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Original Article

Optimum stocking density of adult freshwater crayfish *Procambarus clarkii* (Girard,1852) in recirculation system

Mohamed A. GabAllah and Mohamed Abdelnaser Amer*

Zoology Department, Marine Biology Section, Faculty of Science, Al-Azhar University, Cairo.

ABSTRACT: Crayfishes, like other decapod crustaceans, possess certain biological traits that render them potentially valuable for aquaculture. In this field, stocking density, a key factor influencing crustacean aquaculture, is generally correlated positively with yields. This study aims at determining the optimal stocking density for mature freshwater crayfish, *Procambarus clarkii* collected from River Nile tributaries during March 2023. The specimens were transported and reared in the Marine Invertebrate's Laboratory at the Zoology Department, Faculty of Science, Al-Azhar University.

A total of 520 individuals were maintained at three stocking density levels within an aerated circulation system. The findings revealed that a stocking density of 40 individuals/m2 was optimal for achieving rapid growth, ranging from 12.5% to 15.9% in total weight gain (TWG), with relatively low mortality (0-20%). Furthermore, increasing the stocking density to 80 individuals /m^2 showed promise yield, with a survival rate ranging from 80% to 92.5%. Notably, acceptable survival and growth rates may be achieved, depending on feeding mode and photoperiod. Additionally, the specific growth rate in weight (SGRW) was higher at a stocking density of 40 individuals/m^2 compared to 80 individuals/m^2. However, crayfish, like other freshwater crustaceans, is influenced by other factors that interact with stocking density such as molting, shelter cover, photoperiod, and aquaculture technique. Therefore, it is recommended to conduct further experiments for achievement more investments on this species.

Key word: Freshwater crustacean, Crayfish, aquaculture, stocking density
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1.INTRODUCTION

Crayfishes are freshwater crustaceans that belong to family Cambaridae within order Decapoda which also includes lobsters. Most crayfish cannot tolerate polluted water, although some species like *Procambarus clarkii* are more resilient and help maintain the health of aquatic ecosystems by controlling algal growth and consuming organic matter (Needon *et al.*, 1971; Crandall and Buhay 2008). *P. clarkii* is the most commercial species in the world, and can grow rapidly, reaching up to 15 cm in length as adults (Henttonen and Huner 1999; Loureiro *et al.*, 2015).

Correspondence :
Mohamed Abdelnaser Amer
Zoology Department, Marine Biology Section, Faculty of Science, Al-Azhar University, Cairo
Mail: naser12.7.12.7@gmail.com

It is native to the central southern United States and northeastern Mexico and has been introduced by humans to numerous regions throughout the world (Campos and Rodríguez-Almaraz 1992). It has been introduced to some Central and South American countries, (Magalhães et al. 2005; *Banci et al.,* 2013; Loureiro 2015), central and northern European countries (Huner 2002; Souty-Grosset *et al.,* 2006; Ilhéu et al. 2007); Asia, including China and Japan; and also found in Palestine (Mito and Uesugi 2004; Kawai and Kobayashi 2005).

Moreover, the red swamp crayfish is also established in Africa, with records from Egypt, Kenya, South Africa, Sudan, Uganda, Zambia, and Zimbabwe (Huner 1988; Hobbs *et al.,* 1989).

In Egypt, the first established population of *P. clarkii* was introduced in the early 1980s, probably derived from a commercial aquaculture facility in Giza; within the last few years, it has successfully established in various sites of the river Nile and tributaries (Hamdi 2001; El Zein 2005; Ibrahim and Khalil 2009; Khalil and Sleem 2011). Several investigations have been carried out comprising, ecology (Ibrahim *et al.,* 1995; 1997; Mubarak, 1996; Ahmed 2012), biodiversity (Fishar, 2006), populations dynamics (Emam & Khalil, 1995; Saad *et al.,* 2015; Makkey *et al.,* 2022), biology (Soliman et al. 1998; Hamdi 2001; Sayed 2002 and Ibrahim *et al.,* 2005), using as fishmeal protein (Abd El- Rahman and Badrawy 2007), pollutants (Sharshar & Geasa 1998; Tolba 1999; Aly 2000), management (Aly *et al.,* 2020), molting (Amer *et al.,* 2015a,b,c; 2016; 2017), nutritive value of raw and processed products (Ibrahim 2010; 2017; El-Sherif *et al.,* 2021) and as indicator of the bioavailability of heavy metals and risk assessments (Abdel Gawad *et al.,* 2018).
Global culture yield of crayfish is over two million tons/year (FAO, 2020). Production levels depend on the initial stocking strategy (Daly *et al.,* 2009; Yu *et al.,* 2020). High stocking density can lead to several problems in crustacean aquaculture, including cannibalism (Romano and Zeng 2017), limited food resources (McClain, 1995) and diseases (Nga *et al.,* 2005).

Using shelter increases surface area and reduces physiological stress caused by high stocking density (Morrissy, 1992; Geddes *et al.,* 1993; Pinto and Rouse 1996; Verhoef and Austin 1999; Jones and Ruscoe, 2000). Shelter also helps manage intraspecific interactions (Baird *et al.,* 2006).

Crayfish are nocturnal animals (Bojsen *et al.,* 1998; Lozan, 2000). Therefore, increasing the illumination period may increase growth rate and allow for higher stocking densities. Initial stocking densities have been reported to range from 100 individuals/m² (Nynström 1994; Ulikowiski and Tadeusz 2004) to even 3,000 individuals/m² for early-stage specimens (Policar and Kozak, 2001).
Feeding behavior also affects stocking density, alongside factors like photoperiod and the size of the crayfish being raised. Crayfish larvae and small juveniles cannot utilize artificial feed effectively, but later rearing stages can be provided with this type of food (Strużyński and Niemiec 2001).

The optimum stocking density in closed circulation system for crayfish of early juveniles of red claw crayfish, *Cherax quadricarinatus* was 66 individuals per m2 (Garcia-Ulloa *et al.,* 2012). *P. clarkii* considers as one of crustacean species faces a serious problem of low survivals at high stocking density (Ramalho *et al.,* 2008). Therefore, an adequate natural shelter, and other artificial macrophytes can be used for indoor culture (Yuan *et al.,* 2018; Yu *et al.,* 2020).

P. clarkii is one of the most important crayfish species for aquaculture. It has recently become a flourishing commodity in China due to increasing demand for crayfish as a popular food (Jin *et al.,* 2019; Zhang *et al.,* 2021).
Consequently, China became the world's leading producer of crayfish via integrated aquaculture, with two million tons annually, accounting for 96% of global production (FAO, 2020). However, the marketing for crayfish has expanded in recent years, particularly in northern Europe where several festivals celebrate the consumption of crayfish in the fall.

In Egypt, marketing for crayfish is still limited, and challenges exist in utilizing this species as food. Moreover, the wild catch for the international market has increased in recent years, reaching 8050 tons/year in 2020 (GAFRD, 2020).
Noteworthy, crayfish are considered good animals for culturing, because it exhibits fast growth rates at temperature between 23- 31°C, requires relatively simple spawning technique, and tolerates a wide range of water qualities, (Ibrahim and Khalil 2009; Ibrahim, 2024).

Investment of the freshwater crayfish can contribute to reduce the fish gap with increasing human populations. Therefore, this study was designed to investigate the growth performance and optimum stocking density of *Procambarus clarkii* in closed circulating system for possible aquaculture purposes.

2. MATERIALS AND METHODS

2.1. *Sampling/Rearing*:

The individuals of Procambarus clarkii, were collected from River Nile tributaries at Al-Kanater Al- Khairiya, Qalyoubia Governorate, during March 2023. The collected individuals were transported alive to the Marine Invertebrates Laboratory, Zoology Department, Faculty of Science Al-Azhar University, Naser City, Cairo. All morphometric characters were measured; weighed to the nearest 0.1 g using an electric balance with an accuracy of 0.01 g after blotting excess water with absorbent tissues. All the collected specimens were sexually mature.
The collected individuals of *P. clarkii* were reared in previously prepared aquaria for two weeks before the test for acclimation. Fresh food was used to feed crayfish at acclimation, and water condition during the acclimation and testing were set in closed circulation system (Figure 1).

Figure 1: Circulation system applied for estimation of optimum stocking density of *P. clarkii.*

2.2.Estimation of optimum stocking density

According to (Figler et al., 1999, 2005; Yu *et al.,* 2020), three levels of stocking density (20, 40 and 80 ind. /m2) were set and investigated for 4 weeks with replication for each set. They were fed with proper food supply (artificial food 30% protein) and provided with appropriate refuge (PVC pipes).

A total of 280 individuals of mature crayfish *P. clarkii,* were set in the aquaria and feed once a day at 05:00 PM with ambient photo period. The feeding rate was adjusted according to total biomass of each aquarium (5% of total weight) and faces were removed day by day. The dead crayfish and those with injured carapace were removed once found. Mortality rate and survival rate were recorded. The total biomass of crayfish in each aquarium was weighted at the beginning and end of the experiment.

At this experiment, 240 individuals were maintained at two levels of stocking density (40 and 80 ind./m²) with replication for each aquarium. A combination of artificial food supply 30% protein and fresh fish meat was used. The crayfish individuals were fed twice a day, firstly with fish meat at 08:00 AM and with artificial food with 30% protein at 6:00PM. The feeding rate was adjusted according to the total biomass of each aquarium.

2.3.Calculations

Survival rate (SR), specific growth rate of weight (SGRW) and total weight gain (TWG) were calculated for each aquarium as following:

SR (%) = 100 * (Nf/Ni)

SGRW (%) = 100 * (lnWf - lnWi)/T

TWG (%) = 100 * (TBf - TBi)/ TBi

Where: Nf = final number of crayfish, Ni= initial number of crayfish, Wf = final crayfish weight (g), Wi= initial crayfish weight (g), T= study period (21 days), TBf= total final biomass (g) and TBi= total initial bio- mass (g).

2.4.Feeding behavior:

The feeding behavior and response of *P. clarkii* were determined according to (Kreider and Watts 1998). The movement of maxillipeds, movement of dactyl of walking legs, movement of walking legs to the mouth, and orientation of the body towards the food source were observed and recorded.

2.5.Water quality circulation system:

Water temperature and pH values were measured instrumentally, while dissolved oxygen were measured by titration method. During experiments, the average values of water temperature, TDS, CND and DO were maintained at 23.03±0.60(Cº), 354.73±51.12 (PPM), 462.00±12.97 (PPM), and 10.03±2.49 mg/L, respectively.

2.6.Statistical analyses

All results were statistically analyzed (T-test) for significance difference using the computer program Microsoft Excel 365.

3. RESULTS

A total of 280 individuals were used in two experiments for estimation of optimum stocking density. The values of mortality rate (%), survival rate (%), total weight gain (TWG) and specific growth rate in weight (SGR, %) were estimated for each treatment.

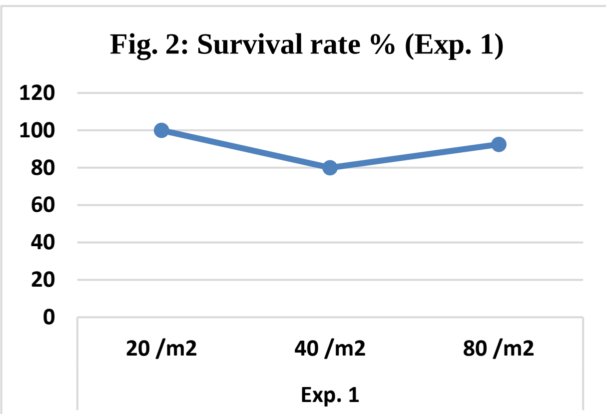
The results in Figures (2,3) indicated that, survival rate of the first stock (20/m^2) was the highest and reached 100%, declined to 80 at 40 ind./m^2 but increased to 92.5% for higher stocks at 80 ind./m^2. On the other hand, the mortality rate reached the maximum (20%) at stocking density of 40/m^2, then declined to 7.5 % at stocking density of (80 ind./m^2).

The total weight gain for each stocking densities was also varied and ranged between 7.34 and 12.5 %. Noteworthy, the best stocking density observed from those three levels considering TWG and growth performance was recorded at 40/m2 but considering survival rate which may affect the total biomass after long period was 80 ind/m^2.

On the other hand, the highest SGR was 0.84, recorded at 40 ind./ m2, but declined to 0.61 at 80 ind./m2 and reached the minimum level (0.5) at 20 ind./ m^2.

Effects of dead fish and artificial feed with protein 30%. The results in Figure (2, 3) show that the survival rate of the first stock at 40 ind./m2 was the highest being 100% but declined to 85% for higher stock (80 ind./m2). Thus, mortality rate was maximized to 15% at that stocking density. However, the total weight gain for each stocking was remarkably varied and increased from 7.4 to 15.9 % at the two levels (80 and 40 ind./m2) respectively.

In contrast, SGR recorded 0.7 at 40 ind./m2 compared with only 0.34 at 80 ind./m2 and indicating that, the stocking density at 40/m2 considered the best for SGRW and survival rate during this experiment.
From the previous results of the two experiments, the best performance, healthy and productive stocking density was 40 ind./m^2. But this stocking can be increased up to 80/m2 with acceptable survival rate in well sheltered ponds.



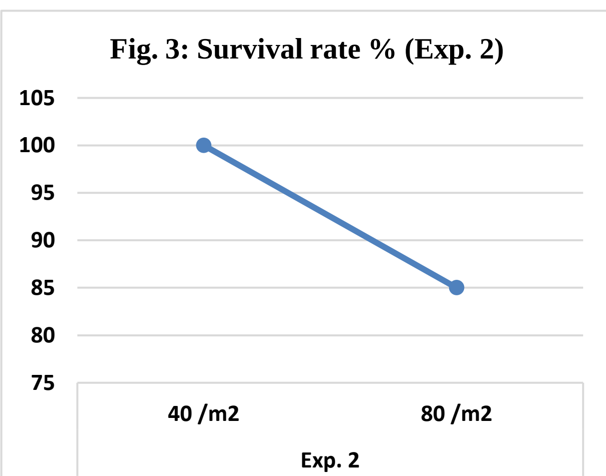
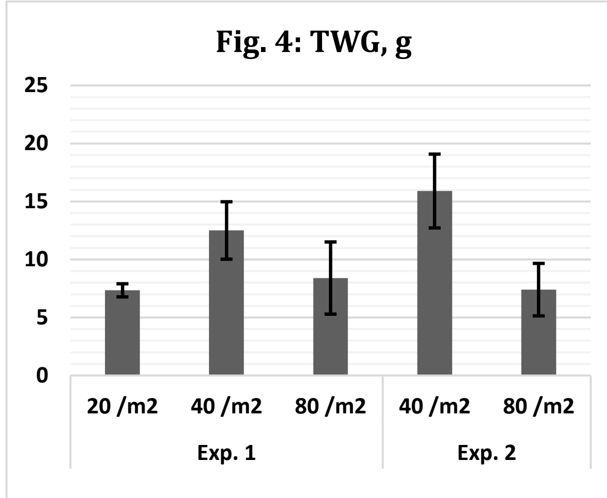
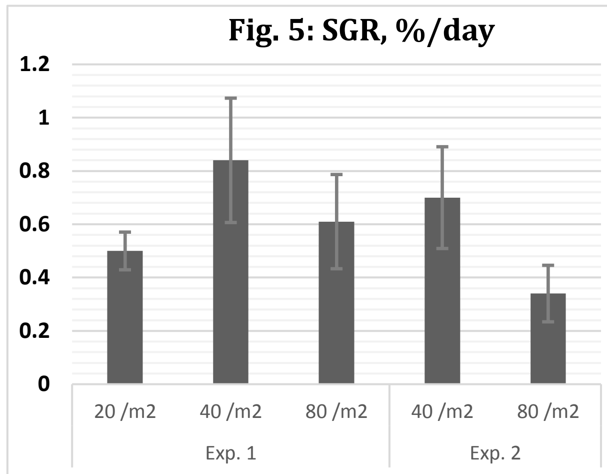


Figure 2, 3. Survival rate (%) at different stocking densities at each experiment for *P. clarkii*





Figures 4, 5. TWG and SGR at different stocking densities for *P. clarkia*.

***Feeding behavior*:**

Feeding response of *Procambarus clarkii* in the aquarium to the dietary items and formulated feed components were observed during this study. *P. clarkii* showed variable feeding response to different food items. The movement of maxillipeds and dactyl of walking legs were noted to both of food types (artificial feeding and fish meat).
Moreover, movement of walking legs to the mouth was noted after a period of time when artificial food was selected by the crayfish. But the orientation of the body towards the food source was noted at once when dead fish was applied to the crayfish.

Thus, the previous behavior indicated that the crayfish attracted by the odor of fish meat rather than artificial feeding.

4. DISCUSSION

Stocking density is one of the major factors that influence aquaculture performance of crustacean species worldwide, while it is generally positively related to aquaculture yields (Lutz and Wolters 1986; Geddes *et al.*, 1993; Verhoef and Austin 1999; Daly *et al.*, 2009). High stocking density of crustacean usually aggravates competition for resources e.g., living spaces, shelters and food (McClain 1995), cannibalism (Romano and Zeng, 2017), and crowding stresses e.g., low appetite, poor physiological state and weak resistance to diseases (Nga *et al.*, 2005), resulting in decreased survival, body size and growth rates, in addition to molting. In closed circulation aquariums, our study found that stocking densities between 40 and 80 ind./m^2 resulted in optimal growth rates (12.5-15.9%) and survival rates (80-92.5%) with no significant difference between these groups ($P<0.05$).

This finding aligns well with previous reports by Goyert (1978) and Yu *et al.*, (2020), who also observed good growth and survival at similar densities. Similarly, Ramalho *et al.*, (2008) and Deng *et al.*, (2010) reported positive results. Furthermore, our results suggest that increasing stocking density to 80 ind./m² could still be acceptable due to the relatively high survival rate (80-92.5%). This aligns with findings in the range of 66 ind/m² for adults and juveniles of red claw crayfish (*Cherax quadricarinatus*) by Naranja *et al.*, (2004) and Garcia (2012).

Noteworthy, the present results showed accepted survival rate which ranged from 80 to 100% for stocking 40 ind./m2 and between 85 and 92.5% for 80ind./m2 depending on feeding mode and photoperiod. These results agree with the findings of Yu *et al.*, (2020) who recorded survival rate between 57.5 and 90%. Moreover, SGRW was higher in stocking density at 40 ind/m^2 than 80 ind./m^2, but without significance difference ($P<0.05$).

The obtained results are within the range of previous studies carried out by Romaire and Villágran (2010), and Yu et al. (2020). Those authors suggested that possible cannibalism habit can be improved at high stoking density by shelter cover and availability of food.

CONCLUSION

In conclusion, the present study recommends carry out further experiments for achievement more investments for this invaded species and its widely distribution in all Egyptian freshwater habitats from Aswan to the northern governorates in Nile Delta.

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