 2015). Right after moulting, the crab's new exoskeleton is still uncalcified and soft, thus movement is greatly hindered with very minimal locomotor activity and large chelipeds are not functional, rendering it highly vulnerable to predators (Priester et al., 2005; Waiho et al., 2015). It is during this stage that they are harvested as soft-shell crabs.

The calcification of the epi- and exocuticle (the two outermost cuticle layers) begins in several hours after moulting—postmoult stage (Shafer et al., 1995). Amorphous calcium carbonate, together with α-chitin and β-keratin, are deposited along the epicuticle and the inner and outer edges of the exocuticle, giving early strength and stability to the newly moulted crab (Roer & Dillaman, 2018). The construction and mineralization of endocuticle come after the formation of the first two cuticle layers, that is epi- and exocuticle (Pratoomchat et al., 2002). Cuticle increases in thickness due to the rapid precipitation of calcium carbonate, which gradually change to calcite deposition (Dillaman et al., 2005). Mineralization of the exocuticle and the concurrent calcification of the endocuticle continues until a non-calcified membranous layer is secreted. This marks the end of exoskeleton mineralization and indicates the transition to intermoult stage (Dillaman et al., 2005; Roer & Dillaman, 2018).

2.2 | Hormonal regulation

As in all crustaceans, portunid crabs experience intermittent growth and moulting is a prerequisite for growth and development. The moulting process is regulated by several crucial hormones. Moult-inhibiting hormone (MIH), a polypeptide neurohormone that negatively regulates moulting from the X-organ sinus gland (XO-SG) complex by reducing the intracellular second messenger cAMP, suppresses the synthesis of moulting hormones known as ecdysteroids, primarily produced in the Y-organ (Nakatsuji et al., 2009; Rodriguez

the physiologically active form 20-hydroxyecdysone (20E) which is activated in the peripheral tissues (Hyun, 2018). Generally, 20E and ecdysone concentrations are low during intermoult, highest during premoult and decrease again during postmoult (Abdullah-Zawawi et al., 2021; Styrishave et al., 2004; Thomton et al., 2006). Apart from 20E, another active form of ecdysteroid, that is ponasterone A (PoA), exists in some crustacean species. For example, PoA is the major type of ecdysteroid rather than 20E in the haemolymph of premoult *C. sapidus* (Chung, 2010) and *C. maenas* (Styrishave et al., 2008). MIH is, in turn, regulated by the extracellular signal-regulated kinases (ERK)/mitogen-activated protein kinases (MAPK) pathway and the moulting process is accelerated when the phosphorylation of ERK is inhibited during early- and mid-postmoult, and intermoult stages (Imayavaramban et al., 2007). In the absence of phosphor ERK, protein kinase C-mediated pathway and subsequently steroidogenic acute regulatory protein (StAR) protein synthesis (an essential ingredient for steroidogenesis, including ecdysteroid biosynthesis) are activated (Imayavaramban et al., 2007; Stocco, 2000).

In addition to ecdysteroids, neuropeptide crustacean cardioactive peptide (CCAP) – produced from the pericardial organs of crab or ventral nerve cord of crayfish – is also shown to be involved in crustacean moulting (Phlippen et al., 2000). The haemolymph level of CCAP increases about 30- to 100-fold in crab (*C. maenas*) and crayfish (*Orconectes limosus*), respectively, during moulting and drops a few hours after postmoult, suggesting their potential involvement in successful termination of the moulting process (Philippen et al., 2000).

Methyl farnesoate (MF) is produced by the mandibular organs (MOs) of crustaceans and is chemically similar to insect juvenile hormone III (Laufer et al., 1987; Xie et al., 2016). MF is negatively regulated by mandibular organ inhibiting peptide hormone (MOIH) produced by the XO-SG complex in the eyestalk (Reddy et al., 2004). It was shown *in vitro* that methyl farnesoate stimulated the secretion of ecdysteroid in Dungeness crab *Cancer magister* (Tamone & Chang, 1993). In addition to inducing ecdysis (Reddy et al., 2004; Rotllant et al., 2000), MF is also involved in gonadal maturation (Laufer et al., 1998; Rotllant et al., 2000), morphogenesis (Rotllant et al., 2000) and protein synthesis (Paulson & Skinner, 1988).

3 | SOFT-SHELL CRAB PRODUCTION PROCESS

Soft-shell crab farming starts with securing suitable sub-adult candidates. In most Asian countries where the production of soft-shell crabs is well established and being conducted in large-scale, seedstocks are still sourced from the wild (Fujaya, 2011; Tobias-Quinitio et al., 2015). This put tremendous pressure on the already decreasing wild crab populations and is not sustainable (Fazhan et al., 2017c; Waiho et al., 2018). With the recent advancement in larval rearing and juvenile culture of crabs, Southeast Asian Fisheries Development Center (SEAFDEC) in the Philippines and Gulf Coast

2015) and blue crabs (*C. sapidus*) (Perry et al., 2010), respectively, using hatchery-reared crablets.

Generally, the common stocking sizes used are 50–80 mm carapace width (CW) (60 – 100 g) for *Scylla* spp. (Fujaya, 2011; Tobias-Quinitio et al., 2015) and 40–50 mm CW for *C. sapidus* (Perry et al., 2010). Crabs are usually stocked individually in cages throughout the rearing period to facilitate easier identification of moulting and harvesting, and to avoid cannibalism (Fazhan et al., 2017c). Feeding is carried out once or twice daily using a wide variety of protein sources such as trash fish, bivalves, and formulated diets at 5%–10% of their initial biomass (Fujaya, 2011; Perry et al., 2010; Shelley & Lovatelli, 2011). Excess feed is often removed an hour after feeding.

Daily visual monitoring is conducted every four to five hours to identify premoult crabs. Newly moulted crabs are retrieved immediately. The identification of premoult period is feasible by visual inspection, that is the visualization of the swimming legs (Freeman et al., 1987; Ostrensky et al., 2015). When a crab is entering the premoult period, new exoskeleton is almost ready and can be seen beneath the old exoskeleton in the form of a white line along the edges of the propodus segment of its swimming legs. When the white line changes to reddish coloration, moulting is expected within 24–48 hr (Kennedy & Cronin, 2007). Since the calcification process of new exoskeleton occurs immediately after moulting, only a short time frame of 1–3 hr is available for soft-shell crab collection before the new exoskeleton starts to exhibit a firm and papery texture, upon which the crab loses its commercial value (Wheatly, 1999) (Figure 2).

After collection, soft-shell crabs are immediately cleaned using freshwater, individually packed and frozen at −20°C while still alive to ensure freshness and quality of the final soft-shell crab products. Storage of frozen soft-shell crabs at −20°C maintains their freshness for at least a year (Shelley & Lovatelli, 2011) and they can be easily stored and transported in the frozen state.

4 | SOFT-SHELL CRAB PRODUCTION SYSTEMS

The high market demand and the scarcity in obtaining soft-shell crabs from wild-caught fisheries drive the need for a continuous production of soft-shell crabs in captivity. The current available soft-shell crab production systems are adequately reviewed by Tavares et al., (2018). Hence, only a brief introduction to the common soft-shell crab production systems is provided in this review.

4.1 | Open systems

The open systems are considered the most traditional systems where a selected coastal zone with tidal influences is enclosed with wooden fences (Oesterling, 1993). Sub-adult crabs are then stocked within the enclosure and newly moulted crabs are harvested as soft-shell crabs. To enable an easier and more efficient monitoring and harvesting of newly moulted crabs, individual boxes or cages, either made of wood or polyethylene, are placed within the enclosure. However, the open systems have several limitations despite its cost-effectiveness in terms of construction and operation. The uncontrollable fluctuations in environmental parameters (e.g. temperature, salinity, dissolved oxygen) would affect the consistency and the overall production of soft-shell crabs (Tavares et al., 2018). Large-scale production of soft-shell crabs would take up large surface area of the coastal zones. Therefore, this will surely have an impact on the coastal ecosystem in addition to the extensive labour needed for the daily management of the open systems (Gaudé & Anderson, 2011; Oesterling, 1988).

4.2 | Semi-closed systems

In semi-closed systems, the whole production set-up of the open systems, for example the use of individual boxes or cages, are incorporated into inland aquaculture ponds to enable a better monitoring and control over the environmental parameters. Waters are often sourced directly from the adjacent brackish environment and walkway structures are constructed perpendicularly across rows of boxes or cages to facilitate easier feeding and monitoring activities (Tavares et al., 2018; Trino et al., 2001). Although the semi-closed systems allow better protection from weather and predators, and provide some control over the environmental parameters, especially

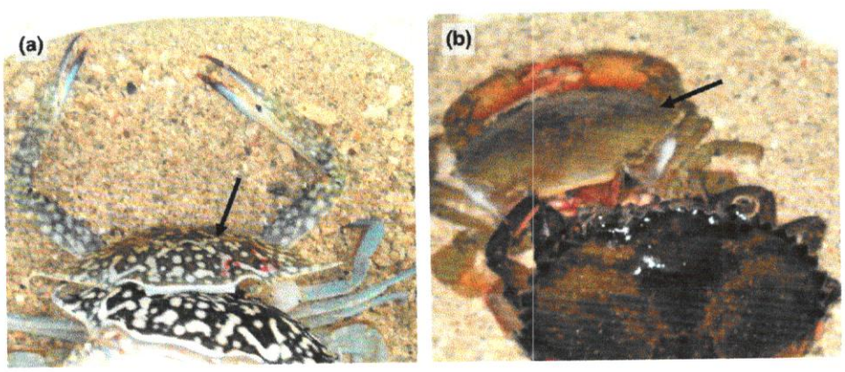

FIGURE 2 Newly moulted *Portunus pelagicus* (a) and *Scylla olivacea* (b).

salinity, they are still very much dependent on the water quality of their source (Kennedy & Cronin, 2007).

4.3 | Closed systems

The closed systems are independent of the natural environment and can be constructed and maintained at any inland indoor facilities, thus giving full control over site selection. In general, closed systems incorporate recirculating systems and crabs are reared in individually connected compartments. The use of stackable individual compartment allows high-density production and enable better monitoring and harvesting. Also, the closed systems allow easy incorporation of other forms of automation, such as the incorporation of monitoring systems to enhance feeding and allow the timely collection of newly moulted crabs. However, the attractive benefits of the closed systems are accompanied by high investment and production costs, and the requirement for skilled labour (Oesterling, 1988; Tavares et al., 2018).

5 | MOULT INDUCTION METHODS

Traditionally, soft-shell crab production involves the rearing of crabs in captivity and retrieve them right after they moult naturally (Oesterling, 1988). However, with the current knowledge on the moulting process of portunid crabs, several methods are commonly practised or potentially useful in soft-shell crab farming.

5.1 | Eyestalk ablation

Eyestalk ablation, the removal of either one (unilateral) or both (bilateral) eyestalks, is among the first methods being applied in crustaceans to induce moulting (Alikunhi et al., 1975; Browdy & Samocha, 1985). Among the common techniques used to perform eyestalk ablation includes (1) direct pinching or cutting of the eyestalk, although this method is prone to leave an open wound (Primavera & Borlongan, 1977); (2) enucleation, that is slicing the distal portion of the eye with a razor and removing the eyestalk contents via the incision (Taylor et al., 2004); (3) cauterizing the eyestalk using electrocautery devices or red-hot forceps (Chu & Chow, 1992); and (4) ligation at the base of the eyestalk using surgical thread (Diarte-Plata et al., 2012). Among these ablation techniques, cauterizing and ligation methods are preferred as they provide immediate wound closure.

Eyestalk ablation essentially removes the XO-SG complex, thus stopping or suppressing the production of MIH and allowing ecdysteroids produced by the Y-organ to act (Stella et al., 2000). Eyestalk ablation successfully reduced the moulting duration of economically important brachyuran species (Table 1). It was found their eyestalk ablated during postmoult stage or without eyestalk ablation (Rana, 2018; Sukardi et al., 2019). Also, bilateral eyestalk ablation induced faster moulting and resulted in higher moulting rate compared with unilateral ablation (Stella et al., 2000). Evidently, haemolymph ecdysteroid levels increased significantly after eyestalk ablation in crabs (Sudha & Anilkumar, 2007; Tamone et al., 2005) and were found to be even higher than the ecdysteroid levels of normal premoult individuals (Abuhagr et al., 2014; Sudha & Anilkumar, 2007).

However, ablation is known to cause adverse sensory experiences (Diarte-Plata et al., 2012), stress (Elwood et al., 2009) and death in crustaceans (Racotta et al., 1998), and these raised concerns among farmers. Eyestalk ablation also induces internal physiological changes, including disrupting the function of crustacean hyperglycaemic hormone (CHH), a stress-indicating hormone, by elevating haemolymph glucose levels (Loredo-Ranjel et al., 2017). CHH is produced in the X-organ and released from the sinus gland, and eyestalk ablation removes these two completely.

5.2 | Limb autotomy

Unlike eyestalk ablation, limb autotomy, or self-amputation of limb, commonly occurs in crustaceans including brachyuran crabs, in their attempt to escape or in response to injury (Emberts et al., 2017; McVean & Findlay, 1979). There is a pre-formed breakage plane between the coxopodite and basi-ishiopodite of the crustacean appendage where limb autotomy could occur (Cooney et al., 2017; McVean, 1982; Sy & Airriess, 2002). It is believed that two separately innervated muscles, the anterior and posterior levator muscles, that are involved in normal walking movements have autotomy-specific motor neurons to initiate and generate the required force to perform limb autotomy in crabs (Emberts et al., 2017; McVean, 1982; Moffett et al., 1987; Vidal-Gadea & Belanger, 2009). New limbs are then fully regenerated during subsequent moulting(s) (Mykles, 2001). Thus, limb autotomy induces moulting in crustaceans (Cooney et al., 2017; Rahman et al., 2020) and has been documented to trigger precocious moults in many brachyuran species (Table 2).

Limb autotomy is one of the practices used by farmers in Southeast Asia to shorten intermoult duration in the production of soft-shell crabs. It is often done by pinching or applying pressure to the merus segment of the appendage (limb) (Smith & Hines, 1991). Patterson et al., (2009) induced autotomy of the chelipeds by making a small cut near the joint of the merus, distal to the breakage plane. This method, however, does not always produce desirable results, as some crabs may not be able to regenerate back the lost limbs to their original size in one moult (Smith, 1990). Some brachyuran species, such as the commercially important blue crab *C. sapidus* would need up to three moults (Smith, 1990) whereas mud crab *Scylla* juveniles need at least two moults to regenerate the missing limbs to the original size (Quinitio & Estepa, 2011). In addi-

1 Eyestalk ablation in commercially important brachyuran species

	Species	Sex	Treatment (*n*)	Ablation method	Days to moult (Mean ± SD)	Moulting rate (%)	Other changes after ablation	References
dae	*Callinectes sapidus*	N/A	UEA (9) C (9)	Direct pinching	31.5 ± 15.50[a] 62.6 ± 17.50[b]	N/A N/A	N/A	Amador del Ángel et al., 1999
	C. sapidus	MF	BEA (15) C (5)	Cauterization	51.6 ± 0.64[^] N/A	80.0 N/A	N/A	Havens & McConaugha, 1990
	Callinectes arcuatus	JF (<60 mm) JF (>70 mm)	UEA (18) C (18) UEA (18) C (18)	Cauterization	15.0 ± 2.00[a] 20.0 ± 2.00[b] 15.0 ± 2.00[A] 30.0 ± 2.00[B]	N/A N/A N/A N/A	N/A	Vega-Villasante et al., 2007
	Ovalipes trimaculatus	J	UEA (8–10) C (8–10)	Cauterization	No moulting 43 – 45	N/A N/A	N/A	Martelli et al., 2019
	Libinia emarginata	MF IF	BEA (43) BEA (51)	Direct cutting	No moulting 14 – 35	0 N/A	- increased spawning	Hinsch, 1972
	L. emarginata	IF	BEA (14) C (7)	Cauterization	27.4 ± 2.31[^,a] 71.6 ± 0.39[^,b]	N/A N/A	- reduced carapace length - increased haemolymph methyl farnesoate level	Laufer et al., 1997
inucidae	*Oziotelphusa senex senex*	MM	BEA (68) C (40)	Cauterization	N/A N/A	17.6[*] 0	- increased in weight of mandibular organs and Y-organs	Sainath & Reddy, 2010
	O. senex senex	F	BEA (40) C (40)	Cauterization	N/A N/A	7.5 0	- high mortality (22.5%) in BEA group versus control (0%).	Reddy, 2019
	O. senex senex	MM MF	BEA (34) C (26) BEA (29) C (37)	Cauterization	N/A N/A N/A N/A	N/A N/A N/A N/A	- increased in weight of mandibular organs and methyl farnesoate levels	Nagaraju et al., 2004

percase alphabets or asterick in each experiment indicate significant difference ($p < 0.05$).

ure females; IF, immature females; M, males; F, females; MM, mature males; IM, immature males; *n*, number of individuals; C, control; UEA, unilateral eyestalk ablation; BEA, bilateral eyestalk

N/A, not available.

es standard error instead of standard deviation (*SD*).

2 Limb autotomy in commercially important brachyuran species.

	Species	Stage/sex	Number of appendages autotomized (n)	Days to moult (Mean ± *SD*)	Moulting rate (%)	Other changes after limb autotomy	References
dae	*Callinectes sapidus*	Im	1 – right CH (26) 4 – CHs, right first WL & left SL (26) C (26)	Intermoult lengths of 1st, 2nd and 3rd moults were not significant among treatments.	N/A	- Multiple limb autotomy resulted in smaller size (lesser CW increment)	Smith, 1990
	Scylla olivacea	Im	1 – right CH (30) 2 – CHs (30) 3 – right WLs (30) 6 – all WLs (30) 4 – right CH and WLs (30) 8 – all appendages except SLs (30) C (30)	Intermoult length of 1st and 2nd moults were shortened when both CHs and when all appendages were removed.	N/A	- 60%–90% showed relatively small claws and walking legs - Unilateral and bilateral CH removal, and the removal of all appendages increased CW - Limb autotomy increased specific growth rate during 1st moult - No effect on survival	Rahman et al., 2020
	Scylla olivacea	IM IF	6 – WLs (48) 8 – all appendages except SLs (48) C (48) 6 – WLs (48) 8 – all appendages except SLs (48) C (48)	28.07 ± 11.71^{b} 19.81 ± 5.87^{a} 59.31 ± 12.43^{c} 26.51 ± 8.00^{b} 19.58 ± 3.45^{a} 57.04 ± 15.28^{c}	87.50 ± 6.25^{a} 89.58 ± 7.22^{a} 54.17 ± 7.22^{b} 81.25 ± 16.54^{ab} 93.75 ± 6.25^{a} 50.00 ± 12.50^{b}	- Removal of all appendages except SLs resulted in lowest size and weight increment - No effect on survival	Fujaya et al., 2020
	Scylla paramamosain	Stage 2 crablets	1 – CH (75) C (75)	6.2 ± 0.1^{a} 5.8 ± 0.1^{b}	98.7 ± 2.3^{a} 97.3 ± 2.3^{a}	N/A	Gong et al., 2015
	Scylla serrata	Im	1 – CH 2 – CHs C	10.3 ± 0.38^ ,a $10.3 \pm 0.52^{\wedge,a}$ $12.5 \pm 0.66^{\wedge,b}$	N/A	- Specific growth rate of crabs with 2 autotomized chelipeds had the lowest specific growth rate after first moult but was the highest after second moult.	de la Cruz-Huervana et al., 2019
lae	*Eriocheir sinensis*	Im	1 (16) 2 (16) 3 (16) 4 (16) 5 (16) C (16)	Intermoult length of 1st (but not 2nd) moult decreased with increasing number of autotomized appendages.	>90 (no significant difference for all treatments)	- Specific growth rate positively correlated with number of appendages autotomized - Fully regenerated appendages after 2nd moult. - No effect on feeding	He, Wu, et al., 2016

ture crabs; IM, immature males; IF, immature females; n, number of individuals; C, control; CH, cheliped; WL, walking leg; SL, swimming leg; CW, carapace width; N/A, not available; uppercase s in each experiment indicate significant difference ($p < 0.05$).

:s standard error instead of standard deviation (S.D.).

s significant when compared to control.

of limbs autotomized. Moulting is hastened if multiple limb autotomy occurs during intermoult stage but no effect or even longer intermoult duration is observed if limbs were autotomized during premoult, especially mid- to late premoult (McCarthy & Skinner, 1977). Thus, for farmers to apply limb autotomy in soft-shell crab production, knowledge on the accurate determination of moult stage is important. The shorter moult cycle induced by limb autotomy is highlighted in the studies of Fujaya et al., (2020) and Rahman et al., (2020) on a commonly used species for soft-shell crab production, that is mud crab *S. olivacea*.

Limb autotomy comes with enormous costs. Crabs subjected to limb autotomy are at risk of huge loss of haemolymph (Fleming et al., 2007), and a feeding and locomotion handicap depending on the number and types of limb lost (Emberts et al., 2017; Smith & Hines, 1991). Also, crabs subjected to multiple limb autotomy exhibited smaller size (carapace width) increment (Fujaya et al., 2020; Smith, 1990) and body weight (Fujaya et al., 2020; He et al., 2016) compared with intact controls post-autotomy moult. The regeneration of appendages, especially chelipeds, after limb autotomy requires even more energy and time. As soft-shell crabs are also classified based on the presence or size of limbs, crabs with smaller newly regenerated limbs upon moulting will not benefit soft-shell crab farmers from an economic viewpoint.

5.3 | Ecdysteroid application

Ecdysteroids are the main hormones that regulate moulting in crustaceans and their levels are positively related to moult stages (Techa & Chung, 2015). Thus, manipulating ecdysteroid levels in haemolymph theoretically would result in faster moulting. After several successful trials of inducing moulting in other crustacean species using 20E (Krishnakumaran & Schneiderman, 1970; Warner et al., 1969), Raghavan and Avanath (2019) showed that when administered during early premoult and intermoult stages, 20E induced shorter premoult and intermoult intervals in the adults of edible freshwater crab *Travancoriana schinerae*. However, as observed in other crustaceans, *T. schinerae* subjected to 20E injections were less likely to survive (up to 43% mortality rate in 20E treated crabs compared with up to 13% in control) (Raghavan & Ayanath, 2019). Interestingly, in mud crab *Scylla olivacea*, Tamsil and Hasnidar (2018) reported that injection of 1.0 μg/ml caused a significant increase in haemolymph ecdysteroid level, higher moulting percentage and body size and weight increment, shorter moulting duration, and survival rate was not negatively affected, whereas higher 20E dosage (1.3 and 1.6 μg/ml) resulted in a negative feedback effect and crabs exhibited lower moulting and growth performance. Due to the high mortality following ecdysteroid application, this method has not been tested in most species used in soft-shell crab production. The use of ecdysteroids on mud crab genus *Scylla*, however, has high potential as shown by the results of Tamsil and Hasnidar (2018). Future research should

5.4 | Phytoecdysteroid application

Phytoecdysteroids are plant-based secondary metabolites analogue to insect's ecdysteroids that are responsible for the regulation of moulting, growth and reproduction (Rharrabe et al., 2010). It is suggested that they form a part of a plant's defence system against insects (Schmelz et al., 2002). Although more than 300 different types of phytoecdysteroids were identified, the most common form is similar to that found in insects and crustaceans, that is 20E (Baltaev, 2000). Phytoecdysteroid levels vary greatly among plant species, and while most contain only minimal amounts, some plant families are known to exhibit high levels of ecdysteroids, such as plants of the family Asteraceae (Odinokov et al., 2002) and Lamiaceae (Rharrabe et al., 2010).

Although generally toxic to insects (induce abnormal moulting leading to developmental defect) and exhibit a strong anti-feedant effect (Dinan, 2009; Rharrabe et al., 2010; Schmelz et al., 1998), phytoecdysteroids are known to induce synchronized development and yield in commercially important arthropods (Chandrakala et al., 1998) and have great potential in arthropod culture and aquaculture (Kanazawa et al., 1972). Research on the use of phytoecdysteroid as a moulting inducer of crustaceans is currently being spearheaded by researchers in Indonesia (Aslamyah & Fujaya, 2010; Fujaya, 2011; Nikhlani & Sukarti, 2017) and Thailand (Sorach et al., 2013), with some phytoecdysteroid products already being used commercially in soft-shell crab production farms (Fujaya, 2011). The most common administration method is injection (Fujaya, 2011; Herlinah et al., 2014, 2015; Sorach et al., 2013) although trials incorporating phytoecdysteroids into feed were also being conducted (Aslamyah & Fujaya, 2010; Fujaya et al., 2011). Based on the available literature (Table 3), phytoecdysteroids extracted from the family Amaranthaceae, Lamiaceae and Moraceae induced higher moulting success and moulting percentage, in addition to promoting higher growth increment and survival rate in portunids. Although further research and optimization are needed, the use of phytoecdysteroids to induce moulting in crabs is promising.

5.5 | Control of physical parameters

5.5.1 | Temperature

Aside from directly manipulating moulting hormones and/or their related organs, changes in physical parameters such as temperature could also affect the production of neuropeptides in the Y-organ (Chung & Webster, 2005; Pitts et al., 2017). As the Y-organ is negatively regulated by MIH and CHH, and CHH is known to increase upon exposure to environmental stress (Rajendiran et al., 2016), therefore, moult inhibition or delay may occur upon stress exposure (Pitts et al., 2017). For example, maximum growth after

3 Phytoecdysteroid application in brachyurans.

of phytoecdysteroid		Tested species		Growth stage (Application method)	Phytoecdysteroid concentration	Effect	Reference
	Species	Family	Species				
ıthaceae	*Amaranthaceae tricolor* (leaf)	Portunidae	*Portunus pelagicus*	Larvae (Feed)	C 1 mg/100 g 2 mg/100 g 4 mg/100 g	- Dose of 4 mg/100 g showed higher survival at crablet stage.	Nikhlani & Sukarti, 2017
	Spinacia oleracea	Portunidae	*Scylla* sp.	Juvenile (Feed)	C A (Pro.: 46.8%; Carb.: 33.3%) B (Pro.: 41.6%; Carb.: 38.3%) C (Pro.: 35.6%; Carb.: 44.3%) D ((Pro.: 30.6%; Carb.: 49.1%) *A-D were enriched with 700 ng/g S. oleracea extract*	- Treatment D achieved 100% moulting success within 60 days. - - Treatment A and B showed higher growth increment after moulting.	Aslamyah & Fujaya, 2010
	Amaranthus spp. (developed into product called *Vitomolt*)	Portunidae	*Scylla* spp.	Juvenile (Injection)	C 9 µg/g 15 µg/g 21 µg/g	- 21 µg/g resulted in highest weight increment after moult. - 15 µg/g resulted in highest moulting percentage after 55 days.	Fujaya, 2011
	Vitomolt	Portunidae	*Scylla olivacea*	Juvenile (Injection & Feed)	C Injection only (15 µg/g) Injection (15 µg/g) + Feed (32.38 mg/g)	- 'Injection only' resulted in highest moulting percentage. - 'Injection +feed' resulted in fastest moulting response. - Both 'injection only' and 'injection +feed' had lower mortality rate than control.	Fujaya et al., 2011
eae	*Vitex glabrata* (bark)	Portunidae	*Portunus pelagicus*	Juvenile (Injection)	C 0.1 µg/g 0.2 µg/g 0.3 µg/g 0.4 µg/g 0.5 µg/g 1.0 µg/g	- Dose of 0.4 µg/g BW injected during postmoult shortened postmoult to premoult stages. - Dose of 0.5 µg/g BW injected during intermoult shortened intermoult to premoult stages. - Survival rates were equal or higher than control when phytoecdysteroids were given at postmoult and intermoult stages.	Sorach et al., 2013
ːae	*Morus* sp. (leaf)	Portunidae	*Scylla olivacea*	Juvenile (feed)	C 1.1 mg/g 1.9 mg/g 2.7 mg/g 3.5 mg/g	- Doses of 1.9 and 2.7 mg/g resulted in faster moulting.	Fujaya et al., 2018
	Morus sp. (leaf)	Portunidae	*Scylla olivacea*	Juvenile (Injection)	C 100 mg/l 125 mg/l 150 mg/l	- Dose of 100 mg/l resulted in higher (50%) moulting percentage compared with other treatments and control (all at 33.3%).	Herlinah et al., 2014, Herlinah et al., 2015

ɔl; conc., concentration; BW, body weight; Pro., protein; Carb., carbohydrate.

that the intermoult period of *C. sapidus* decreased as temperature increased, although temperature did not affect their growth per moult. *Scylla serrata* juveniles held in a constant temperature of 29°C and 32°C exhibited significant shorter moult interval (32 ± 0.8 days and 28 ± 1.1 days respectively) than those reared at ambient temperature (39 ± 0.9 days) whereas all crabs reared in 35°C died 11 days after stocking due to incomplete moulting (de la Cruz-Huervana et al., 2019). Further, crabs reared in 32°C had significantly higher specific growth rate compared with those reared in 29°C and ambient temperature in both first and second moult, although no difference was observed in weight and body size increment after each moult (de la Cruz-Huervana et al., 2019). Also, Ruscoe et al., (2004) showed that *S. serrata* instar 2 crablets exhibited shorter intermoult duration when held at high temperature (30 and 35°C) compared with at 25°C and had the highest mean weight at harvest at 30°C in salinities of 5–20 ppt. A similar observation of shorter intermoult duration in higher temperature (up to 32–34°C) was also observed in *S. serrata* larvae (Nurdiani & Zeng, 2007) and in stage 1 crablets of other *Scylla* species, that is *Scylla paramamosain* (Gong et al., 2015).

5.5.2 | Salinity

Low salinity (5 ppt) resulted in low moulting success rate (74.7 ± 2.3%) but shorter moulting interval (4.7 ± 0.1 days) compared with higher salinities (10 – 40 ppt with moulting success rate >96.0%, moulting intervals >5.0 days) in stage 1 crablets of *S. paramamosain* (Gong et al., 2015). Thus, to a certain extent within the acceptable zone, an increase in salinity induces moulting in brachyurans. Salinity has a lesser effect on moulting compared with temperature and the optimal salinity is mostly species dependent.

5.6 | Other potential moult induction methods

5.6.1 | Biogenic amines application

Biogenic amines are biogenic substances with at least one amine group. They are neuroregulatory molecules involved in various biological functions of crustaceans (Fingerman, 1994). For example, serotonin and melatonin are known regulators of crustaceans' reproduction (Fingerman, 1997; Tinikul et al., 2008), glucose homeostasis (Reddy & Pushpalatha, 2007; Tilden et al., 2001, 2003) and moulting (Reddy, 1990; Sainath & Reddy, 2010; Tilden et al., 1997). By using freshwater edible crab *Oziotelphusa senex senex* as a model, Sainath and Reddy (2010) showed that injection of melatonin (10^{-7} mol/crab) into intact crabs (n = 60) resulted in faster moulting (42 in premoult stage, 6 in postmoult stage after 28-day experimental period) compared with control (n = 40; all in intermoult stage after 28-day experimental period) and induced heavier mandibular organs and Y-organs by 29.9%

moulting. Further, no significant variations in moulting duration, and weights of mandibular organs and Y-organs were observed in crabs when serotonin and melatonin were injected after eyestalk ablation compared with crabs subjected to only eyestalk ablation (Sainath & Reddy, 2010). Thus, it was postulated that melatonin induces moulting in crabs by inhibiting the release of MIH and/or MOIH (Sainath & Reddy, 2010). In a separate study on mud crab *Scylla serrata*, injection with 0.4 ng/g body weight of serotonin resulted in significantly higher methyl farnesoate (86.5%) and ecdysteroid (132%) levels in haemolymph (Girish et al., 2017). Therefore, the effect of serotonin in moult induction of crabs appears to be species-specific.

5.6.2 | Methyl farnesoate application

As mentioned in 3.2, the sesquiterpenoid methyl farnesoate is synthesized in the MOs (Laufer et al., 1998). Its role in moulting is tightly linked with its stimulatory effect on the secretion of ecdysteroids (Homola & Chang, 1997). An earlier study showed that the length between moults of white shrimp *Penaeus setiferus* significantly reduced (average days to moult = 32.2 days) when implanted with the MOs of blue crab *C. sapidus* compared with intact controls (average days to moult was 87.6 days), those only jabbed with syringes (average days to moult = 76.5 days) and those implanted with muscle tissues (average days to moult = 70.6 days) (Yudin et al., 1980). Injection of methyl farnesoate into *T. schinerae* (10, 30, 50 ng/10 µl per injection) during intermoult and early premoult stages significantly shortened their intermoult and premoult intervals, although it also resulted in high mortality rate (up to 47% compared with control of only up to 13%) (Raghavan & Ayanath, 2019). The positive effect of methyl farnesoate on moulting was also validated in freshwater crab *O. senex senex* when 64% and 16% of females injected with methyl farnesoate (10 ng/crab) were in premoult stage and moulted respectively. Also, 76% and 24% of methyl farnesoate-injected (10 ng/crab) males were in premoult stage and moulted respectively (Reddy et al., 2004). Comparatively, the controls (intact controls and controls injected with 10% ethanol) of both sexes remained in intermoult stage (Reddy et al., 2004). Administration of methyl farnesoate also resulted in significant increase in ovarian and testicular indexes, and mean oocyte and testicular follicle diameters (Reddy et al., 2004). Based on these results, Reddy (2019) then tested the effect of dietary methyl farnesoate on the growth of female *O. senex senex*. It was found that dietary methyl farnesoate of 10^{-8} mole/crab, 10^{-9} mole/crab and 10^{-7} mole/crab resulted in higher moult percentage (25.0%, 12.5% and 10.0% respectively) and no mortality compared with eyestalk-ablated controls (moult percentage = 7.5%; mortality = 22.5%) (Reddy, 2019).

5.6.3 | Moult-inhibiting hormone (MIH) inhibition

Y-organs. Thus, theoretically, inhibition of MIH would enhance ecdysteroid synthesis and subsequently promote ecdysis. This is feasible using RNA interference (RNAi) technique. With the aid of double-strand RNA (dsRNA), Techa and Chung (2015) showed that multiple injections of CasMIH-dsRNA (15 μg per injection, approximately 30 times within 60 days) successfully reduced the expression of MIH in *C. sapidus* by about 50 times. However, the significant reduction of MIH levels in *C. sapidus* did not result in shorter moult intervals compared with control (14.5 ± 3.0 days versus 20.0 ± 3.2 days respectively). In contrast, *in vivo* injections of Cq-MIH dsRNA significantly reduced moult interval up to 32% in Australian red claw crayfish *Cherax quadricarinatus* and comparable to treatments injected with ecdysone (Pamuru et al., 2012). Similar significant reduction of moult interval in both sexes was also reported in the oriental river prawn, *Macrobrachium nipponense* after inhibition of MIH by RNAi (Qiao et al., 2018). These suggest that the effect or extent of MIH on ecdysteroid synthesis might be species-specific. In mud crabs (*Scylla serrata*), it has been shown that MIH suppressed ecdysteroidogenesis via the induction of ERK activation through Ras/Raf pathway (Imayavaramban et al., 2007). Therefore, it is highly possible that the inhibition of MIH might promote ecdysteroid synthesis and leads to shorter moulting interval in *Scylla* species.

6 | COMPARISON BETWEEN MOULT INDUCTION METHODS

Although the efficiency of a technique to induce moulting would be the main criterion during the selection of moult induction methods, farmers and aquaculturists would also need to consider other cost- and profit-related factors such as the ease of application, application cost, crab survival rate, effect on size and weight, and limb completeness (Table 4). Among the methods described in this study, manipulation of physical parameters, that is temperature and salinity would be the easiest to apply. Eyestalk ablation, limb autotomy, and the administration of moult inducers such as ecdysteroid, phytoecdysteroid and methyl farnesoate would require some form of prior knowledge and handling practice.

In terms of costing, eyestalk ablation, limb autotomy and salinity manipulation are the cheapest. In comparison to salinity control, heater and chiller are needed to manipulate temperature, and their purchase and maintenance are pricier. The cost of phytoecdysteroids is considered as moderate because these plant-derived moult inducers can be extracted in high quantity and their raw materials (plants) are readily available (Fujaya, 2011). Comparatively, pure synthetic ecdysteroid, biogenic amines, methyl farnesoate and the manipulation of MIH are more expensive and could be a deterring factor for small-scale farmers.

The ability to induce moulting is obvious in most moult induc-

4 Comparative analysis of the characteristics of different moult induction methods for soft-shell crab production

teristics	Eyestalk ablation	Limb autotomy	Ecdysteroid	Phytoecdysteroid	Physical parameters		Biogenic amines	Methyl farnesoate	Moult-inhibiting hormone inhibition
					Temperature	Salinity			
application	●	●	●	●	●	●	○	●	○
tion cost	○	○	●	●	●	○	●	●	●
nduction	●	●	●	●	●	○	●	●	NA
l	●	●	●	●	●	NA	NA	○	NA
increment	NA	○	●	●	●	NA	NA	NA	NA
rement	NA	○	●	●	●	NA	NA	NA	NA
mpleteness	●	○	●	●	●	●	●	●	●

fficult; ●, medium; ●, high/easy; NA, no data available.
etween species.

ecdysteroids (Qiau et al., 2018; Stella et al., 2000). The administration of ecdysteroid and phytoecdysteroid into crab directly increase its ecdysteroid level and accelerate the moulting process (Fujaya, 2011; Raghavan & Ayanath, 2019). The increase in methyl farnesoate levels stimulates the production of ecdysteroids (Reddy et al., 2004) whereas different biogenic amines regulate physiological processes such as moulting differently, with melatonin might inhibit the release of MIH or mandibular organ inhibiting hormone to promote the production of ecdysteroids and subsequent promote moulting (Sainath & Reddy, 2010).

Among all moult induction methods, limb autotomy consistently showed no significant effect on crab survival (Table 2) as limb loss and regeneration is common in crabs (Edwards, 1972; Hopkins, 2001) whereas eyestalk ablation moderately affects survival of crabs (Table 1) as the process is stressful and eyestalk-ablated crabs are more prone to infection (Taylor et al., 2004). In comparison, the negative effect of methyl farnesoate on survival has been reported in crabs (Raghavan & Ayanath, 2019) and other commercial crustacean species such as crayfish (Abdu et al., 2001) and shrimps (Alnawafleh et al., 2014). The administration of ecdysteroids and phytoecdysteroids are known to enhance the size and weight of crabs after moulting (Fujaya, 2011; Tamsil & Hasnidar, 2018). The size and weight increments in soft-shell crabs were less obvious, with smaller increments being reported in limb autotomized crabs compared with controls (Table 2).

All moult induction methods except limb autotomy do not require the removal of limbs, thus have no effect on the aesthetic value of the soft-shell crabs produced. However, crabs subjected to limb autotomy often presented with incomplete or significantly smaller new appendages (Fujaya et al., 2020). This greatly affects the market value of soft-shell crabs, in addition to lighter average body weight. Although aesthetic value could be considered as a secondary factor in selecting moult induction methods, its effect on grading and final market price makes limb autotomy unsuitable to be promoted as the ideal moult induction methods for soft-shell crab production.

7 | CONCLUSION AND FUTURE PROSPECTS

With its increasing world market demand, soft-shell crab production is among the promising revenue-generating industry for commercially important crab species aside from them being sold off as it is. Progress has been made in understanding the moulting mechanism of crabs, the production process of soft-shell crabs and moult induction methods that would effectively shorten moult intervals and allow more synchronized moulting. Induce moulting via eyestalk ablation for soft-shell crab production should be avoided as it is considered inhumane and causes significant stress towards the animal. Limb autotomy, although occurs naturally, should be used minimally as well as this method produces soft-shell crabs of uneven body size and increase in risk of infection from the open wound generated if limbs are not autotomized properly.

The main challenge of the current soft-shell crab production industry is the lack of a suitable moult-inducing method that exert minimal stress towards the animal yet still able to hasten moulting. The various methods that are able to induce faster moulting in crabs reviewed here are promising, although most of them are still in their early experimental stages. Until a mature technique is developed, soft-shell crab farmers are selective in using moult induction methods and most still rely on traditional rearing and harvesting soft-shell crabs right after moulting. Future research on the mechanism of limb autotomy is warranted as this would allow moulting to be induced without physically removing crab's appendages. Also, the use of ecdysteroid and phytoecdysteroid is promising, although subsequent studies should focus on their optimization of administered dosages and reduction of production costs.

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DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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