Effect of in situ water on the survival rate and behavior of the crab *Callinectes amnicola* (de Rochebrune, 1883) from Lake Nokoué in captivity

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Abstract. In order to contribute to the diversification of aquaculture species and especially to the sustainability of the natural aquatic stock, a study focused on the acclimatization and ethology of the crab *Callinectes amnicola* from Lake Nokoué in captivity over a period of 42 days. The objective of the study was to determine the optimal conditions for the maintenance and survival of the crab *C. amnicola* in a controlled environment, as well as their ethology. The experiment was carried out in three repetitions in 9 tanks measuring 42.5 x 33 x 21.3 (cm) each. The tanks contained white sand from marshy environments to a height of 5 cm and they were filled with 16 liters of water of different types. The density was 5 crabs per tank with an average biomass of 9.07±1.02 g and an average size of 4.6±0.99 cm. Treatment T1 contained water from the crab collection medium, treatment T2 contained water-containing salt of the same concentration as that of the crab collection medium (17.5‰) and treatment T3 contained fresh water (0.3‰). The subjects were fed twice daily with a ration of 8% of their biomass with *Macrobrachium macrobrachion*. An aeration device (ACO-009; 120L/Mn – 120W) was provided for each tank and a video surveillance camera (Reolink; model: RLC-811A/4K-8MP) was used for the observation and description of the behavior of crabs. At the end of the trial, the results in terms of average survival time were 528 hours (22 days), 14 hours and 13 hours for treatments T1, T2 and T3 respectively. The best performance recorded during this trial was observed at treatment T1 with a survival rate of 53.33% on the 28th day. The ethograms established after observing the crabs revealed that under stress conditions, these crabs are more agitated and move more in the environment. Conversely, when they are in a preferential environment, they spend most of the day buried in the sand and feed late in the evening. Maintaining *C. amnicola* in captivity was able to do with water from the collection environment where they preferred to feed late in the evening. Therefore, to successfully domesticate *C. amnicola*, efforts must be made to best reproduce the living environment of crabs under controlled conditions.
Key Words: acclimatization, *Callinectes amnicola*, Lake Nokoué, survival.

Introduction. The development of new techniques and production methods in the agricultural sector and particularly in aquaculture in recent decades have enabled it to rise to the rank of key sectors favorable to socio-economic development. Unlike northern and Asian countries which already raise several aquaculture species, developing countries only have two or three species. This is not without consequences on the natural fish stock which is under enormous pressure due to the constantly increasing population. The quantity intended for human consumption (excluding seaweed) is 20.2 kg per capita, more than double the average of 9.9 kg per capita recorded in the 1960s (FAO 2022). While total aquaculture production in Africa was estimated at 1.9 million tonnes in 2016, that of crustaceans in particular was estimated at 5 thousand tonnes (FAO 2018).

Furthermore, many authors have indicated the interest in breeding the crab *Callinectes amnicola* (Sankaré 2007; d'Almeida & Fiogbé 2008; Hinvi et al 2013) present in our fresh or brackish water reserves. It is a crab species highly valued by the population in terms of consumption and economic resource (Gruvel 1913; Kwei 1978; Akin-Oriola et al 2005; Sankaré 2007; Babatunde 2008; Gnimadi et al 2008; Lawal-Are 2009). More than 90% of it is sold mainly in fresh form both on the local market and in neighboring countries where it is massively imported (Dessouassi et al 2018).

Given its socio-economic importance and the data available in the literature, this species of crab is victim of overexploitation in Benin because the size of first capture is smaller than the size of first sexual maturity according to Goussanou et al (2017). These crabs also play a vital role in the transfer of energy through the food chain and contribute to food recycling (Santhanam 2018) because they are also scavengers.

Despite its importance in ecology, to date there is little work related to its breeding in a controlled environment in our regions. Scientific work has also generated biological and ecological knowledge on this species which shows that it can be a candidate for domestication (Dessouassi et al 2019). This is what justifies the present study, the main objective of which is to contribute to the domestication of the crab through the establishment of the fundamental bases for the survival and breeding of juveniles of the crab *C. amnicola* in captivity.

Material and Method

Crab collection medium*.* The crabs used were collected in Lake Nokoué located in the southeast of Benin with geographic coordinates 6°26'00'' N, 2°27'00'' E. The crabs collected come from artisanal fishing methods and techniques using fishing gear such as crab scales, Ghana traps, cast nets, gill nets, fyke nets and palanzas. Crabs without fouling or unusual coloring and without damage to the legs or claws, with an average biomass of 9.07±1.02 g and inter-spine width 4.6±0.99 cm were collected. They were then transferred into baskets for transport to the experiment site within 30 minutes.

Experimental site*.* The study was carried out over a period of six weeks or 42 days (from January 22, 2024 to March 4, 2024) at the hatchery of the Territorial Agency for Agricultural Development-7 (6.466592, 2.355402) in the commune of Abomey-Calavi in Benin.

Methods used*.* The experiment was carried out in three repetitions in nine tanks of dimensions: 42.5 x 33 x 21.3 (cm) which served as breeding tanks for 42 days. Each tank was filled with white sand from marshy environments to a height of 5 cm. The arrangement of these tanks was done following the complete randomization model. Each tank was filled with 16 liters of water of different types depending on the treatment; treatments T1, T2 and T3 respectively contained water from the crab collection environment, water containing salt of the same concentration as that of the natural environment (17.5‰) and fresh water (0.3‰). The stocking density was 5 crabs with an average body mass of 9.07 ± 1.02 g and an average size of 4.6±0.99 cm per tank. 10% of the volume of water contained in each tank was taken and replaced with another of the same quantity and of the same nature every 24 hours (Kohinoor et al 2019). The device was equipped with an aerator (ACO-009; 120L/Mn – 120W) and a video surveillance camera (Reolink; model: RLC-811A/4K-8MP) for the supply of oxygen to the environment and the description of the subjects' behavior respectively.

The crabs were fed twice a day (Shelley & Lovatelli 2011), early in the morning and late in the evening (9:00 a.m. and 7:30 p.m.) with a ration of 8% of their biomass (Ponniah 2009) with *Macrobrachium macrobrachion*. Water quality control consisted of taking physicochemical characteristics such as: temperature (TP3001 digital thermometer), salinity (salinity-meter Hanna HI98192) and pH (pH meter Portlab pH-100) three times a day, morning, noon and evening (8 a.m., 1 p.m. and 6 p.m.) according to Griessenger et al (1991).

Three parameters were taken into account for the ethology of crabs: social and dietary behavior and stress; and two groups of crabs served as a comparison for ethology: those from treatment T1 (the control) and those from treatment T3 (the stressful environment considering water concentration). The crabs were observed using the "behavioral sampling" method followed by formal description (Tinbergen 1963; Dawkins 1989). The observation of the crabs took place in three periods over an interval of 15

minutes per period (Mégevand 2022): 1st period (5 minutes after loading) 10:05 a.m. – 10:20 a.m.; 2nd period 1:00 p.m. – 1:15 p.m.; 3rd period 4:00 p.m. – 4:15 p.m.
• Collection of data relating to stress: measurement of the distance traveled and the mobility time of crabs (Mégevand 2022).
• Collection of data relating to the social and feeding behavior of crabs: duration of agglutination and preferential feeding period (day or night).

Data analysis. Mathematical formulas were used to calculate the numerical data recorded during the experiment. These include:

$$\text{Survival rate (\%)} = \frac{\text{Final number}}{\text{Initial number}} \times 100$$

Survival time (hours): Time of death - Time of loading

$$\text{Mobility time (\%)} = \frac{\text{Subject mobility duration}}{\text{Length of observation period}} \times 100$$

$$\text{Coefficient of variation} = \frac{\text{Standard deviation}}{\text{Average}} \times 100$$

Normality tests were performed on the variables followed by a Fisher test for variables that follow a normal distribution and a Mann-Whitney test for those that do not follow a normal distribution. The statistically different variables ($p < 0.05$) were determined using Microsoft Excel 2016 and XLStat 2024 software. Wondershare Filmora 13 software allowed the processing of images and video recorded by the camera.

Results

Physico-chemical parameters. The minimum temperature of 28.18°C was recorded at treatment T2 and the maximum of 28.74°C at treatment T1. At the 5% threshold, the Fisher test revealed that there was no significant difference between the average temperatures of the three treatments ($p > 0.05$). Regarding the pH, the Mann-Whitney test revealed at the 5% threshold, a non-significant difference between the means of the T1 and T2 treatments ($p > 0.05$) and a significant difference ($p < 0.05$) between treatments T1 and T3. The minimum value recorded was 6.98 at treatment T3 compared to a maximum of 8.72 at treatment T2. As for salinity, the minimum value recorded was 0 at treatment T3 compared to a maximum of 16.05 at treatment T1. The Mann-Whitney test at the 5% threshold revealed that there was no significant difference between the means of treatments T1 and T2. On the other hand, a significant difference ($p < 0.05$) was recorded between the means of treatments T1 and T3 (Figure 1).

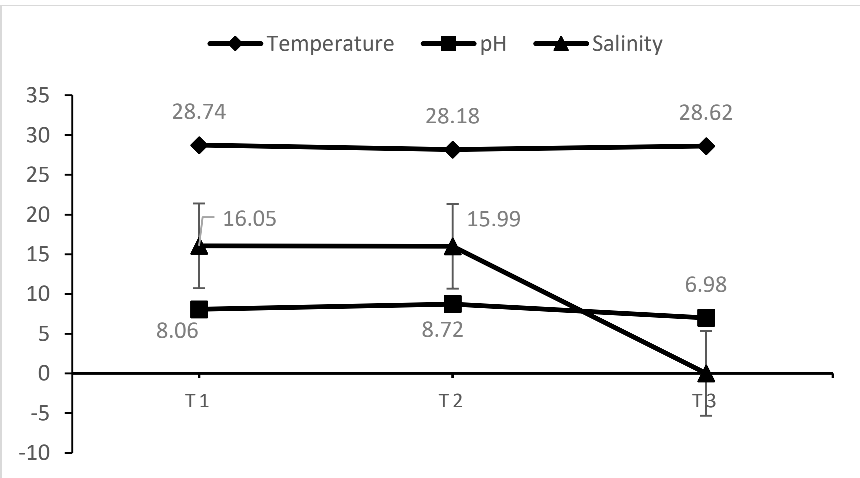

Figure 1. Average variation of physicochemical parameters.

***Survival time**.* The lowest and highest survival times recorded during this experiment were observed respectively at treatment T2 and T1 with the respective values 3 hours and 672 hours. At the 5% threshold, the Mann-Whitney test does not reveal a significant difference ($p > 0.05$) between the means of the T2 and T3 treatments. On the other hand, significant differences ($p < 0.05$) were recorded between the mean of treatment T1 compared to that of T2 and T3 (Table 1).

Table 1

Survival time of crabs (in hours) depending on treatments

	T1	*T2*	*T3*
Minimum	288	3	7
Maximum	672	21	52
Average	531.2 ± 173.50^{a}	13.93 ± 7.31^{b}	13.13 ± 11.76^{b}
CV	32.66	52.49	89.57

Average with the same superscript letters are statistically identical ($p > 0.05$).

***Survival rate**.* The survival rate recorded 24 hours after loading the crabs is 0%, 20% and 100% respectively for treatments T2, T3 and T1. Fifty-two (52) hours after loading, only the crabs from treatment T1 were alive with a survival rate of 100%. Five hundred and fifty-two (552) hours and one thousand eight (1008) hours after loading, the survival rates were 53.33% and 34.66%, respectively. The lowest average survival rate recorded during this trial was 5.85% for a maximum of 87.68% for treatments T2 and T1 respectively. According to the Mann-Whitney test, there is no significant difference ($P > 0.05$) between the means of treatments T2 and T3 (Figure 2).

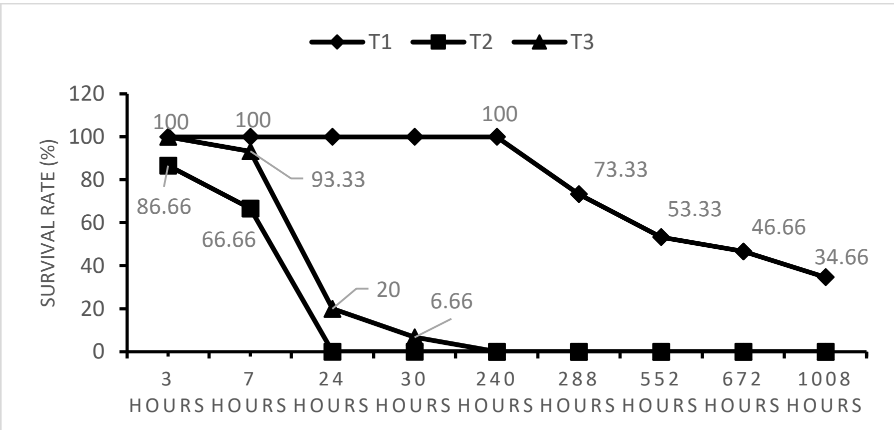

Figure 2. Evolution of the survival rate of crabs by time interval and by treatment.

***Evolution of stress over time**.* The observation of two groups of crabs, one of which placed in a preferential environment T1 (water from the crab collection environment) and the other in fresh water without added salt (T3) allowed us to identify differential behavioral traits. The burying of the crabs in the sand immediately after loading (10:00 GMT+1) is observed in almost all of the crabs in treatment T1. This behavior is usual among crabs in the natural environment, especially during the day (in the presence of solar radiation). The agility and mobility (Figures 3 and 4) of these crabs were noted in the absence of light in the environment from 7:30 p.m. GMT+1. Conversely, those in T3 remained very mobile from loading until nightfall for the majority of them. Three hours after loading, certain subjects in the T3 treatment presented signs of physiological dysfunction such as: unusual swimming postures, imbalance or inversion.

The ethogram of the distance traveled reveals that the crabs in the T1 treatment traveled less distance than those in T3 over the three observation periods. The Mann-Whitney and Fisher tests revealed significant differences ($p < 0.05$) respectively for periods 1 and 2. On the other hand, there was no significant difference in the third period (Mann-Whitney test $p = 0.12 > 0.05$).

The ethogram of the duration of mobility reveals a similarity of data between the first and the third periods of the observation for the two groups of crabs and a significant difference in the duration of mobility in favor of the crabs from the T3 treatment to the second period of observation. These observations were confirmed by the Mann-Whitney test, which revealed a significant difference ($p < 0.05$) between the means recorded in the second period. Furthermore, the same test revealed that there was no significant difference ($p > 0.05$) between the means of the first period and those of the third period.

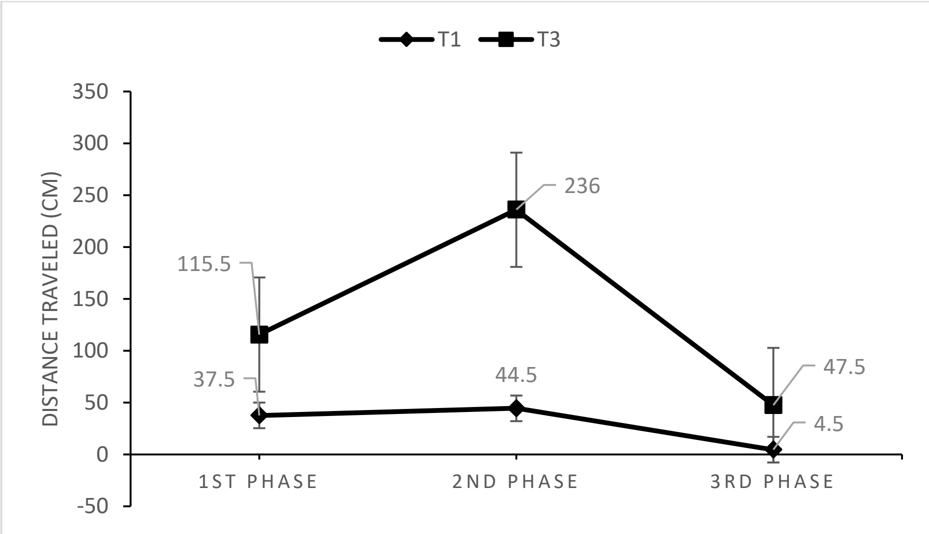

Figure 3. Ethogram of the distance traveled by the two groups of crabs.

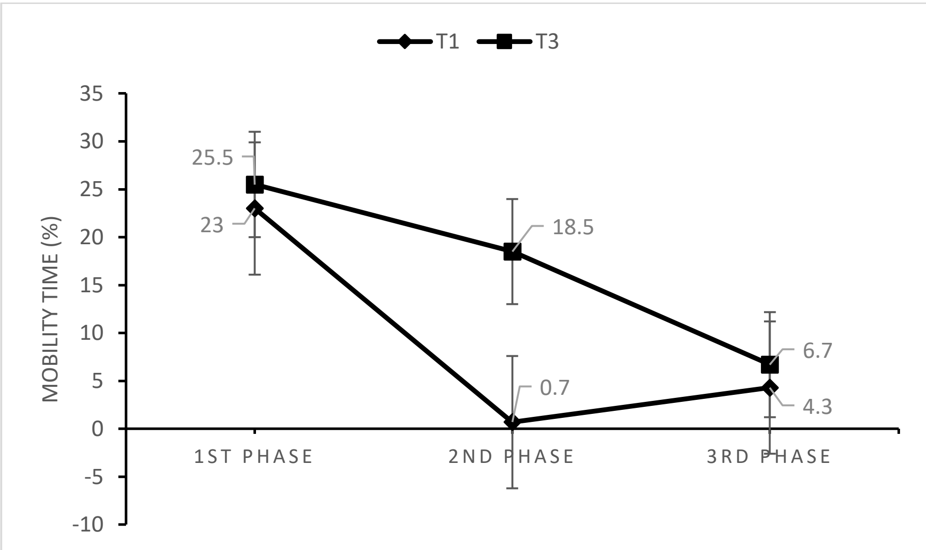

Figure 4. Ethogram of mobility duration by the two groups of crabs.

Social and eating behavior. The crabs moved freely without any social interaction; they rarely clumped together (T3 treatment) and did not move in groups. There was no fighting among them and sexual behavior was not observed as well. Their feeding during the day where they prefer to bury themselves in the sand has proven unsuccessful in the majority of cases. Conversely, they happily agree to feed at nightfall (7:30 p.m. GMT+1) when they all come out of their hiding places.

Discussion

Physico-chemical parameters. The statistical results obtained for the averages of treatments T1, T2 and T3 with regard to temperature and pH show that these parameters did not have a major impact on the performances recorded in terms of survival time and survival rate. The values of temperature (28.18-28.74°C) and pH (6.98-8.72) meet the standards recommended by Ponniah (2009), Ikhwanuddin et al (2012) and Kohinoor et al (2019) for rearing in a controlled environment with juvenile crabs (inter-spine width 4.6±0.99 cm). As for salinity, significant differences obtained between the means of treatments T1 and T3 lead us to deduce that salinity is a factor which influences the survival time as well as the survival rate of crabs. These results correlate with the results of the work of Nurdiani & Zeng (2007) and Ikhwanuddin et al (2012) who estimated that the salinity rate is a factor which conditions a better quality of the living environment of swimming crabs.

***Duration and survival rate**.* Baylon & Suzuki (2007) and Nurdiani & Zeng (2007) stated that the survival rate of portunid crabs is affected not only by salinity, but also by temperature. In the present study, despite the similarity of the physicochemical parameters in terms of temperature, pH and salinity of treatments T1 (water from the crab collection medium) and T2 (fresh water + salt of concentration equal to that of the crab collection medium crabs), the best performances obtained for duration and survival rate were recorded at treatment T1. The statistical results confirm that there were significant differences ($p < 0.05$) between the mean of treatment T1 compared to that of treatment T2. The low survival rate recorded in the T2 treatment leads us to say that the salinity of the environment as well as its temperature are not the only physicochemical parameters which promote a better quality of the living environment of the crab *C. amnicola*. According to Ikhwanuddin et al (2012), Anger (2001) and Mia & Shah (2010) salinity not only affects the survival rate but also growth.

***Evolution of stress over time**.* It emerges from these observations and the statistical analyzes recorded on the one hand that the sudden change in the nature (physicochemical parameters) of the water in the living environment is a stressful factor and on the other hand that the response to this imbalance physiological is the wandering of crabs in all directions seeking a more comfortable environment. These observations support the statement of Anger (2001) when he asserted that the salinity level is a stressful factor for crustaceans and which delays their development, reduces their survival rate, their feeding frequency and the exuviation process. These results confirm the words of Thornton & Lukas (2012), when they say that the environment in which animals are raised can affect their cognitive abilities.

***Social and eating behavior**.* The majority of crabs from the two treatments observed behaved as if there were no conspecifics near them. The absence of fighting, much less courtship throughout the observation period, is due to their uniform size and sexual immaturity.

Their eating behavior is conditioned by their lifestyle; they prefer to bury themselves in the sand during the day and leave their hiding places once night falls. Making contact with the food is very aggressive, this character is similar to the predator which catches its prey. The preference of *C. amnicola* to bury itself in the sand and to want to feed in the dark confirms their belonging to demersal species (Eldredge 2000). Their preference for eating shrimp confirms the works of Arimoro & Idoro (2007) and Lawal-Are (2009) who claimed to have identified in the digestive tract of *C. amnicola* a predominance of detritus, crustaceans, fish, filamentous algae, diatoms and grains of sand.

Conclusions. Like most crustaceans, the swimming crab *Callinectes amnicola* is affected by the salinity level of its living environment. This is a stress factor whose physiological response of the body manifests itself through unusual swimming postures and imbalance. It is an unsociable crab which does not move in groups and which burrows in the sand in the presence of light and prefers to feed late in the evening when environmental conditions are favorable for it. Maintaining *C. amnicola* in captivity has been done with water from the crab collection medium, so to further achieve acclimatization of the crabs, efforts must be made to best reproduce the living environment of crabs under controlled conditions.

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Conflict of interest. The authors declare that there is no conflict of interest.

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