5.5 – CRAYFISH POPULATIONS IN THE DIFFERENT EXPERIMENTS

5.5.1 – FIRST EXPERIMENT

In the first experiment performed between July 1st and August 2nd, when the average daily temperature fluctuated between 22.9°C and 31°C the crayfish population had two main peaks (Fig.102). The first bigger peak occurs on the first day (average population feeding on a single carcass: 60.2 specimens), while the second peak occurs 7 days after the positioning in water (average population feeding on a single carcass: 20.4 specimens). The second peak corresponds to the end of the floating stage and the beginning of the sunken stage, when the carcasses sank to the pond floor.
5.5.2 – SECOND EXPERIMENT

In the second experiment performed between August 2\textsuperscript{nd} and August 22\textsuperscript{nd}, when the average daily temperature fluctuated between 21,5°C and 30,8°C, the crayfish population had three main peaks (Fig.103). The first bigger peak occurred on the first day (average population feeding on a single carcass: 31,8 specimens), the second smaller peak occurred on the seventh day after the carcasses positioning in water (average population feeding on a single carcass: 13,6 specimens), while the third bigger peak occurred 17 days after the carcasses positioning in water (average population feeding on a single carcass: 31,4 specimens). In particular, the second peak corresponds with the end of the floating stage and the beginning of the sunken stage.
5.5.3 – THIRD EXPERIMENT

In the third experiment performed between November 3rd and June 19th, when the average daily temperature fluctuated between 1,0°C and 27,8°C, the crayfish population detected inside the lobster pots was on average very low, being on average lower than 15 individuals feeding on a single carcass during winter time, with a minimum of 4,2, occurred on December 17th, when the average temperature was 3,2°C. The bigger peak occurred on the last day of the experiment (average population feeding on a single carcass: 22 specimens).
5.5.4 – FOURTH EXPERIMENT

In the fourth experiment performed between June 20\textsuperscript{th} and July 10\textsuperscript{th}, when the average daily temperature fluctuated between 20.5°C and 27.4°C, the crayfish population had two main peaks. The first peak occurred on the third day after the carcasses positioning in water (average population feeding on a single carcass: 41.4 specimens), the second peak occurred on the tenth day after the carcasses positioning in water (average population feeding on a single carcass: 37.6 specimens. The second peak corresponds with the end of the floating stage and the beginning of the sunken stage.

Table 58 - Population dynamics in the fourth experiment
6 - DISCUSSION

6.1 – IMPORTANCE OF THE STUDY

The presence and activity of animals on dead bodies can accelerate the processes of decomposition because of dismemberment, tissue laceration and consumption. The animal feeding activity on a cadaver mainly depends on its accessibility, the decomposition stage, and on death or concealment modalities (Smith, 1986; Campobasso et al., 2001). Most of the published studies on the post-mortem modifications by animals on carrions and cadavers were carried out in terrestrial environments, and very little information is available in the case of dead bodies in aquatic environments (e.g. lakes, rivers, sea) (Holzer, 1939; Mottonen, Nuutila, 1977). It is common to find bodies in freshwater systems, whether drowned or dropped in water after death and it is important to know how the decomposition process takes place, in order to be able to recognize the cause of death and to estimate the minPMI and the PMSI. Furthermore, P. clarkii has become an important element in the European freshwater systems and for this reason, it has to be taken in account when investigation human or animal decomposition.

6.2 – CRAYFISH COLONISATION IN RELATION TO THE PERIOD OF THE YEAR

The three experiments performed during summer, showed that P. clarkii plays a major role in the carcass decomposition in freshwater environments. The crayfish appeared very active, feeding on the carcasses until their complete dismemberment. Crayfish feed for their own sustenance and to collect energies for their reproductive events: the first occurring in spring and the second at the end of summer (Gherardi, 1999b). Compared to the summer experiments, the one performed in autumn-winter proceeded with a completely different dynamics, as the crayfish feeding activity was extremely reduced and the specimens found inside the lobster pots were not actually feeding on the carcasses. As a consequence, the carcasses dismemberment was delayed, as shown by the duration of the experiments: the third experiment lasted 225 days (November 1\textsuperscript{st} 2013 – June 19\textsuperscript{th} 2014) before the carcass were completely consumed, whereas the other experiments lasted a maximum of 30 days. A study conducted on a crayfish population in Italy showed that the hibernation period of P. clarkii lasts for the entire winter, from November to March (Gherardi et al., 1999b) and this explains the low numbers of crayfish detected as well as interrupted feeding activity. On the other side, as the winter came to an end and the temperature gradually increased,
an increasing number of crawfish was detected on the carcass remains and the crayfish activity was completely resumed by actively feeding on the carcasses until their full consumption.

In addition, another factor that slowed the carcasses decay in the winter experiment was the absence of a Diptera colonisation on the carcass parts projecting above the water surface. In fact, in the summer experiments the presence of fly maggots actively feeding on the emerged parts greatly contributed to the acceleration of the decomposition.

6.3 – CRAYFISH COLONISATION IN RELATION TO THE POSITION IN THE COLUMN OF WATER

The position of the body in the column of water showed a great importance in relation to the crayfish colonisation. The results of the experiments performed in summer (first, second and fourth) showed two crayfish population peaks: the first one occurred immediately after the deposition of the carcasses inside the pond, while the second one occurred 7-10 days after the placement, which corresponded to the end of the floating stage. After this moment, the crayfish population showed further peaks which occurred during the submerged stages of decomposition. This peculiarity is probably connected with a difficulty for the crayfish to reach the carcasses inside the lobster pots during the floating stage, because of their poor swimming ability. Both peaks occurred in the submerged stage, with the carcass lying on the pond bottom, where it was easily attainable by the crayfish. Also, it must be taken in account that during the floating stage crayfish specimens were observed feeding from the outside of the lobster pots, placed above the structure. This feature is important for the estimation of the minPMI and the PMSI.

6.4 – POST-MORTEM INJURIES MADE BY THE CRAYFISH FEEDING ACTIVITY

*Procambarus clarkii* displays generalist and opportunistic feeding habits (Gutierrez-Yurrita et al. 1998) in proportion to the food availability, and its diet can change with habitats. A few hours after the carcasses positioning in water, the crayfish entered the lobster pots and started to feed on the bodies, mainly on the areas where skin is thinner (abdomen, posterior area and underarm). The crayfish feeding activity damaged the epidermal layer with lacerations up to 20-30mm, generally ascribable to round shape wounds. Post-mortem animal feeding activity may cause considerable damage to bodies resulting in the modification of wounds, loss of identifying
features, and injury or removal of internal organs. Certain postmortem lesions may appear inflicted or non-inflicted ante-mortem injuries, with consequent potential problems in investigation interpretation (Vanin and Zancaner, 2011). In the following days the size of the wounds increased reaching up to 50mm on the second day and 60mm in the third one. Apart from abdomen, posterior area and underarm, the crayfish feeding activity was concentrated on head, hind legs, forearm and torso. In particular, the injuries inflicted on the face could be relevant in the identification of bodies recovered from water by the analysis of the facial features. Moreover, dismemberment and disarticulation of hind legs and forearm was described in the first days, together with the removal of internal organs (guts). After a variable period of time, the crayfish activity reached the body cavity; from that moment, the feeding activity continued mostly in the internal part of the carcass, with the attack of the internal organs, until the flesh was completely consumed and only bones remained. Yet, together with the bones, parts of saponified tissues remained not decomposed in the lobster pots. Adipocere is a hard gray-white waxy substance formed during decomposition of bodies in particular conditions like submersion. It consists of fatty acids formed by the post-mortem hydrolysis and hydrogenation of body fats (Mant and Furbank, 1957). The hardening takes a few months to fully develop and then the body remains relatively well-preserved for months or years (Dix and Graham, 2000). In all the experiments, the crayfish did not show any interest on these saponified tissues, which remained un-eaten together with the bones.

The wounds made by the crayfish feeding activity might have a great importance in the forensic investigations. In fact, injuries inflicted by the crayfish are typically round-shaped, and could be easily mistaken for sharp force injuries, subsequently leading to a wrong report on the causes of death. Also, during the recovery of a body from a freshwater system contaminated by this species, the measurement of the wounds on the body could help in the estimation of the PMSI.

Moreover, the decomposition is accelerated in a freshwater environment colonised by *P.clarkii* because of its great influence on the tissues consumption. For this reason *P.clarkii'*s activity must be taken into account when evaluating the minPMI and the PMSI because it might affect the normal estimation.
6.5 – IMPORTANCE OF FURTHER STUDIES

It would be important to continue the study of the influence of the crayfish *P.clarkii* on the decomposition of bodies in water, with a deeper analysis of the population dynamics in relation to different parameters and the examination of wounds made by its feeding activity. Further studies are being conducted on the bone remains collected during the present experiment to analyse the type and features of the scratches that the crayfish feeding activity might have left on the bones. The results of this study might be useful in the investigation on body remains recovered after a long period.
7 – CONCLUSIONS

Water deaths are frequently the most difficult deaths to investigate, especially when the decedent has been immersed for a prolonged period of time. As well, homicide victims are frequently disposed of in water. Postmortem interval determinations are vital to an investigation, but are particularly difficult in water deaths. (Hobischak and Anderson, 1999). This study provides useful information about the great influence that the crayfish *Procambarus clarkii* has in the forensic investigations related to freshwater systems. It was demonstrated that the crayfish voraciously feed on a carcass put in water, with tissues laceration and consumption, until the complete dismemberment of the body. With its activity, the crayfish inflicts typical round-shaped wounds that could be useful in the estimation of the PMSI. The size and distribution of wounds could also be used for the estimation of the minPMI. Besides that, this typical wounds shape could be important in the estimation of the manner of death because could be mistaken for sharp force injuries. Further evidences were observed, like the removal of internal organs and the damage of the facial features, an important identification means. At the end of the body dismemberment, together with the bones, only saponified body parts remained, and *P.clarkii* did not show any interest in these tissues. Important differences were observed in the colonization of the body in relation to its position in the water column and the season in which the decomposition takes place. The analysis of *P.clarkii* population on the carcass showed differences between the colonisation during the floating stage and the submerged stages: due to the crayfish low swimming ability, the number of *P.clarkii* feeding on the carcass decreased when it was floating. Furthermore, significant differences were observed during the winter season: the crayfish enter a hibernation period that moderates the feeding activity, delaying the decomposition process. All these information could be useful for the forensic investigations, in particular the estimation of the minPMI, PMSI and manner of death. For all these reasons, the crayfish *Procambarus clarkii* is an important feature in the investigation on bodies recovered in freshwater systems.


