Egyptian Journal of Aquatic Biology & Fisheries Zoology Department, Faculty of Science, Ain Shams University, Cairo, Egypt. ISSN 1110 – 6131 Vol. 24(2): 69 – 82 (2020) www.ejabf.journals.ekb.eg



Isolation and cultivation of the freshwater amphipod *Gammarus pulex* (Linnaeus, 1758), with an evaluation of its chemical and nutritional content

Hamdy A. Abo-Taleb^{1*}, Amr F. Zeina¹, Mohamed Ashour², Mohamed M. Mabrouk³, Ahmed E. Sallam² and Mohamed MM. El-feky².

1- Faculty of Science, Al-Azhar University, Cairo, Egypt.

2- National Institute of Oceanography and Fisheries, Alexandria, Egypt.

3- Faculty of Agriculture, Al-Azhar University, Cairo, Egypt.

*Corresponding author: <u>hamdy.ali.hamdy@gmail.com</u>

ARTICLE INFO

Article History:

Received: Feb. 14, 2020 Accepted: March 18, 2020 Online: March 21, 2020

Keywords:

Gammarus pulex Cultivation Nutritional components, Fish-farms, Nests,

ABSTRACT

In recent years, global aquaculture has increased significantly, and therefore the demand for animal protein used to feed farmed fish has increased, this has led to the search for other sources of animal protein instead of fish meal. Amphipods as *Gammarus* are among those possible alternatives due to its abundant productivity and appropriate nutritional content, as the current study showed that its protein content was 40%, carbohydrates 27.4%, fat 5.5%, and it also contained antioxidants such as tannic acid (223.146 mg/100g) and β -carotene (21602.964 IU/100g) in addition to many essential vitamins in considerable values such as Vitamin B2 338.3842 mg/100g, Vitamin B6 (635.616 mg/100g), Vitamin B12 (419.5017 mg/100g), Vitamin A (19623.98 IU/100g), Vitamin E (177.958 mg/100g), Vitamin D (59.672 mg/100g), and Folic Acid (521.185 µg/100g).

Gammarus pulex has been isolated from the natural resource (Lake Mariout), and after 90 days of rearing in tanks (initial stock: 30 individuals/liter) supplied with four different types of nests: pored wood pieces, balls of palm fibers, balls of palm leaves, and balls of nylon net "Haba net", one type for each tank. The obtained data revealed that balls of palm leaves are the best nest for rearing as it gave the highest yields (127 individuals/liter), the average obtained numbers of the different rearing tanks was 108 individuals/liter with average wet weight 2.16g/l, which means 1m3 water can produce approximately 2160g of *Gammarus*.

A new nutritional formula was used in the current study to feed the cultivated *Gammarus* in its various stages, which gave good results that were reflected in its productivity and the analysis of its chemical components.

IUCAT

INTRODUCTION

Indexed in Scopus

Gammarus species amphipod crustaceans have widespread and very common worldwide, they are frequent and abundant in marine and freshwater, some species are

ELSEVIER DOA

characteristic of the surface waters (Sutcliffe, 1991) and others are benthic. Gammarid amphipods can be considered as the dominant macroinvertebrate (in abundance and biomass) in many freshwater ecosystems (Shaw, 1979; MacNeil et al., 1999) or marine water (Farrag et al., 2019). They exhibit a range of feeding patterns including grazing, detritus feeding, predation including cannibalizing, while they are preferable prey for many other macroinvertebrates and fish (MacNeil et al., 1997; Wilhelm and Schindler, **1999**). They have a principal position in the freshwater food webs for tearing and mixing all kinds of decomposing leaves remains, hence their classified as detritivores (Sutcliffe, 1991; Karlson et al., 2007). The mean brood of the freshwater Gammarus pulex increased from 6 eggs, in 6-7 mm females, to 29 eggs in 11-12 mm females. Gammarus contains a high level of protein and fats and appropriate values of the essential amino acid (Köprücü and Özdemir, 2005), as well as the Gammarus meal, enhances the feed intake in fish and increase its immune response, stress resistance and growth performance (Köprücü and Özdemir 2005; Rufchaei et al., 2017). Due to all these reasons, it is desirable to use this organism as a feed supplement in aquaculture although it is not yet been carried out in such industries (Harlioğlu and Farhadi, 2018), and it can be used as an excellent and cheap alternative to fish meal in fish diets (Navlor et al., 2009). Gammarus was recently used as a substitute for animal protein in fish diets used to feed some of the most important high-value fish such as Caspian roach (Rufchaei et al., 2017), African jewelfish (Adhami et al., 2017), Siberian sturgeon (Razgardani Sharahi et al., 2016), Caspian salmon (Darhani et al., 2014), common carp (Navazandeh et al., 2013), rainbow trout (Azimi et al., 2011), and Nile tilapia (Köprücü and Özdemir 2005; Ashour et al., 2018).

The objective of the current study is to isolate and cultivate *Gammarus pulex* and determines the preferable nests that can use as habitat for these organisms in mass culture to improve its growth performance and biomass production and create suitable food mixture used in feeding these organisms. Additionally, analysis of their nutritional components to determine its suitability for use as an alternative to animal protein in fish diets. This work is considered to be the first of its kind, as an extensive culture of *Gammarus* under controlled conditions for commercial purposes has not yet been carried out, as indicated by **Harlıoğlu and Farhadi (2018)**.

MATERIALS AND METHODS

New feed mixture for the cultivated Gammarus

Since the *Gammarus* feed on detritus, which includes leaves material that is decomposed by natural factors and microbial degradation, as well as leftovers of macroinvertebrate food as mentioned by **Cummins and Klug (1979)**, **Sutcliffe** *et al.* (1981) and **Webster and Benfield (1986)**, while **Moore (1975)** noted that adults can be fed on algae. The young feed on organic matter (faeces of adults) (McCahonand Pascoe 1988b). Hence, the food used in the current study has been chosen with great care to suit what was previously mentioned, as well as to contain the nutrients necessary for high growth performance. The food mixture used in the present experiment is a new of its kind that was not previously used in feeding *Gammarus* or any of the other invertebrates. The

nutritional mixture consists of 33.3% spirogyras algae, 33.3% berries leaves, 16.7% rice bran powder, and 16.7% soybean powder (1:1:0.5:0.5), respectively, the mixture was roughly mixed using a hand mixer (to produce coarse mixture).

Conditioned process of the mixture

To do these steps (Sutcliffe et al. 1981) was consulted and the following steps created:

- The dried mixture items have been mixed in the form of a coarse mixture.
- Organic water has been added to the mixture and left in indoor condition for 6 days to stimulus bacteria and fungi to do partial breakdown to the diet mixture.
- After 6 days the conditioned mixture used directly to feed the cultivated *Gammarus*.

Feeding process

Weighted quantities, 50g/100l culture water, of feed were directly added manually two times a day. Siphonation was performed every two days to excluding the precipitations from the bottom of the tanks to prevent the anaerobic conditions.

Collection and isolation of Gammarus

Lake Mariout is one of the Egyptian northern lakes was chosen to collect *Gammarus*. A small plankton net with 200-micron mesh size was lowered vertically from bottom to surface and the benthic substrate just beneath the net was gently agitated before towing. Amphipods in the water and from the substrate have been collected in the net through dragging. Furthermore, all amphipods that are encountered were collected opportunistically.

The contents of the net were investigated, *Gammarus* was identified and isolated, while other invertebrates were carefully excluded. *Gammarus* were identified with a dissecting microscope and the collected specimens were separated into males and females and placed into plastic containers filled with lake water.

Then the *Gammarus* placed into 100l transparent plastic holding containers filled with dechlorinated tap water (modified to be 8% salinity, like that in the wild) for 24h acclimatization before starting the cultivation experiments.

Cultivation conditions

Cultivation conditions resembled what measured in the natural environment where the collected organisms were lived, and adjusted to be constant at all experiment tanks where pH was 7.8, salinity was 8‰, the temperature was 27 °C and concentration of dissolved oxygen was 5.5 mg/l. The rearing was in transparent tanks (51) 12-hour daylight and 12-hour fluorescent light tubes (600 lux). Oxygenation of the tanks was assured by air bubbling, aeration cause water agitation which helps in regular redistribution of the organisms overall the culture water body. 25% of water replacement was performed on a weekly scale.

The individuals of *Gammarus pulex* were moved and adapted in 100l tanks contains dechlorinated tap water for 24 hours, then transferred and distributed on the culture tanks

(51). The individuals have been stored at density 30 individual/liter (10 males "distinguished by visible genital papillae" and 20 females with fully developed oostegites with long fringed bristles which tangle together to form the brood pouch) for 90 days at environmental conditions resemble what measured in the wild where the species was collected.

Amphipods prefer to live and reproduce in nests contain pores and grooves and interstitial pathways as the habitats where they gathered. Hence we provided every cultivation tank with one different type of nests to determine which one is the preferable nest for the cultivation of this species. Four types of nests were used, one inside every single tank (pored wood pieces, balls of palm fibers, balls of palm leaves, and balls of nylon net "Haba net") three replicates for each type.

Chemical and biological analysis

• Determination Physicochemical characters of the culture medium

Water temperature was measured by using a mercury thermometer 110 °C graduated to 0.1 °C. The turbidity of water was measured by turbidimeter bench HACH 2100N (SN 08040CO2711). Hydrogen ion concentration (pH) values of the water were measured by using a digital pH meter levibond sensodirect pH200 SN (0814375). The dissolved oxygen was measured by DO-meter (HQ30d Flexi meter) HACH S.N 080600022236.

• Analysis of bacterial and fungal content

The bacterial content of the harvested *Gammarus* was analyzed to ensure that it was free of pathogens and its suitability for use in feeding fish larvae. The total count of bacteria in the yields was calculated and their contents of *Salmonella*, *Clostridium*, *Listeria*, and *Escherichia coli* were analyzed, as well as their Yeast and moulds content.

• Analysis of vitamins and toxins content

Vitamins B2, B6, B12, A, D, E, and folic acid, as well as the antioxidants as tannic acid and β -carotene contents, have been analyzed in the cultivated *Gammarus* to evaluate its nutritional value. On the other hand, the carcinogenic Aflatoxin has been analyzed to find out whether it is present in internationally permitted limits by the World Health Organization or not because it is one of the most dangerous known toxins, and therefore determine whether these organisms are actually valid for use in feeding of fish larvae or not.

• Statistical analysis

The obtained results were statistically analyzed using SPSS (version 20) for a oneway analysis of variance. Differences between individual treatments were tested with Duncan Multiple range test at a probability level of 0.05% when the test was significant.

RESULTS

After 90 days of *Gammarus pulex* rearing in four different types of nests: pored wood pieces, balls of palm fibers, balls of palm leaves, and balls of nylon net "Haba net". The obtained results showed that, as general, most of these individuals appear to have

been mature because after 16 days the female's individuals had been produced two to five broods containing a mean of 13 eggs (range 8-27 eggs) resulted in a large number of offspring (average of 108 individuals/liter). Each month, irritation of the water in each cultivation tank has been done to ensure the organisms were distributed evenly and a random sample was taken to count the individuals in them to represent the number in liters. This process was done three times during three consecutive months. The highest density, mean of 127 individuals/l, was recorded from the tanks contain balls of palm leaves, while the lowest population density was recorded from that contains balls of palm fibers and pored wood pieces (**Table 1**).

contain different nests typesDaysPalm fibersPalm leavesNylon netWood piecesAverage

Table 1. Average numbers of cultivated *Gammarus* (individuals/liter) at cultivation tanks

Days	Palm fibers	Palm leaves	Nylon net	Wood pieces	Average
Day 0	30	30	30	30	30
Day 30	44	75	63	50	58
Day 60	76	106	95	79	89
Day 100	92	127	115	98	108

The nutritional content of *Gammarus* that was isolated was analyzed to determine its efficacy as food for fish larvae, as well as the difference between the concentrations of its contents from those used in other studies. The obtained data, in percentage, showed that the protein content of *Gammarus pulex* was 40 and fats were 5.5 while calories recorded 391.1 (**Table 2**).

Chemical compositions	Values
Moisture %	5.7
Crude protein (CP)%	40.0
Ether extract (EE)%	5.5
Ash %	21.4
Total carbohydrate %	27.4
Calories	319.1
Fibers%	2.9

Table 2. Nutritional values of Gammarus pulex (% of its dry weight "DW")

Biomass and yields

The average weight of the harvested individuals was 1 ± 0.13 g wet weight/50 individuals. While the average harvest was 108 individuals/liter with average weight gain 2.16g wet weight. The difference in the numbers of yields resulting in each treatment was reflected in the total biomass product (**Table 3**), where overall experiment period (90 days) the average amount of the harvest was ranged between 2.54g/l (tanks contain palm leaves) and 1.84 (palm fibers balls). The total average harvest from different tanks (51 tanks) was 2.16g/l (which means approximately 2160g wet weight/m³).

Days	Palm fibers	Palm leaves	Nylon net	Wood pieces	Average
Day 0		0	.6		0.6
Day 30	0.88	1.5	1.26	1	1.16
Day 60	1.52	2.12	1.9	1.58	1.78
Day 100	1.84	2.54	2.3	1.96	2.16

Table 3. Monthly changing in average weight (g/l) at different experiments

Microbial content

Analysis of the bacterial content of the harvested *Gammarus* showed a total bacterial count $>6500*10^2$ CFU/gm, while the harvest has no dangerous bacteria like *Salmonella* sp., *Clostridium* sp., and *Escherichia coli*, while *Listeria* sp. was found in a relatively low count, 65 CFU/gm (**Table 4**).

Table 4. Total bacterial count and content of the harvest from pathogenic bacteria, as well as yeast and moulds (CFU is a colony-forming unit that means: the number of viable bacteria or fungal cells in a sample)

Count
>6500*10 ² cfu/gm
0.0 CFU /gm
0.0 CFU/gm
0.0 CFU/25gm
0.0 CFU/25gm
65 CFU/gm

Vitamins and toxins content

.....

The obtained results showed that the farmed *Gammarus pulex* contained most of the vitamins necessary for the growth and improvement of the health status of fish larvae as they contained vitamins B2, B6, B12, A, D, E and folic acid in high proportions. It contains high values of the antioxidants tannic acid and beta-carotene, respectively. It was also observed that these organisms contain low levels, 6.933 ppb, of the toxic compound aflatoxin (**Table 5**).

Table 5. Different values of vitamins, antioxidants, and aflatoxin inside *Gammarus pulex*.

Items	Values		
Vitamin B2	338.3842 mg/100g		
Vitamin B6	635.616 mg/100g		
Vitamin B12	419.5017 mg/100g		
Vitamin A	19623.98 IU/100g		
Vitamin E	177.958 mg/100g		
Vitamin D	59.672 mg/100g		
Folic Acid	521.185 µg/100g		
β-carotene	21602.964 IU/100g		
Tannic acid	223.146 mg/100g		
Aflatoxin B1	6.933 ppb		

DISCUSSION

For a successful culture of this type of macroinvertebrate, a brief reference should be made to its life cycle. The *Gammarus* undergoes an impressive reproductive cycle that begins when the male (larger than the female unlike all other arthropods) began to hold several females before deciding which one will carry the most eggs (Dick and Elwood 1996; Zielinski 1998). After the male determines the appropriate female, and holds it using his first pair of gnathopods in the lower side of his body and parallels to him (Borowski, 1984) and becomes responsible for the movement (Bollache and Cezilly, **2004**). The pair stay in this position for up to 2 weeks as a precopula stage (Hartnoll and Smith, 1980), mating occurs as soon as the female sheds her skin, the pair is separated after mating where the female becomes carries the developing eggs in her brooding pouch. After 1 to 3 weeks, the eggs hatch juveniles which remain in the brooding pouch 4-6 weeks until the next moult for the mother female. Then the young go out of the brooding pouch to swim away from the mother, the first food for the young is faeces of adults (coprophagy) (McCahonand Pascoe, 1988b). The nutrition type gradually turns into conditioned leaves and detritus, which becomes the main nutrition after about a month. These young reach sexual maturity after 10 moults that take 90-120 days (approximately 3-4 months) (McCahon and Pascoe, 1988a). The average age of Gammarus is from one to two years.

The idea of this study raised from the facts that *Gammarus* is a cheap protein source in comparison to fishmeal and as it a live food characterized by high nutritional value (Harlıoğlu and Farhadi, 2018), besides, Köprücü and Özdemir (2005), Rufchaei *et al.* (2017) confirmed that using these organisms as a feed supplement for fish has a great impact on increasing their feed intake and enhancing the immune response, hence additional studies on the rearing and cultivation of *Gammarus* sp. should be conducted in large scale.

To create the new food needed to feed cultured *Gammaus*, the former researchers' experiences and observations were consulted, where **Sutcliffe** *et al.* (1981) stated that *Gammaus* could feed on conditioned plant leaves with organic water or leaves that had partial microbial degradation (Cummins and Klug, 1979). McCahon and Pascoe (1988b) noted that the young feed on the faeces of adults, and accordingly a new nutritional formula was made as the water in the fish farms ponds was used as organic water, dried berries leaves were crushed as well as dried spirogyra algae and put them in this organic water for six days and adding powder soybean and rice bran to make a conditioned mixture that used directly in feeding the *Gammarus*, and thus we had obtained a mixture suitable for the individuals of all sizes and ages. The cultured *Gammarus* is harvested using nets with holes of varying sizes, so the larger individuals harvested to use as feed additives and the small sizes returned to the culture ponds again.

There is a severe lack of data about the culture of *Gammarus* as only a few publications are detailing *G. pulex* culture include **McCahon and Pascoe** (1988a; 1988b) and the later **Bloor** (2009) in addition to a review of **Kunz** *et al.* (2010) who confirmed the lack of publications except the previously mentioned ones. The plastic containers that supplied with aerated dechlorinated tap water and illumination periods 12 hours by artificial light was previously used in this purpose by **Sutcliffe** *et al.* (1981) and **Kunz** *et al.* (2010), the authors used conditioned common leaves for feeding the cultivated *Gammarus*, this feeding procedure is the only known method used by (**Kaushik and Hynes, 1971; Willoughby and Sutcliffe, 1976**) for feeding the adults.

In the present study, most of the collected Gammarus individuals appear to have been mature because after 16 days of cultivation the female's individuals had been produced two to five brood pouches containing a mean of 13 eggs (range 8-27 eggs) resulted in a large number of offspring (average of 108 individuals/liter). This result was resembled what reported by Kunz et al. (2010), who mentioned that 200 visibly gravid G. pulex females and 100 precopula can produce 500-1000 newly hatched individuals after 5 days. Youngsters begin feeding on the faeces of adults and after 25 days they turn completely into nutrition as adults on the conditioned leaves of the plant. We assumed that the individuals were mature in the wild before the cultivation because of the freshwater *Gammarus* almost mature after an average of 90-120 days (Hynes, 1955; Welton and Clarke, 1980). Selective harvesting large sizes is a stressful and timeconsuming process that carries benefits as it gives an opportunity to growth of the young as well as improving the characteristics of the culture environment and. This procedure was conducted by McCahonand Pascoe (1988a) on laboratory cultivated Gammarus. The young *Gammarus* can be distinguished from adults as juveniles possess a primary flagellum of each antenna consists of five segments, the number of these segments increases with age and growth, reaching 14-16 segments after 10 moults (McCahon and Pascoe, 1988b).

Data revealed that among the four used nests, the balls of palm-leaf nests were preferable for rearing as it gave the highest yields (127 individuals/liter), this may be because these mesoherbivores natural behaviour is built their nests by rolling up a selected plant leaves into tubes as reported by **Barnard** *et al.* (1991) and **Fukui** (2001). The nest-building behaviour for these organisms has been first reported by **Holmes** (1901) and then by **Skutch** (1926). These publications revealed that the nest as being connected together by silk threads surround the used leaves that are secreted from special glands in the pereopods. Additionally, in the current study, the palm leaves nest differs from the other three nests since it composed of leaves that can be used by the organisms in the feeding, this may be a reason why the tanks contain palm leaves nests gave the highest *Gammarus* productivity. This may be confirmed by **Jones** (1971), Griffiths

(1979), Poore and Steinberg (1999) who reported that most amphipods consume the algal tissues that build up the walls of their nests.

The time taken to reach sexual maturity for *Gammarus pulex* is influenced by temperature; in the present experiment maturity time taken 16 days at 27°C, while it takes 120 days at 15-20 °C (**Hynes, 1955**), 130 days at 13 °C (**McCahon and Pascoe, 1988b**), and 133 days at 15°C (**Welton and Clarke, 1980**). Harloğlu and Farhadi (2018) reported that "by increasing rearing temperature and providing excess food, it is possible to culture animals throughout the year and attains a reduced time to sexual maturity".

In the present study, chemical analysis of the present species indicated that it contains 40% protein, 5.5% lipid, 27.4% carbohydrate, 21.4% ash, 2.9% fibers, 3191cal/g energy, and 5.7 moisture. The values of protein and carbohydrates are in the range of recorded values for the chemical content of different *Gammarus* species in previous studies (Mathias *et al.*, 1982; Seyfabadi *et al.*, 2004; Köprücü and Özdemir, 2005; Alavi Yeganeh *et al.*, 2008; Shamsaei and Khodami, 2009; Azimi *et al.*, 2011; Baeza-Rojano *et al.*, 2014) as reported by Harlıoğlu and Farhadi (2018) where the protein content was varied between 40-45%, lipid was 5-10%, 25-35% ash, and 2700-3500 cal/g energy. While the value of carbohydrates was 6-15%, which is a lower value than recorded in our study, perhaps this difference is due to the diet used in the current study, as it contained rice bran and soy powder, which are known to contain carbohydrates in high proportions. Additionally, several factors can affect the chemical composition of *Gammarus* as enumerated by Harlıoğlu and Farhadi (2018) which include age, habitats, season, region, life stage, and drying process after harvesting.

Gammarus is a very suitable food that provide the protein requirements for fish larvae in the early stages of life than large fish (2-3 cm) (Mathias *et al.*, 1982). The aflatoxin B1 value within the cultivated *Gammarus* was very low, 6.933 ppb, it less than the permitted values of the United States Food and Drug Administration which ranged from 20-300 ppb (FDA, 2013). Its content of aflatoxin B1 is resembled what measured by El-feky and Abo-Taleb (2020) for another cultivated crustacean *Daphnia magna*.

These organisms also contained high levels of the most important vitamins, as well as two of the most important antioxidants, tannic acid, and beta-carotene, additionally, these organisms are considered free from many pathogenic bacteria, which makes the use of these organisms as food for fish very beneficial and safe for animals and human health.

CONCLUSION

Based on the obtained data in the current work, *Gammarus pulex* is one of the most important invertebrates has high levels of protein and other nutritional content that is recommended for use as a partial replacement for fish meal in feeding fish larvae. Also, many nutrients of low economic value can be used to form a mixture used for feeding these organisms in mass culture. It is preferable to supply the culture with nests characterized by the presence of holes and interstitial pathways, as well as composed of plant leaves materials like palm leaves to improve the cultured *Gammarus* production.

ACKNOWLEDGEMENT

The researchers extend their sincere thanks to the Egyptian Ministry of Higher Education and Scientific Research for its continuous support, and all the results in the current research are some of the outputs of a project funded from the budget of the Minister of Scientific Research office (a project of national strategy program for genetic engineering and biotechnology, phase III).

REFERENCES

- A.O.A.C. (1990). Official methods of analysis, 16th edition. AOAC, Arlington, Virginia.
- Adhami, B.; Yeganeh, S. and Kenari SJ. (2017): Influence of Caspian Gammarus on growth parameters, survival and serum biochemical factors in jewel fish (*Hemichromis bimaculatus*). Exp. Anim. Biol., 19: 31–37 (In Persian with English abstract)
- Alavi Yeganeh, M.S.; Abedian Kenari, A.M. and Rezaii, M. (2008): Effect of Gammarus powder as a supplementary diet on growth and survival of rainbow trout larvae (Oncorhynchus mykiss). Pajouhesh & Sazandegi, 77:113–123 (In Persian with English abstract).
- Ashour, M.; Abo-Taleb H.A.; Abou-Mahmoud M.M. and El-Feky M.M. (2018): Effect of the integration between plankton natural productivity and environmental assessment of irrigation water, El-Mahmoudia Canal, on aquaculture potential of *Oreochromis niloticus*. Turk. J. Fish. Aquat. Sc., 18:1163-1175.
- Azimi, A.; Hosseini, S.A. and Sudagar, M. (2011): Effect of replacement of Caspian Sea *Gammarus* meal by partial kilka fish meal on growth performance, feed conversion ratio and survival of juveniles of rainbow trout (*Oncorhynchus mykiss*). Iran. Fish. Sci. J., 20: 63–74 (In Persian with English abstract).
- **Baeza-Rojano, E.; Hachero-Cruzado, I. and Guerra-García, J.M. (2014):** Nutritional analysis of freshwater and marine amphipods from the strait of Gibraltar and potential aquaculture applications. J. Sea Res., 85: 29–36.
- Barnard, J.L.; Sandved, K. and Thomas, J.D. (1991): Tube-building behaviorin *Grandidierella*, and twospeciesof *Cerapus*. Hydrobiol., 223: 239–254.
- **Bloor, M. (2009):** Aquatic pollution: Case study of landfill leachate toxicity and remediation. VDM Verlag, Germany pp. 1–176. ISBN 978-3-639-14699–8.
- **Bollache, L. and Cezilly, F. (2004):** State-dependent pairing behaviour in male *Gammarus pulex* (L.) (Crustacea, Amphipoda): effects of time left to moult and prior pairing status. Behav. Process., 66: 131–137.
- **Borowski, B. (1984):** The use of the males' gnathopods during precopulation in some gammaridean amphipods. Crustaceana, 47:245–250.
- Cummins, K.W. and Klug M.J. (1979): Feeding ecology of stream invertebrates. Ann. Rev. Ecol. Syst., 10:147–172.

- **Darhani, M.; Mohammadi, M. and Shamsaei, M., et al (2014):** Investigation on the effects of live and commercial food on growth and survival rate of Caspian Salmon (*Salmo trutta caspius*). Breed. Aquacult. Sci. Qr, 2: 23–34
- **Dick, J.T.A. and Elwood, R.W. (1996):** Effects of natural variation in sex ratio and habitat structure on mate-guarding decisions in amphipods (Crustacea). Behav. Process., 133: 985-996.
- El-feky, M.M. and Abo-Taleb, H.A.(2020): Effect of feeding with different types of nutrients on intensive culture of the water flea, *Daphnia magna* Straus, 1820. Egypt. J. Aquat. Biol. Fish., 24(1): 655–666
- Farrag, M.M.; El-Naggar, H.A.; Abou-Mahmoud, M.M.; Alabssawy, A.N.; Ahmed H.O.; Abo-Taleb H.A. and Kostas, K. (2019): Marine biodiversity patterns off Alexandria area, southeastern Mediterranean Sea, Egypt. Egypt. Environ. Monit. Assess., 191: 367.
- **Fukui, A. (2001):** Indirect interactions mediated by leaves shelters in animal-plant communities. Popul. Ecol., 43: 31–40.
- Griffiths, C.L. (1979): A redescription of the kelp curler *Ampithoe humeralis* from South Africa and its relationship with *Macropisthopous*. Ann. S. Afr. Mus., 79: 131–138.
- Harlioğlu, M. and Farhadi, A. (2018): Importance of *Gammarus* in aquaculture. Aquac. Int., 26: 1327–1338.
- Hartnoll, R.G. and Smith M. (1980): An experimental study of sex discrimination and pair formation in *Gammarus duebeni*. Crustaceana, 38:253–264.
- Holmes, S.J. (1901): Observations on the habits and natural history of *Amphithoe longimana* Smith. Biol. Bull., 2: 165–193.
- Hynes, H.B.N. (1955): The reproductive cycle of some British freshwater Gammaridae. J. Anim. Ecol., 24:352–387.
- Jones, L.G. (1971): Studies on selected small herbivorous invertebrates inhabiting *Macrocystis* canopies and holdfasts in southern California kelp beds. Pp. 343–367 in The Biology of Giant Kelp Beds (Macro- cystis) in California, W. J. North, ed. Series: Nova Hedwigia 32. J. Cramer, Lehre, Germany.
- Karlson, K.; Bonsdorff, E. and Rosenberg, R. (2007): The impact of benthic macrofauna for nutrient fluxes from Baltic Sea sediments. AMBIO: A. J. Hum. Environ., 36: 161-167.
- Kaushik N.K., Hynes H.B.N. (1971): The fate of dead leaves that fall into streams. Arch Hydrobiol., 68: 465–515.
- Köprücü, K. and Özdemir, Y. (2005): Apparent digestibility of selected feed ingredients for Nile tilapia (*Oreochromis niloticus*). Aquaculture, 250: 308–316.
- Kunz, P.Y.; Kienle, C. and Gerhardt, A. (2010): *Gammarus* spp. in aquatic ecotoxicology and water quality assessment: toward integrated multilevel tests. In Reviews of Environmental Contamination and Toxicology Volume 205 (pp. 1-76). Springer New York.

- MacNeil, C.; Dick, J.T.A. and Elwood, R.W. (1997): The trophic ecology of freshwater *Gammarus* spp. (Crustacea: Amphipoda): problems and perspectives concerning the functional feeding group concept. Biol. Rev., 72: 349-364.
- MacNeil, C.; Dick, J.T.A. and Elwood, R.W. (1999): The dynamics of predation on *Gammarus* spp. (Crustacea: Amphipoda). Biology Review 74: 375-395. Marchant, R., and H. B. N. Hynes. 1981. The distribution of *Gammarus pseudolimnaeus* (Crustacea: Amphipoda) along a reach of the Credit River, Ontario. Freshw. Biol., 11: 169–182.
- Mathias, J.A; Martin, J.; Yurkowski, M.; Lark, J.G.I.; Papst, M. and Tabachek, J.L. (1982): Harvest and nutritional quality of *Gammarus lacustris* for trout culture. Trans. Am. Fish. Soc., 111: 83–89.
- McCahon, C.P. and Pascoe, D. (1988a): Culture techniques for three freshwater macroinvertebrate species and their use in toxicity tests. Chemosphere, 17: 2471–2480.
- McCahon, C.P. and Pascoe, D. (1988b): Use of *Gammarus pulex* (L.) in safety evaluation tests: Culture and selection of a sensitive life stage. Ecotoxicol. Environ. Saf., 15: 245–252.
- Moore, J.W. (1975): The role of algae in the diet of *Asellus aquaticus* L. and *Gammarus pulex* L. J. Anim. Ecol., 44: 719–730.
- Navazandeh, A.Z.; Yousefian, M. and Hedayatifar, M. et al (2013): Effect of *Gammarus* meal and different enzymes on growth performance and biochemistry of *Cyprinus carpio* larvae. Breed. Aquacult. Sci. Qr., 1:79–90 (In Persian with English abstract).
- Naylor, R.L.; Hardy, R.W.; Bureau, D.P.; Chiu, A.; Elliott, M.; Farrell, A.P.; Forster, I.; Gatlin, D.M.; Goldburg, R.J.; Hua, K. and Nichols, P.D. (2009): Feeding aquaculture in an era of finite resources. Proc. Natl. Acad. Sci., 106: 15103–15110
- **Poore, A.G. and Steinberg P.D. (1999):** Preference-performance relationships and effects of host plant choice in a herbivorous marine amphipod. Ecol. Monogr., 69: 443–464.
- Razgardani Sharahi, A.; Falahatkar, B. and Efatpanah I. (2016): Effect of fish meal replacement with *Gammarus* meal on growth and body composition of juvenile Siberian sturgeon, Acipenser baerii (Brandt, 1869). J. Aquat. Ecol., 6: 102–113 (In Persian).
- Rufchaei, R.; Hoseinifar, S.H.; Mirzajani, A. and van Doan, H. (2017): Dietary administration of *Pontogammarus maeoticus* extract affects immune responses, stress resistance, feed intake and growth performance of Caspian roach (*Rutilus caspicus*) fingerlings. Fish Shellfish Immun., 63:196–200.
- Seyfabadi, S.J.; Negarestan, H. and Moghadasi, B. (2004): Chemical components of amphipod, *Pontogammarous meoticus*, along the southern coast of the Caspian Sea. J Mar Sci Technol 3:51–56 (In Persian with English abstract).

- Shamsaei, M.M. and Khodami, S. (2009): The effects of different drying methods on quality and food factor of *Gammarus (Pontogammarous meoticus)*. J.fish., 5:1–12 (In Persian with English abstract).
- Shaw, G. (1979): Prey selection by breeding dippers. Bird Study 26: 66-67. Taylor, S. J., and D. W. Webb. 2000. Subterranean Amphipoda (Crustacea) of Illinois' Salem Plateau: spatial and temporal components of microdistribution. Illinois Nat. Hist. Survey Techni Rep. INHS., 2000 27: 1-62
- Skutch, A.F. (1926): On the habits and ecology of the tube-building amphipod *Amphithoe rubricata*. Ecology, 7: 481–502.
- Sutcliffe, D.W. (1991): Reproduction in *Gammarus* (Crustacea, Amphipoda): Basic processes. Freshw. Forum, 2: 102-128.
- Sutcliffe, D.W.; Carrick, T.R. and Willoughby, L.G. (1981): Effects of diet, body size, ageand temperatureon growthrates in the amphipod *Gammarus pulex*. Freshw. Biol., 11: 183–214
- Webster, J.R. and Benfield, E.F. (1986): Vascular plant breakdown in freshwater ecosystems. Ann. Rev. Ecol. System., 17: 567–594.
- Welton, J.S. and Clarke, R.T. (1980): Laboratory studies on the reproduction and growth of the amphipod, *Gammarus pulex* (L.). J. Anim. Ecol., 49:581–592.
- Wilhelm, F.M. and Schindler, D.W. (1999): Effects of *Gammarus lacustris* (Crustacea: Amphipoda) on plankton community structure in an alpine lake. Canadian. J. Fish. Aquat. Sci., 56: 1401-1408.
- Willoughby, L.G. and Sutcliffe, D.W. (1976): Experiments on feeding and growth of the amphipod *Gammarus pulex* (L.) related to its distribution in the River Duddon. Freshw. Biol., 6:577–586.

Zielinski, D. (1998): Life cycle and altitude range of *Gammarus leopoliensis* Jazdzewski & Konopacka, 1989 (Amphipoda) in south-eastern Poland. Crustaceana, 71:129–143.

ARABIC SUMMARY

عزل واستزراع أحد أنواع مزدوجات أرجل المياه العنبة (Gammarus pulex) في الموائل المختلفة مع تقييم محتواها الكيميائي والتغذوي

حمدى أبوطالب ، عمرو زينة ، محمد عاشور ، محمد مبروك ، أحمد سلام ، محمد الفقي .

١- كلية العلوم، جامعة الأز هر، القاهرة، مصر.

٢- المعهد القومي لعلوم البحار والمصايد، الأسكندرية، مصر.

٣- كلية الزراعة، جامعة الأز هر، القاهرة، مصر.

في السنوات الأخيرة ، ازداد الاستزراع المائي بشكل كبير في جميع أنحاء العالم ، مما أدى إلى زيادة الطلب على البروتين الحيواني المستخدم في تغذية الأسماك المستزرعة، ولذلك أصبح من الضرورى البحث عن مصادر أخرى للبروتين الحيواني بديلاً عن مسحوق السمك. تعد مزدوجات الأرجل والتى تشمل الجامارس من بين تلك البدائل المحتملة وذلك بسبب إنتاجيتها الوفيرة ومحتواها الغذائى المناسب ، حيث أظهرت الدراسة الحالية أن نسبة البروتين بها كانت ٤٠ ٪ بينما بلغت الكربو هيدرات بها ٢٧.٤ ٪ ، وكان محتواها من الدهون ٥.٥ ٪ ، كما أنها تحتوي على مضادات الأكسدة مثل حمض التانيك (٢٤.٢٢٦ / ٢٢ معم/ ١٠٠ جرام) ، وبيتاكاروتين (٢٤.٣٢٩ معن مضادات الأكسدة مثل حمض التانيك (٦٤.٢٢ / ٢٠ ملغم/ ١٠٠ جم)، وبيتاكاروتين (٤٩.٢٠ ٣٣٨ ملغم/ ١٠٠ جرام) بالإضافة إلى الكثير من الفيتامينات الأساسية مثل فيتامين ب٢ (٢٩.٢٩٦٤ معم/ ١٠٠ جم) ، وفيتامين ب٦ (٦٢٠ ٣٣٨ ملغم/ ١٠٠ جم)، وفيتامين ب٥ (١٣.٥ ٢٣ ملغم/ ١٠٠ جم)، وبيتاكاروتين (١٩.١٣٩ ملغم/ ١٠٠ جم) ، وفيتامين ب١ (١٣.٩ ٢٠ ٢٣ ملغم/ ١٠٠ جم) ، وفيتامين ب٢ (١٣.٥ ٣٢٠ ملغم/ ١٠٠ جم) ، وفيتامين ب٢ (١٩.٥ ٣٠ منهم معمرات الأساسية مثل حمض التانيك (١٤٠ ٢٣٠ مكرا الأساسية مثل فيتامين ب١ (١٩.٥ ٣٠ ملغم/ ١٠٠ جم) ، وفيتامين ب١ (١٩.٥ ٣٠ ملغم/ ١٠٠ جم) ، وفيتامين ب١ (١٩.٥ ٣٠ ملغم/ ١٠٠ جم) ، وفيتامين ب١ (١٩.٥ ٢٠ ملغم/ ١٠٠ جم) ، وفيتامين ب١ (١٩.٥ ٣٠ معمرات ملغم/ ١٠٠ جم) ، وفيتامين ب٢ (١٩.٥ ٣٠ جم) ، وفيتامين ب١ (١٩.٥ ٣٠ ملغم/ ١٠٠ جم) ، وفيتامين ب١ (١٩.٥ ٣٠ ملغم/ ١٠٠ جم) ، وفيتامين ب١ (١٩.٥ ٣٠ جم) ، وفيتامين ما ملغم/ ١٩٠ جم) ، وفيتامين ب١ (١٩.٥ ملغم/ ١٠٠ جم) ، وفيتامين ما مرار ٩٠ جمع الفوليك (١٩.٥ ملغم/ ١٠٠ جم) ، وخيامين ما ملغم/ ١٩٠ جم) ، وحمض الفوليك (١٩.٥ ٣٠ مرار ٩٠ جمع المغرار ٩٠ جمر الفوليك (١٩.٥ ٩٠ جم) ، وخيتامين أمر ٩٠ جمار جمار ما يا مرار ٩٠ جمر الفوليك (١٩٠ مرار ٩٠ جمر) ، وفيتامين ما مرار ٩٠ جمار جمار ، وفيتامين ما مرار ٩٠ جمار ما يا ولفوليك (١٩.٥ ٩٠ جمر) ، وحمض الفوليك (١٩٠ مرار ٩٠ جمر) . ميكرو غرام/ ١٠ جم) ، وحمض الفوليك (١٩.٥ ٩٠ جمر) ميكرو غرام/ ١٠ جمار .

تم عزل Gammarus pulex من أحد الموارد الطبيعية (بحيرة مريوط) حيث تعد تلك البحيرات مصدرا لثروات عديدة لم يتم استغلالها الإستغلال العلمى الأمثل والمستدام حتى الان، وبعد ٩٠ يومًا من التربية في الخزانات (معدل تسكين أولى: ٣٠ فردًا / لتر) مزودًا بأربعة أنواع مختلفة من الأعشاش: قطع خشبية مثقبة ، وكرات من ألياف النخيل ، وكرات من أوراق النخيل ، وكرات من شباك النايلون "هابا نت" ، نوع واحد لكل خزان. أظهرت البيانات التي تم الحصول عليها أن كرات أوراق النخيل هي أفضل عشوش التربية حيث أعطت أعلى إنتاجية (١٢٧ فرد / لتر) ، وبلغ متوسط الكثافات التي تم الحصول عليها من مواض التربية حيث أعطت أعلى إنتاجية (١٢٧ فرد / لتر) ، وبلغ متوسط الكثافات التي تم الحصول عليها من أحواض التربية المختلفة ١٠٨ فرد/لتر، بمتوسط وزن رطب بلغ ٢١٦ جم/لتر وهو ما يعني أن كل ١٩ من مياه الإستزراع يمكن أن يصل إنتاجه إلى حوالي ٢١٦٠ جرام من الجامارس الحى وهو ما يعني أن كل ١٩