Plant Archives Vol. 20, Supplement 2, 2020 pp. 17-24 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

MOULTING CYCLE RELATED CHANGES IN BIOCHEMICAL, ANTIOXIDANTS GENES AND FEEDING RATES IN COMMERCIAL PENAEIDAE SHRIMPS *METAPENAEUS AFFINIS* (H. MILNE EDWARDS, 1837)

Saba Anis Alhajee*, Sajed S. Alnoor and Entisar Sultan
Department of fishers and marine resources, College of Agriculture, University of Basra, Iraq
E. mail: sabaanis60@yahoo.com

Abstract

There is an urgent need to understand the behaviors of important species in aquaculture, to facilitate their reproduction. *Metapenaeus affinis* is the most commercially important species. The hallmark of crustacean physiology is the periodic removal of its old exoskeleton, which was achieved by moulting. All processes such as crustacean metabolism, reproduction and behavior are affected by the periodic removal of the exoskeleton and the characteristics of the moulting cycle. The present study explained assess the components of biochemical structure and to estimate the antioxidant enzymes as well as to determine the feeding behavior during the different stages of moulting in *M. affinis*. The maximum level of total protein in muscle tissue (62.83%) mg/g in stage (postmoult) and the lowest level (62.05%) mg/g in stage (premoult), While A sharp reduction in total lipid level (3.01%) mg/g at stage (postmoult) was observed compared with the high levels (6.08%) mg/g at stage (moult), It had the highest value of moisture 3.80% at the stage (ecdysis) while the lowest 1.86% at the stage (premoult), the highest level of ash (0.105%) in the (intermoult) then the lowest value (0.01%) at stage (moult). The contents of glutathione, catalase and reactive oxygen species were determined in muscle tissue in the studied species, the results obtained showed the types of parameters examined different ranges within the stages of the moulting cycle. This work represents the first study of its kind and has shown that these parameters are considered biological markers suitable for determining the state of cellular oxidation in different stages of moulting in commercial shrimp *M. affinis*. Relative important index IRI was adopted to evaluate the importance of different nutrients during different stages of moulting in the studied species. The results of the study showed different nutritional ratios in the Premoult, Intermoult and Postmoult stages, while the ecdysis stage, this stage was characterized by microscopic examination 243 animals that the foregut of the gastrointestinal tract was empty. This study documents and expands our understanding of the physiological and biochemical changes that occur in *M. affinis* shrimp during four different stages of moulting, through which useful criteria can be provided in the life cycle of this commercial shrimp.
***Keyword*:** Ecdysis, biochemical constituents, glutathione, catalase, ROS, feeding, jinga shrimp.

Introduction

The shrimp is one of the largest groups of crustaceans, which contains more than 3000 species but only commercially exploits 40 species (Khodanazary, 2019). Shrimp has various benefits that are important in the economies of many East Asian countries. It is a nutritious source of meat protein and has a high economic importance for humans as an exportable commodity (Shokoohmand *et al.,* 2018). Crustaceans are characterized by continued moulting and growth even after sexual maturity (Kamaruding *et al.,* 2018). In many shrimp, the process of moulting can occur during the cycle of oogenic compared with the large crustaceans, such as the female crab. The cycle of reproduction is completed during the period of intermoult, and the moulting process occurs only after the cessation of reproduction (Promwikorn *et al.,* 2004).

The biochemical changes happen in crustaceans during molting, are indicators of their nutritional requirements and are an essential basis for determining suitable diets (Yan *et al.,* 2017). Details on seasonal changes in biochemical structure is therefore important in the study of crustacean reproductive biology (Yan *et al.,* 2017). Comparative biochemistry has evolved in crustaceans in recent years due to interest in aquaculture. Several preliminary hypotheses have been taken in the field of biochemistry of insects because of their evolutionary affinity with crustaceans specifically (Sugumar *et al.,* 2013), so that the biochemistry of crustaceans can benefit from the knowledge available about insects (Zhang *et al.,* 2017).

Arthropods do not have an adaptive immune system, which relies on highly specific antibodies and receptors (Wang *et al.,* 2012). They must rely on effective innate immunity to protect themselves against invaders (Iwanaga and Lee, 2005). Because of the large economic demand of Penaeid shrimp, which leads to research on their innate immunity, shrimp aquaculture is still limited due to infectious diseases. Phagocytosis is an important immune response to the defense when the organism is attacked by bacteria and fungi. During the phagocytosis, oxygen consumption increases and stimulates the production of a mass of reactive oxygen species (ROS). Although (ROS) plays an important role in antimicrobial defense, its excessive expression and residual (ROS) can cause cell damage (smith *et al.,* 2003).

Most cells acquire the relevant antioxidant mechanisms to maintain the lowest possible level of ROS within the cell. Protection mechanisms include both enzyme oxidation systems such as glutathione and non-enzymatic antioxidant systems such as catalase (Jyoti *et al.,* 2018). The importance of enzymes in the production of different types of chemical and biological products has become a well-established technique in the food and other industries, because processes containing enzymes usually reduce process time, waste quantity and shorten the reaction steps (Kaushal *et al.,* 2018). Moulting is likely to affect shrimp feeding behavior, as well as overall activity (such as swimming and movement) (*Tran et al.,* 2019). Penaeid shrimp stops feeding activity immediately before and after ecdysis, and can exhibit different sensitivities to environmental stresses depending on the moulting stage (*Tran et al.,* 2019). Deprivation of nutrition and moulting can affect all behaviors, and contribute to individual variation in feeding behavior, emphasizing the importance of knowledge of individual variation in aquaculture.

Some studies have been conducted such as the study of both Abdul-Sahib and Sultan (1996) possible relation of the moulting of *M. affinis* with the tidal phase was investigated. Abdul-Sahib and Ajeel (2005) studied the biochemical structure (proteins, lipids, carbohydrates, ash) and caloric contents in the males and females of the commercial shrimp *M. affinis*. The study of (Saud *et al.,* 2014) examined the nutritional value and physical condition of *M. affinis*, *Parapenaeopsis stylifera* by studying the chemical composition of the two species. Al-Maliki and Al- Khafaji (2018) studied the effect human consumption of contaminated shrimps on human health, As well as studied the use of marine shrimp as a bio-indicator of heavy metal pollution in the environmental monitoring studies. Corgos *et al.* (2007) studied is to quantify growth at moult of *Maja brachydactyla* in the Rı´a de A Corun˜a (NW Spain) using data from a mark-recapture experiment, and compare these data with two previous studies performed in the laboratory and extensive culture. Wang *et al.* (2012) illustrated the relative expression of Prx, CAT, and GPx in the cephalothorax of Chinsese shrimp. *Fenneropenaeus chinensis* was studied at transcription and translation level after one of them was silenced by double-strand RNA (dsRNA) injection. A study of jung *et al.* (2013) showed that muscle growth in crustaceans accompanied moulting and included periodic changes in size, muscle protein recovery and shrinkage characteristics during evolution. Xue *et al.* (2017) studied moulting in the crustaceans is controlled by ecdysteroids and the MIH hormon of x-organ/sinus gland and in turn prevents the moulting process. This species is the most important economic species in the decapods common in our territorial and inland waters, so it is necessary to understand the changes associated with the moulting cycle.

Hence, the main objective of this study is to assess some of the biochemical structures and feeding behaviour, As well as understand the relationship of antioxidant reactions in shrimp. During the different stages of moulting in this penaeid shrimp *M. affinis*.

Materials and Methods

Collection of Shrimps

Fresh shrimp *M. affinis* were obtained for this study from the River Al masahab East South of Al Hammar marsh Basrah Governorate. It was stored in glass basins, their capacity ponds (60 x 60 x 30 cm) with recirculating system and two aerators for each bin

Then category was approved for samples by length (5.5–8.5 cm) to monitor the phase-out moulting stages within 24 hours. During the experiment, salinity was maintained at 3.5% both in the bins and aquaria and temperature ranged from 28–30 ºC. 50% of the water is replaced daily for one month. Experimental animals are fed with 45% protein, 4% raw fat, 6% raw fiber, 11% moisture) twice a day at 07:00 and 14:00. The waste was siphoned from the aquaria once every two days, the animals were weighed to the nearest 0.01 gm using an electric balance with an accuracy of 0.01 g after disposal of excess water with absorbent tissues. The total body length, were measured with a Caliper Vernier with an accuracy of 0.01 mm.

Analysis of moulting stage

Setal development of *M. affinis* was observed at the posterior median part of the uropods. Uropods were examined and photographed under a light microscope (Olympus BX40) connected to a digital camera (DCE-2). The criteria used for moult staging were used according to Promwikorn, *et al.* (2004). For P. clarkii individuals molting cycle were classified into four stages according to Drach (1939), modified by Warner (1977) and Lowery (1988).

Estimation of total protein, total lipids, ash

- Protein (%) was estimated by calculation the amount of nitrogen by micro-Kjedahle method to determine the amount of nitrogen, and the total amount of protein obtained N * 6.25 (A.O.A.C., 2000)
- Lipids (%) were determined by sexhlet extraction method for intermittent extraction to measure lipid ratio using solvent cyclohexane for 6 hours (Folch *et al.,* 1957).
- Moisture (%) The moisture content was determined by drying a known weight of the sample using an oven at 105 ° C until the weight was constant (A.O.A.C., 2000).
- Ash (%) Determination of ash content by combustion in the oven (550 °C) from 3-4 hours (Jafri *et al.,* 1964).

Estimation of glutathione, catalase, reactive oxygen species

Reduced glutathione was measured following the method of Sedlak and Lindsay (1968). Estimation of total, protein-bound and nonprotein sulfhydryl groups in tissues with Ellmans reagent.

Catalase activity has been estimated following the method of Hadwan and Abed (2016). Data supporting the spectrophotometric method for the estimation of catalase activity.

Reactive oxygen species (ROS) were estimated using a method by Erel (2005). Anew automated colorimetric method for measuring total oxidant status.

Food

The first third of the gastrointestinal tract was adopted for species that do not have distinct stomachs, in order to study their nutritional contents after measuring their lengths to the nearest millimeter using Vernia or ruler and weighing in grams using a laboratory scale. Sartorius of German origin. The digestive tract was extracted and the first part was separated and its contents were placed in a petri dish and the sample was taken from the food after being mixed well with water and placed on a glass slide and examined by a microscope installed under a magnification of 10x and 40x. It was filled with 3/4 full, 1/2 full and 1/4 full and gave her points 0, 5,10,15,20 respectively. Points were distributed to different food ingredients depending on their suitable size, points were collected for each food component and the percentage of total points was calculated, Adopted in the classification of food ingredients as Edmondson (1959) was used to classify crustaceans and aquatic insects, while Hadi etal. (1984) and Wehr and Sheath (2003) were classified as phytoplankton. Two methods were used to determine the composition of food according to the nature of the food of the studied species, the point's method (p) and the method of repetition of presence (o) according to Hyslop (1980), Hansson (1998).

Results

Describe the stages of the moulting cycle

A detailed explanation of the moulting stages of *M. affinis*, moulting stages were characterized by morphological changes such as cuticle hardness or changes in the development of setae in pleopod and europod. On the basis of setogenesis, the moulting cycle was classified into four specific stages: postmoult, intermolt, premoult, Moult as shown in Table 1.

Table 1 : Characteristics of moulting cycle stages in *M.affinis*

Stages	Diagnostic characters
Postmoult	Granular protoplasmic matrix, setae thin walled, carapace hard and rigid with development of cuticular nodes, pleopod soft
Intermoult	Setal cone formation, setal lumens narrow, exocuticle remains hard, setal wall translucent.
Premoult	Beginning of epidermal retraction (apolysis), cuticle remains soft, retraction zone between old cuticle and epidermis widens, new setae clearly visible, epidermal retraction continues.
Ecdysis (moult)	The animal withdraws from the old cuticle and for a short time absorbs water rapidly.

Moult cycle stages based on (Drach, 1939)

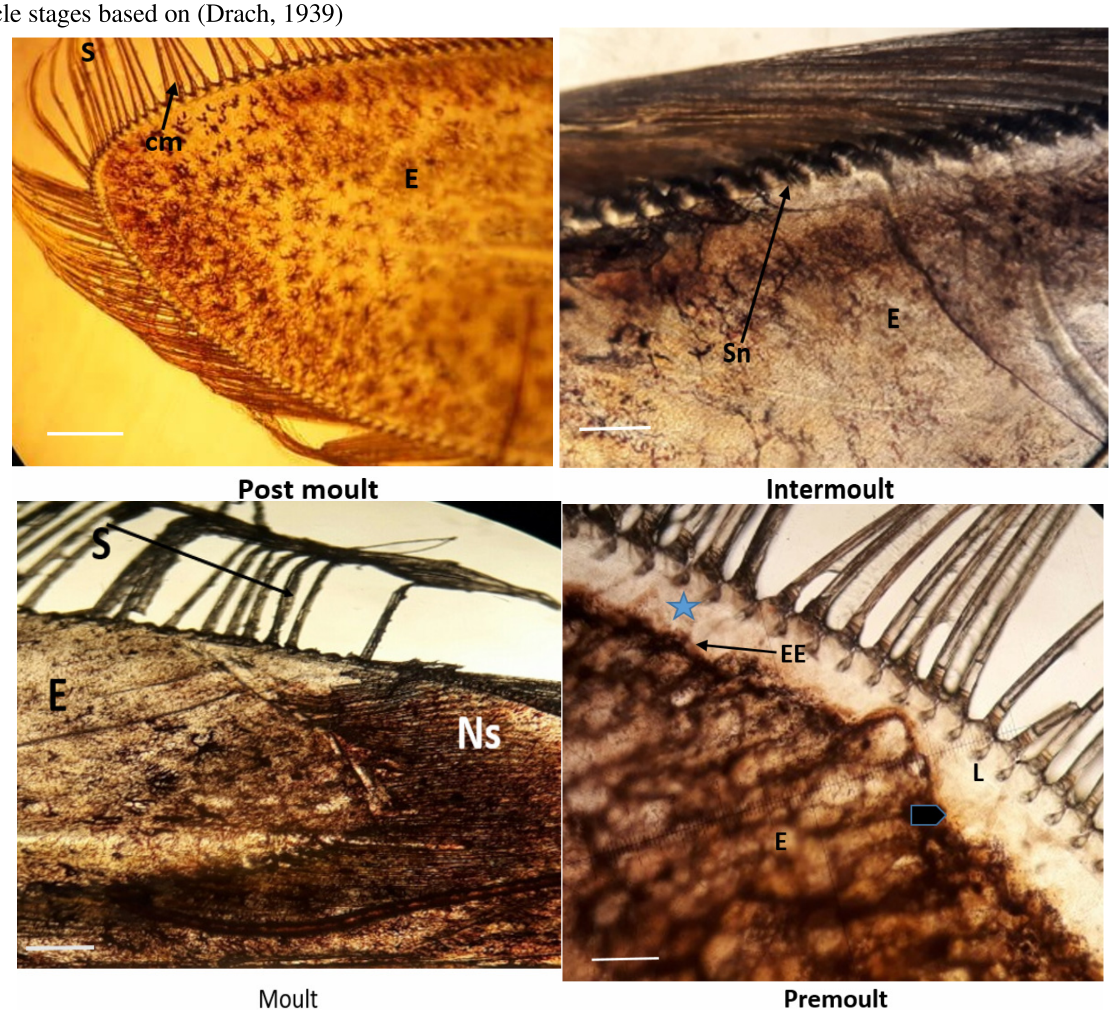

Fig. 1 : Morphological variation in different stages of moulting in uropods *M. affinis* shrimps were examined and photographed under a light microscope (Olympus BX40) connected to a digital camera (DCE-2). The criteria used for moult staging followed Drach staging. Scale Bar =50 µm. EE=epidermal edge, L= white layer, S= setae, SC= seta cone, Sn=setal node, Ns= new setae, E=epiderme.

➡ = wavy edge of epidermis.

★ = clear zone between cuticle and epidermis.

Protein

The results showed a high percentage of total protein in muscle tissue during different stages of the moulting cycle, the highest concentration (62.83%) mg/g in postmoult stage and the lowest level 62.05% mg/g in premoult stage were observed, differences in total protein levels in muscles were evident (Figure 2).

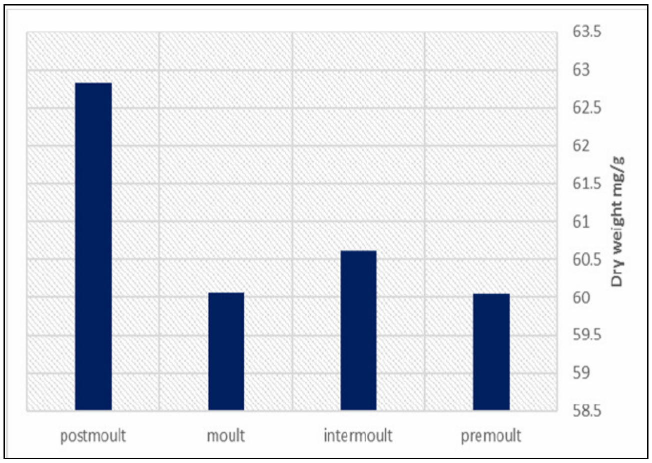

Fig. 2 : Variation in total protein level in muscle of *M. affinis* in different moulting stages

Lipid

The results showed differences in total lipid levels in muscle tissue during the different stages of moulting. A sharp reduction in total lipid level 3.01% mg/g at postmoult stage was observed compared with the high levels 6.08% mg/g at stage moult (Figure, 3).

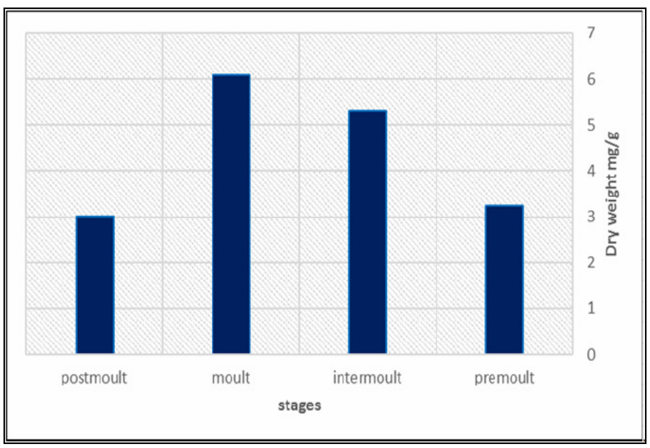

Fig. 3 : Variation in total lipids level in muscle of *M. affinis* in different moulting stages

Moisture

The moisture ranged from 1.86% - 3.80% in muscle tissue during the different stages of moulting (Figure 4).

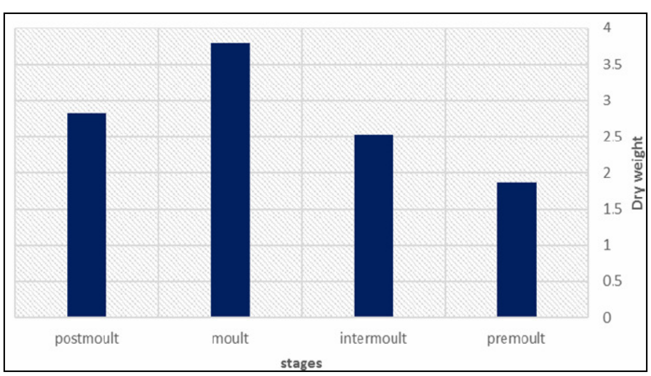

Fig. 4 : Variation in moisture level in muscle of *M. affinis* in different moulting stages

Ash

The ash percentage showed the highest value (0.105%) was in the intermoult stage then the lowest value 0.01% at moult stage (Figure 5). It shows the percentages of ash at different stages of the moulting cycle.

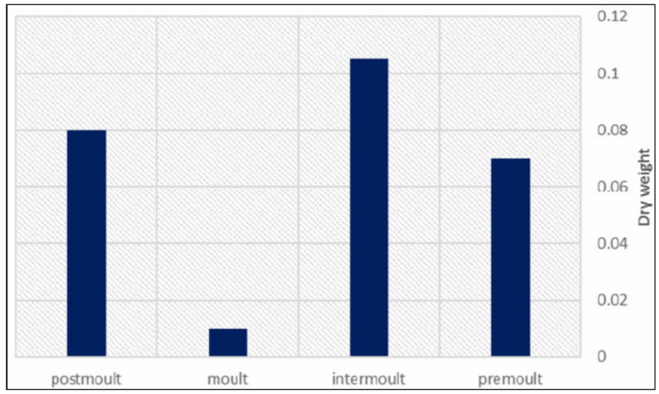

Fig. 5 : Variation in ash level in muscle of *M. affinis* in different moulting stages

Antioxidant enzymes

Glutathione (µg/mg.protein)

The results showed significant differences in glutathione levels in muscle tissue during the different stages of moulting, the highest value 13.26 µg/mg protein was in the intermoult then the lowest value 3.28 µg/mg protein at premoult stage (Figure 6).

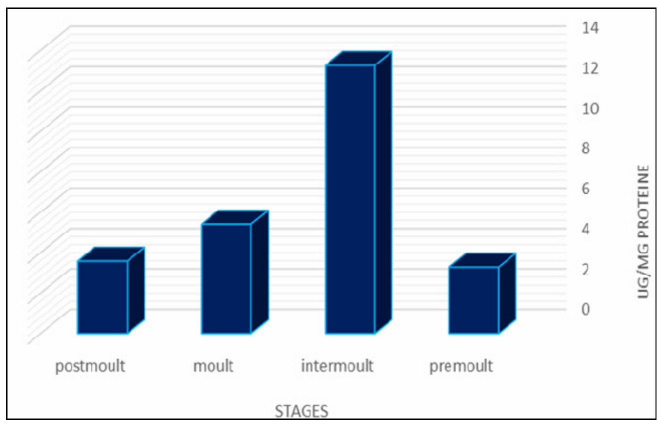

Fig. 6 : Variation in glutathione level in muscle of *M. affinis* in different moulting stages

Catalase activity KU/L

Figure 7 shows the changes in the level of catalase activity during the different stages of moulting, as the maximum 154.04 ku/L in the intermoult stage and the lowest value 46.21 ku/L in postmoult stage.

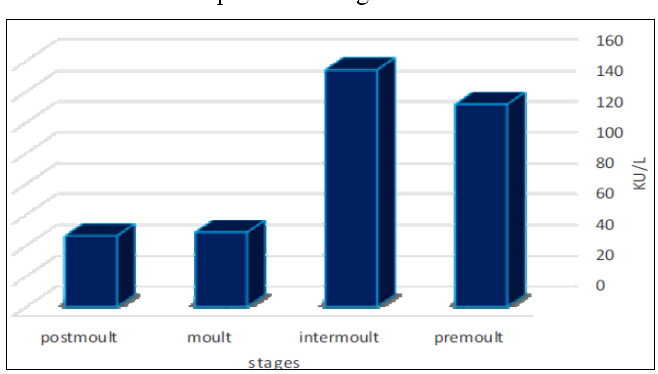

Fig. 7 : Variation in catalase level in muscle of *M. affinis* in different moulting stages

ROS (Micromol/gm)

The results showed differences in reactive oxygen species levels in muscle tissue during the different stages of moulting, the highest value (52.95) micromole/gm in the premoult stage and the lowest value (32.68) micromole/gm in the moult stage (Figure 8).

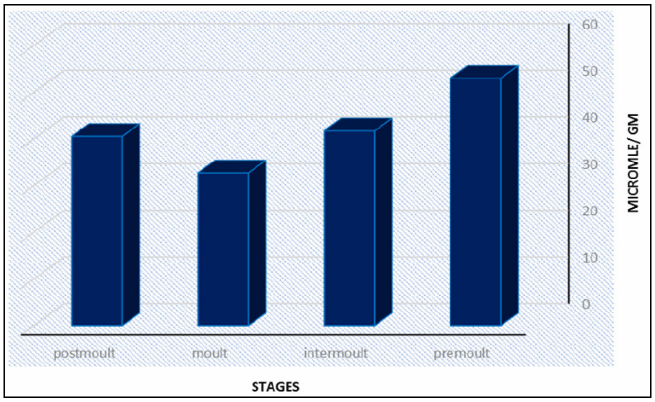

Fig. 8 : Variation in (ROS) level in muscle of *M. affinis* in different moulting stages

Food preference

Chavanich *et al.* (2015) when analyzing the components of the stomach of penaeid shrimp species, diets including phytoplankton, crustacean's appendages, zooplankton, organic matter were found. Some studies have indicated that penaeid shrimp are considered carnivores, and may show cannibal behavior (Zhang *et al.,* 2010).

Food composition

Food items examined on the basis of abundance observed in shrimp foregut of gastrointestinal tract, zooplanktons were, ranked first among the foodstuffs that present in the foregut throughout the study period, while organic matter ranked second among foodstuffs and phytoplankton constituted the third nutrient, and finally green algae were found in the final rank among contents of the forgut.

Figure (9) shows the percentages of relative importance index (IRI) of the food ingredients in foregut of gastrointestinal tract of *M. affinis* in the Premoult stage. The diet consists of five food ingredients, with zooplankton ranked first with 44.95%, organic matter came second with 28.88%, and algae ranked third with 18.27%. Diatoms ranked fourth with 4.17%, and phytoplankton ranked fifth with 3.74%.

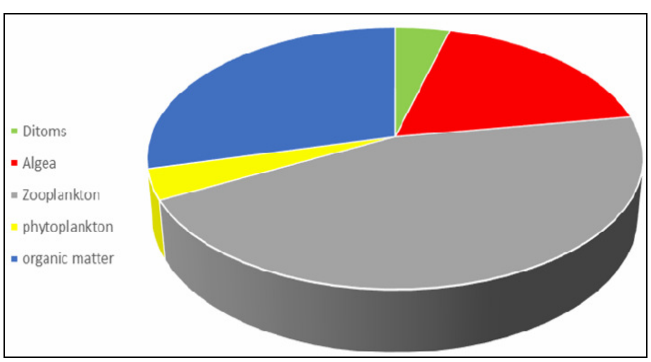

Fig. 9 : Relative importance index (IRI) of the food ingredients in the Premoult stage

While illustrating figure (10) the percentages of relative importance index (IRI) of the food ingredients in foregut of gastrointestinal tract of *M. affinis* in the Postmoult stage. The diet consists of five food ingredients, these are zooplankton, organic matter, algae, phytoplankton and diatoms and their ratios are as follows 41.51%, 34.40%, 15.29%, 7.35%, 1.45% respectively.

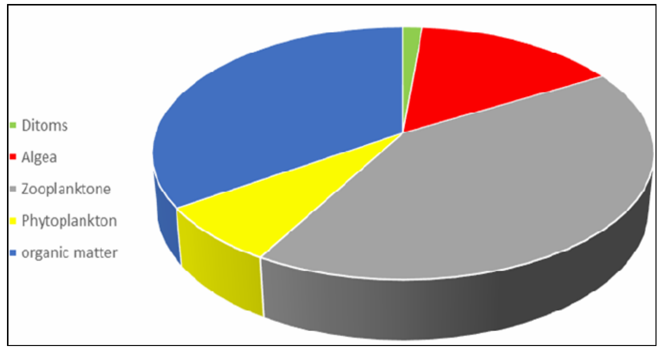

Fig. 10 : Relative importance index (IRI) of the food ingredients in the Postmoult stage

On the other hand, the figure (11) shows the percentages of relative importance index (IRI) of the food ingredients in foregut of gastrointestinal tract of *M. affinis* in the intermoult stage. The contents of the foregut of the gastrointestinal tract consisted of five food ingredients, it is represented by organic matter, zooplankton, phytoplankton, algae and diatoms. Organic matter ranked first with 42.72%, zooplankton ranked second with 38.85%, phytoplankton ranked third with 12.93%, algae ranked fourth with 5.25%, and diatoms ranked fifth with 4.25%.

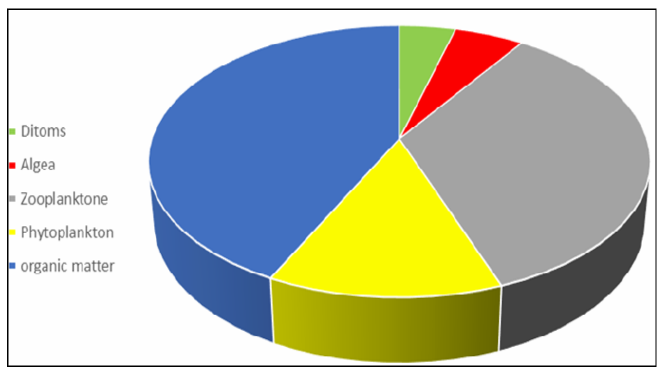

Fig. 11 : Relative importance index (IRI) of the food ingredients in the intermoult stage

Results showed *M. affinis* in the ecdysis stage, this stage was characterized by microscopic examination 243 animals that the foregut of the gastrointestinal tract was empty.

Discussion

Changes in the exoskeleton, reflect the regular cyclical growth that occurs in crustaceans during the moulting cycle (El– sayed *et al.,* 2015). Although setogenesis has been used as a criterion for moulting for many years (Drach, 1939), these criteria include discernment in the internal cones and setal organs, setal lumen, Similar criteria have been used to determine stages for the penaeidal shrimp (Paray *et al.,* 2014).

Differences in species related to setae morphology and development lead to crustaceans variation in both the gradient criteria and in the sub-divisions defined in the moulting stages. The results of the present study showed apparent phenotypic changes in the uropod setae of the *M.*

affinis during the moulting cycle, especially during the sub phases of pre-moult stage. In the Intermoult stage, the setae cones were arranged in one row on the bases of the setae without the withdrawal of pigments, and the epidermal tissue appeared to be attached to the setal cones. These characteristics were consistent with a study by Promwikorn, et al. (2004) on the black tiger shrimp (*Penaeus monodon*), On the other hand, Zilli *et al*., 2003 pointed out that the pigment seemed a thick line at the bases of the setal nodes in some shrimp species. Withdrawal of epidermal pigments during the D1-D2 sub phases within the Premoult stage, where the undulating edge of the epidermal tissue and the white area between the epidermis and the old setae bases have been agreed (Stevenson 1971 ;Skinner 1985; Zilli *et al.,* 2003; Promwikorn *et al.,* 2004; El sayed *et al.,* 2015) On the other hand, the level of matrices in the setal lumens varies within the sub-stages during the pre-moulting cycle, These are agree with that demonstrated by (El sayed *et al.,* 2015; Chansiu, *et al.,* 1988). Europods was used to determine the stages of moulting because removal of other appendages leads to shock or death (Paray *et al.,* 2014).

The process of moulting results in periodic changes in the chemical composition (Suod *et al.,* 2014; Khodanazary, 2019), so we need more chemical analyzes of commercial aquatic organisms, because it gives importance to know the nutritional value and understanding metabolism, to obtain a healthy balanced food (Abdul-Sahib and Ajeel 2005), as well as it reflects the physiological status of the organism. On the other hand to show the negative effects of pollution in the environment and the extent of its impact on the components of the chemical composition, to see whether this sea food is still suitable for human consumption or not (Khodanazary, 2019).

The results were obtained for total protein levels in muscle tissue collected during the different stages of moulting. The maximum concentration in the postmoult stage was attributed to the formation of kitten in the new epidermis. While the total protein level was observed to be lower in the primoult stage, it may be attributed to the re-adsorption of kitten and protein in the epidermis in the old exoskeleton and the degradation of proteins in energy production (paray *et al.,* 2014). Protein levels ranged from 62.83% -60.05% a study but higher than that recorded (Abdul-Sahib and Ajeel, 2005; Al-Maliki and Al- Khafaji, 2018), The high percentages of total protein in the present study are attributed to different sampling time or sampling location. Total lipids levels in muscle tissue showed a sharp decrease in postmoult and premoult, compared to high levels in ecdysis and Intermolt stages, This is agree with the observed in penaeid shrimp species (Paray *et al.,* 2014). High ash levels in the Intermoult stage, may be attributed to kitten compared to the low percentage in ecdysis.

The results of the study show the percentages of moisture in jinga shrimp *Metapenaeus affinis* 3.80%, 2.82%, 2.53%, 1.86 in ecdysid, postmoult, intermoult and premoult respectively, that the increase in moisture content in the moulting stages can be explained that the increase in volume when crustacean moulting is, due to water absorption (Oconnor and Gilbert, 1969; NorFaadila *et al.,* 2013) and this is consistent with a study (Travis, 1957) during the moulting cycle involved the water-to-mass ratio is greater in the moulting stage and early postmolt stage.

The role of the antioxidant defense system in invertebrates can be more important than in vertebrates, because during the phagocytosis process involving the production of (ROS) this is a major defense mechanism against invading microorganisms, due to their lack of antibodies and acquired immunity (Johansson *et al.,* 1999; Wang *et al.,* 2012). On the other hand, the radicals peroxide produced after the stress of the cell are converted to hydrogen peroxide and oxygen by superoxide dismutase (SOD), and the excess of hydrogen peroxide is stimulated into harmless water and oxygen by catalase and glutathione (Holmblad and Soderhall, 1999; Wang *et al.,* 2012). illustrate Yeh *et al.* (2010) Increased respiratory activity in the giant freshwater shrimp *Macrobrachium rosenbergii* to eliminate invasive pathogens, so antioxidant enzyme activity and RNA transcription were regulated in order to convert (ROS) excess from pathogenic infections. Confirmed Liu *et al.* (2010) in the black tiger shrimp *Penaeus monodon* associated with increased respiratory bursts significantly with *Photobacterium damselae* and WSSV-injected shrimp. Finally therefore, antioxidant enzymes may have key roles in the innate immunity of shrimp. The highest concentration of the antioxidant enzyme glutathione during the intramoult stage to protect cells from damage, while decreasing during the ecdysis, this applies to the enzyme Catalase, This is due to the biological and chemical processes active in the intermoult stage, while the ROS level is high during the premoult and postmoult stages to prepare for any microbial attack. It is noted that shrimp are not fed during the moulting stage. A clear understanding of food preference and behavior is important in aquaculture and for efficient nutrition management, which in turn helps reduce food wastage and thus protect the ecosystem. During the moulting stage, the exoskeleton is so thin that it does not allow the activity of searching for food. On the other hand, shortly after the moulting stage, shrimp consumes exuvia (Phlippen *et al*., 2000), which is rich in chitin, which in turn will compensate for the loss of minerals due to moulting. The natural food of the animal during the moulting stage includes ditoms, algea, phytoplankton, zooplanktone and organic matter, but the lack of nutrition during the moulting stage is noted, because the animal is busy with moulting. The postmoult and premoult phases observe the similarity of food types at almost the same proportions, this is agree with (Nguyen *et al*., 2014)

Conclusion

The developmental stage in setae provides a quick and accurate indication of the moulting stage in *M. affinis*, which would be without sacrificing of the animal, when it is intended the repetition of measurements taken from the same animal in order to monitor the rate of development. This study provides a detailed explanation of moulting cycle such as epidermal retraction and related setal formation in the epidermis, this present study confirms differences in the components of biochemical composition as well as the measurement of three antioxidant enzymes in muscle tissue during different stages of moulting cycle, which contribute to our understanding of physiology during the ecdysis of *M. affinis*. On the other hand, the reduction of feeding rates before and after moulting compared to excessive rates during the intermoult stage.

Acknowledgements

Authors present all thanks to university of Basrah, college agriculture, department of fisheries and marine resources for supporting this research.

References

A.O.A.C. (2000). Official Methods of analysis (17th edn). A.O.A.C. International. Gaithersburg. Maryland. USA.

Abdul-Sahib, I. and Ajeel, S. (2005). Biochemical constituents and nutritional values for the males and females of the commercial penaeid shrimp *Metapenaeus affinis* (H. Milne –Edwards 1837). J. Basrah Researches (Sciences), 31(1): 35–40.

Abdul–Sahib, I.M. and Sultan, E.N. (1996). Moult Stage and Fortnightly Moulting of the Penaeid Shrimp *Metapenaeus affinis*. Marina Mesopotamica. 11(1): 79-87.

Ainaz, K. (2019). Freshness assessment of shrimp *Metapenaeus affinis* by quality index method and estimation of its shelf life. Journal of food properties, 22(1): 309–319.

Al-Maliki, G.M. and Al-Khafaji, kh. (2018). Comparative Study of Chemical Composition of Three Species of Commercial Shrimp *Penaeus semisulcatus* (De Haan, 1844), *Metapenaeus affinis* and *Parapenaeopsis stylifera* (H. Milne -Edward) in North West Arabian Gulf Research Article Volume 7 Issue 5-June

Amer, M.; El-Sayed, A.; Al-Damhougy, K.; Zaakouk, S. and Ghanem, M. (2015). Changes in uropod setae during molting of the freshwater crayfish, *Procambarus clarkii* (Cambaridae) from the River Nile, Egypt International Journal of Advanced Research, 3(8): 360-368.

Bilal, A.; Jawahar, A.; Mehrajuddin, W. and Kumar, M. (2014).Variation in the Biochemical Constituents during Different Moulting Stages in Green Tiger Shrimp, *Peneaus semisulcatus*. Annual Research & Review in Biology, Sciencedomain international 4(6): 985-997.

Chan Siu, S.; Ming, C. and Rankin Larry, M. (1988). Characterization of the Molt Stages in *Penaeus vannamei*: Setogenesis and Hemolymph Levels of Total Protein, Ecdysteroids, and Glucose Biological Bulletin 175(2): 18.

Chan, S.; Rankin S.M. and Keeley, L. (1988). Characterization of the Molt Stages in *Penaeus vannamei*: Setogenesis and Hemolymph Levels of Total Protein, Ecdysteroids, and Glucose, BioLBull. 175: 185-192.

Chavanich, S.; Voranop, V.; Wansuk, S. and Suwanna, P. (2016). Laboratory assessment of feeding-behavior interactions between the introduced Pacific white shrimp *Litopenaeus vannamei* (Boone, 1931) (Penaeidae) and five native shrimps plus a crab species in Thailand. Aquatic Invasions, 11(1): 67–74.

Corgos, A.; Sampedro, M.; Gonza´lez-Gurriara´n, E. and Freire, J. (2007). Growth at moult, intermoult period, and moulting seasonality of the spider crab *Maja brachydactyla*: combining information from mark-recapture and experimental studies. Journal of crustacean biology, 27(2): 255–262.

Drach, P. (1939). Mue ET cycle d'intermue chez les crustaces Decapodes. Annls. Inst. Oceanogr. 19: 103-391.

Edmondson, W.T. (1959). Fresh water biology. John Wiley and Sons Inc. New York, 1248p.

Erel, O. (2005). A new automated colorimetric method for measuring total oxidant status. Clin Biochem, 38: 1103–1111.

Folch, J.; Lee, M. and Stanley, G. (1957). A simple method for the isolation and purification of total lipid from animal tissues. J. Biol. Chem. 266: 494-509.

Galindo, C.; Gaxiola, G.; Cuzon, G. and Chiappa-Carrara X. (2009). Physiological and biochemical variations during the molt cycle in juvenile *Litopenaeus vannamei* under laboratory conditions. Journal of Crustacean Biology, 29: 544–549.

Hadi, R.A.M.; Al-Saboonchi, A.A. and Haroon, A.K.Y. (1984). Diatoms of Shatt Al-Arab River, Iraq. Nova Hedwigia, 39: 513-557.

Hadwan, M.H. and Abed, H.N. (2016). Data supporting the spectrophotometric method for the estimation of catalase activity. Data in Brief, 6: 194-199.

Hansson, S. (1998). Methods of studying fish feeding: a comment, Canadian Journal of Fisheries and Aquatic Sciences, 55: 2706-2707.

Holmblad, T. and Soderhall, K. (1999). Cell adhesion molecules and antioxidative enzymes in a crustacean, possible role in immunity. Aquaculture 172(1–2): 111–123.

Hyslop, E.J. (1980). Stomach contents analysis -a review of method and their application. Journal of Fish Biology, 17: 413-422.

Iwanaga, S. and Lee, B.L. (2005). Recent advances in the innate immunity of invertebrate animals. J Biochem Mol Biol., 38(2): 128–150.

Jafri, A.K.; Khawaja, D.K. and Qasim, S.Z. (1964). Studies on the biochemical composition of some freshwater fishes. Technol., 1: 148-157.

Johansson, M.; Holmblad, T.; Thornqvist, P.; Cammarata, M.; Parrinello, N. and Soderhall, K. (1999). A cell-surface superoxide dismutase is a binding protein for peroxinectin, a cell-adhesive peroxidase in crayfish. J Cell Sci., 112(6): 917–925.

Jung, H.; Lyons, R.E.; Hurwood, D.A. and Mather, P.B. (2013). Genes and growth performance in crustacean species: a review of relevant genomic studies in crustaceans and other taxa. J. Sci. Technol., 16: 34-52.

Kamaruding, N.; Noraznawati, I. and Mhd, I. (2018). Characterization of molting stages in the giant freshwater prawn, *Macrobrachium rosenbergii* using setagenesis of pleopod Songklanakarin J. Sci. Technol., 40. (2).

Kaushal, J.; Seema, M.; Gursharan, S.; Arun, R. and Shailendra, K. (2018). Catalase enzyme: Application in bioremediation and food industry. India Biocatalysis and Agricultural Biotechnology 16: 192–199.

Khodanazary, A. (2019). Freshness assessment of shrimp *Metapenaeus affinis* by quality index method and estimation of its shelf life. International Journal of Food Properties, 22(1): 309–319.

Liu, K.; Yeh, S.; Kou, G.; Cheng, W. and Lo, C. (2010). Identification and cloning of a selenium-dependent glutathione peroxidase from tiger shrimp, *Penaeus monodon*, and its transcription following pathogen infection and related to the molt stages. Dev Comp Immunol, 34(9): 935–944.

Lowery, R.S. (1988). Growth, molting and reproduction. In: Holdich, D.M., Lowery, R.S. (Eds.), Freshwater Crayfish, Biology, Management and Exploitation. Croom Helm Press, London, 83–113.

Markus, K.; Simon, G.; Sook, J. and Heinrich, D. (2000). Ecdysis of Decapod Crustaceans is Associated with a Dramatic Release of Crustacean Cardioactive Peptide into the Haemolymph. The Journal of Experimental Biology, 203: 521–536.

Maryam, S.; Hossein, Z.; Sakineh, M.; Faramarz, L.; Ali, M. and Ahmad, S. (2018). Diversity of White Shrimp (*Metapenaeus affinis*) from the Northwest of the Persian Gulf Using Microsatellite Markers. Turkish Journal of Fisheries and Aquatic Sciences, 18: 385-394.

Nguyen, N.; Chimb, L.; Pierrette, L. and Laurent, W. (2014). Feed intake, molt frequency, tissue growth, feed efficiency and energy budget during a molt cycle of mud crab juveniles, *Scylla serrata* (Forskål, 1775), fed on different practical diets with graded levels of soy protein concentrate as main source of protein. Aquaculture, 434: 499–509.

Noor, K.; Noraznawati, I. and Ikhwanuddin, M. (2018). Characterization of molting stages in the giant freshwater prawn, *Macrobrachium rosenbergii* using setagenesis of pleopod. Songklanakarin J. Sci. Technol. 40 (2), Mar.-Apr. 2018.

Nor Faadila, M.I.; Harivaindaran, K.V. and Tajul, A.Y. (2013). Biochemical and texture property changes during molting process of tiger prawn, *Penaeus monodon*, International Food Research Journal, 20(2): 751-758.

O'Connor, J.D. and Gilbert, L.I. (1969). Alterations in *Limulus polynya* sps. lipid metabolism associated with premolt activity in a land crab and a freshwater crayfish. Comparative Biochemistry and Physiology, 29: 889-904.

Paray, B. and Al-Sadoon, M.K. (2014). Molt cycle related changes and effect of short term starvation on the biochemical constituents of the blue swimmer crab *Portunus pelagicus* Zoology Department, College of Science, King Saud University. PO Box 2455, Riyadh 7 7 45 7, (bparay@ksu.edu.sa) ZOOLOGIA 34: e11923 ISSN 1984-4689.

Promwikorn, W.; Kirirat, P. and Thaweethamsewee, P. (2004). Index of molt staging in the black tiger shrimp (*Penaeus monodon*), Songklanakarin J. Sci. Technol., 26(5): 765-772.

Saoud, D.; Al-Hamdany, Q. and Al-Hasson, A. (2014). Comparative Study of Chemical Composition of Two Species of Commercial Shrimp *Metapenaeus affinis* and *Parapenaeopsis stylifera* (H.Milne -Edward) in North West Arabian Gulf.

Sedlak, J. and Lindsay, R. (1968). Estimation of total, protein-bound and nonprotein sulfhydryl groups in tissues with Ellmans reagent. Analytical Biochemistry, 25:192-205.

Shokoohmand, M.; Hossein, Z.; Sakineh, M.; Faramarz, L.; Ali, M.F. and Ahmad, S. (2018). Analysis of Genetic Diversity of White Shrimp (*Metapenaeus affinis*) from the Northwest of the Persian Gulf Using Microsatellite Markers. Turkish Journal of Fisheries and Aquatic Sciences, 18: 385-394.

Skinner, D.M. (1985). Molting and regeneration. In: Bliss, D.E. and Mantel, L.H. The biology of crustacea, Academic Press, New York, 9: 43-128.

Smith, J.; Brown, H. and Hauton, C. (2003). Immune stimulation in crustaceans: does it really protect against infection? Fish Shellfish Imanol., 15: 71-90.

Souza, D.M.; Kutter, M.T.; Furtado, P.S.; Romano, L.A.; Junior, W.W.; Monserrat, J.M. and Garcia, L. (2019). Growth, antioxidant system, and immunological status of shrimp in bioflocs and clear water culture systems. Pesquisa Agropecuária Brasileira, v.54, e00363.

Stevenson, J.R. and Henry, B.A. (1971). Correlation between the molt cycle and limb regeneration in the crayfish *Orconectes sariborni* (Hagen) (decapoda, Astacidea). Crustaceana, 20: 301-307.

Sugumar, V.; Vijayalakshmi, G. and Saranya, K. (2013). Molt cycle related changes and effect of short term starvation on the biochemical constituents of the blue swimmer crab *Portunus pelagicus*. Saudi Journal of Biological Sciences, 20: 93-103.

Tran, M.; Donald, L.M.; Abigail, E. and Tomer, V. (2019). Characterization of G-protein coupled receptors from the black back land crab *Gecarcinus lateralis* Y organ transcriptome over the molt cycle. BMC Genomics, 20: 47-69.

Travis, D.F. (1957). The molting cycle of the spiny lobster, *Panulirus argus* latreille. 1V. Postecdysial histological and histochemical changes in the hepatopancrease and integumental tissues. Biological Bulletin, 1: 451-479.

Wang, D.; Fuhua, L. and Yanhong, C. (2012). Potential relationship among three antioxidant enzymes in eliminating hydrogen peroxide in penaeid shrimp Cell Stress and Chaperones, 17: 423–433.

Warner, G.F. (1977). The Biology of crabs. Elek Science, London.

Wehr, J.D. and Sheath, R.G. (2003). Freshwater algae of North America, ecology classification. San Diego Academic press, 918p.

Xue, B.; Zhang, P.; Zhi, H.; Zhao, L.; Wan, Y.; Jin, Q.; Hang, K.; Bin, L. and Huan, G. (2017). Characterization of an MHC gene in *Palaemon carinicauda* (Holthuis, 1950) (Caridea: Palaemonidae) and its expression profiles at different post-molt time points Journal of Crustacean Biology Advance Access published 13 July 2017S.

Yan, H.; Mei, X.; Haiying, L.; Lianshun, W.; Qi, L. and Linan, J. (2017). Energy reserves and gonad steroid levels during the reproductive cycle of the Japanese mantis shrimp *Oratosquilla oratoria* De Haan, 1844 (Stomatopoda: Squillidae) in Pikou Bay, Dalian, China. Journal of Crustacean Biology, 37(1): 99–108.

Yeh, S.; Liu, K.; Chiu, S.; Jian, S.; Cheng, W. and Liu, C. (2010). Identification and cloning of a selenium dependent glutathione peroxidase from giant freshwater prawn, *Macrobrachium rosenbergii*. Fish Shellfish Immunol, 27(2):181–191.

Zhang, Q.; Chong-Yu, X. and David Chen, Y. (2010). Variability of water levels and impacts of stream flow changes and human activity within the Pearl River Delta, China', Hydrological Sciences Journal, 55(4): 512-525.

Zhang. J.; Zhou, X.; Wu, W.; Wang, J.; Xie, H.; and Wu, Z. (2017). "Regeneration of glutathione by α-lipoic acid via Nrf2/ARE signaling pathway alleviates cadmium-induced HepG2 cell toxicity". Environ Toxicol Pharmacol., 51: 30–37.

Zilli, L.; Schiavone, R.; Vilella, S.; Scordella, G. and Zonno, V. (2003). Researchers Capture Images For Practical Molt Stage Analysis in Shrimp, Frigole-Lecce, Lecce, Italy Global Aquaculture Advocate.