

■ REVIEW ARTICLE

Forensic Investigation of Aquatic Organisms

¹Ekampreet Kaur, ²Jaskarn Singh, ³Jasmeet Kaur, ⁴Tilak Raj

ABSTRACT

Insects play an imperative role in the criminal investigations. The association of the insects with that of the corpse in the criminal investigation is forensic entomology. These insects invade the cadaver and ameliorate in decomposition process and hence aid in criminal investigation. Number of research has been conducted on terrestrial entomological insects regarding their role in criminal and forensic investigation but not enough work has been done on aquatic forensics. There are many environmental factors that may act upon the colonization of an organism on a dead or decaying matter. While this is a known factor in terrestrial settings, this aspect is also observed in the decomposition process in aquatic environment as well. The water and its physical and chemical aspects, involving parameters such as temperature and oxygen content, may not only have a significant role to play in the process of decomposition but may also impact the core process of decomposition. This review paper summarizes the studies on aquatic forensics, its importance, forensically substantial aquatic insects, and their collection, preservation and decomposition studies of human cadavers in aquatic habitats.

KEY MESSAGES: This article discusses the importance of insects as forensic evidence.

KEYWORDS | forensic entomology, aquatic forensics, metamorphosis, decomposition

Author's Credentials:

^{1,3-4}Intern, Toxicology Division, Regional Testing Forensic Science Laboratory, Ludhiana, Punjab 141008, India.

²Assistant Professor, School of Allied Health Sciences, Sharda University, Greater Noida 244001, Uttar Pradesh, India.

Corresponding Author:

Ekampreet Kaur, Intern, Toxicology Division, Regional Testing Forensic Science Laboratory, Ludhiana, Punjab 141008, India.

Email:

ekampreet2409@gmail.com



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INTRODUCTION

FORENSIC, ENTOMOLOGY IS A PROMISING TOOL used in criminalistics for determination of time since death and analysis of carrion insects and other arthropods that invade them. Biological organisms like plants, fungi, insects, mammals etc. are of great forensic importance as they are useful in establishing the linkage between the victim, suspect and the crime scene. This forensic discipline is also termed as medico-criminal forensic entomology. The insects colonize the corpse in a predictable ecological succession which relies on the environmental factors as well as body decomposition. The succession of insects plays a key role in determining the stage of decomposition along with the season in

which the death took place and the movement of corpse from one location to another.¹

Insects are most commonly the first witness to the death. Their action and sequence is determined by variety of instant and complex chemical, biological and physical reactions as the corpse degrades from fresh stage to a skeleton stage. Arthropods and invertebrates invade the corpse and the resulting community provides clues to the victim's death and post-mortem history thus provide great aid in forensic investigation. Therefore, they act as an important tool in criminal investigations. The insects act on the corpse in a specific order by forming a biological clock this helps in determining the age of the developing fly

progeny and hence, the PMI (Post mortem interval) can be analysed. The wide diversity of organisms exploit the corpse, these organisms range from microbes to vertebrates (scavengers). In case of terrestrial habitats, arthropods and insects are present in diverse amount and are constant but in marine habitats, the arthropods are replaced by crustaceans.

Researchers have claimed that the species composition as well as the insect succession differs from each other with respect to the geo-location and the season. There are several factors that play role in determining the time since death. These include:

- Temperature
- Season
- Humidity
- Insect or animal activity

The ages of insect can be inferred by analysing the immature stages found on the dead body. This could help in estimating minimum post mortem interval.¹ The study or investigation of the corpse recovered from aquatic habitat is quite different from the terrestrial one. There are no specific sacrophagous aquatic insects that are functionally evolved to feed or invade the carrion alone. A like terrestrial succession, there is no specific predictable time frame of succession in aquatic habitat. The findings in aquatic forensic may vary from one another depending upon the nature of the resting site (pool, river, ocean, etc). When the corpse is submerged in water, the worthy evidences often get demolished by the water submersion and the integrity of the evidence is lost during the recovery. Aquatic insects are the imperative tools for assembling valuable information from the corpse.¹

The corpse become an eventual source of food for the aquatic insects like fishes and for wide variety of invertebrates. It also acts as a sheltered microhabitat for small non-scavenging species secondary predatory species also get attracted to the original scavengers. The corpse also acts as a substrate for primary producers like algae or other periphytons,

there organisms colonize on the corpse and grow. At the advanced stage, which is highest stage of decomposition, there is formation for bacterial or algal biofilms on the bones, skin or the clothes and hence is attracted by invertebrate grazers. In the case of aquatic forensic investigation, it is imperative for forensic investigators and police officials have to keen knowledge of aquatic organisms that participate in colonization and decomposition process of the human corpse. Along with this, the investigators must have knowledge regarding the factors (environmental) that influence or affect the decomposition process. Time of Submergence (TOS) can be estimated by the aquatic invertebrate succession as well as the decomposition changes.

Aquatic Insects

Aquatic macro-invertebrate investigation is the first and foremost step for the basic understanding of the key role of these insects in death investigation. The variation in morphology, physiology and behavioral adaptations in the aquatic organisms allows them to inhabit in all bodies of water virtually. True aquatic organisms and semi-aquatic ones inhabit in every conceivable habitat in which human body can be found. Human corpse is tend to be found in hot and cold springs, pools, streams, ponds, saline lakes etc. In cases, when the insects are virtually absent in the open sea, it can be elucidated that other marine organisms are more important for forensic investigation in that habitat.¹

Chironomid midges in their immature stage are considered predominantly for forensic investigations. These aquatic insects are ubiquitous in the aquatic environment and have tendency to colonize the submerged body in short duration for the sake of food and shelter. Along with this, these insects are commendable environmental indicators as they require specific conditions like pH, salinity, temperature, oxygen concentrations, pollution etc. Chironomids are good forensic indicators as they play crucial role in determination and investigation of submerged corpse therefore,

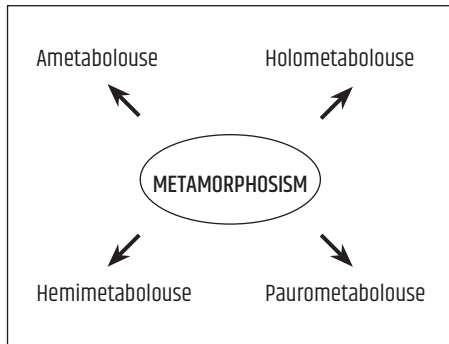


Figure 1: Types of Metamorphosis.

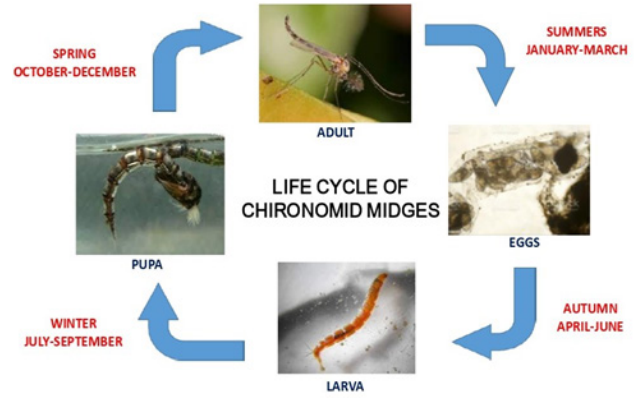


Figure 2: Lifecycle of Chronomid midge

it helps in depicting the location of the corpse. Chironomids are considered as PMSI indicators, because the larvae of these species are not at all affected by the scavengers in submerged corpse.¹

How do insects reproduce?

Insects undergo series of stages when they develop from egg stage to an adult. The appearance and the time taken for the same varies from one species to other. Studies have revealed that the development cycle accelerates with the enhancement of temperature. The procedure of undergoing physical changes from one stage to another is called “metamorphosis”.¹

Aquatic insects undergo paurometabolous or holometabolous metamorphosis. Each stage takes specified time depending on the species type, season, clothing, temperature. These parameters helps in estimating the PMSI (Post mortem submersion interval).

Caddisflies also play an imperative role in calculating PMSI. (Post mortem submergence interval) These insects should always be kept into consideration as they can provide plenty of information for forensic investigation . Chironomid midges possess four distinctive

life stages: Egg, Larvae, Pupa and Adult. The duration of the entire life span may vary from 2 weeks to several years. Parameters like species and environmental conditions play a vital role in the life cycle. With the separation of larva from the substrate that is pupal integument, there is beginning of the pupal stage. Chironomid adults genuinely survive for few days, except for some species which remain alive for several weeks. Adults deny feeding in this particular stage and they only reproduce and disperse. Copulation occurs in aerial swarms, especially on the surface of water where they sink towards the bottom or they release some gelatinous egg masses either on the open water or emergent vegetation.

Aquatic and Semiaquatic Insects associated with Corpses:

- Mayflies (Order: Ephemeroptera)*
- Stoneflies (Order: Plecoptera)*
- Caddisflies (Order: Trichoptera)*
- Trueflies (Order: Diptera)*

METHOD

Collection and Rearing of Aquatic Evidences

Collection, Rearing and preservation of

INSECTS	ORDER	METAMORPHOSIS	CHARACTERISTIC FEATURES
Mayflies	Ephemeroptera	Hemimetabolous	<ul style="list-style-type: none"> • Occur in freshwater exclusively. • Duration of adult stage is 2 to 3 days. That's why these insects possess ephemeral nature. • Species of these flies are found feeding on and around the pig carcass. • Proved to be an important entomological evidence that aids in forensic investigation.
Stoneflies	Plecoptera	Hemimetabolous	<ul style="list-style-type: none"> • These species are found in clean and cool running water. • Eggs hatch as soon as the decomposition process initiates. • Some species of this order were found colonizing and feeding on the carcass of pig and carrions. • Presence of such species on the corpse indicates that the body remained in the riffle zone of the lotic environment.
Caddisflies	Trichoptera	Holometabolous	<ul style="list-style-type: none"> • Occurs in freshwater, brackish water or sometimes in marine habitats. • Presence of caddisflies on the corpse might indicate whether it had moved from one specific lotic habitat to another or it is transported to different location via water currents.
True flies	Diptera	Holometabolous	<ul style="list-style-type: none"> • Largest family of freshwater insects therefore are forensically important. • Most successful colonizers and can survive in extreme environmental conditions. • One of the first colonizers that invade the corpse. • These flies aid in determining PMSI, linking corpse with specific aquatic habitat, ascertaining the time of the year as well as the duration of submersion.

Table 1: A list of aquatic and semi aquatic insects associated with corpse and their salient features. ²

aquatic insects is different from terrestrial insects. Aquatic insects have varied survival adaptation depending upon their species and the environment in which they are residing. Specific insects aid in forensic investigation by providing information regarding the geographical location of the time of the year. Aquatic arthropods like crawfish, crabs will feed extensively on the human tissue that leads to post-mortem artefacts. In some aquatic insects, entire life span as well as the stages are in water, others grow and feed in water but reproduce in terrestrial habitat that is why, and only larvae, pupa and other immature stages are found on submerged bodies. During summer, there is probability to encounter newly emerged adult stages having wings to be found on corpse either on the surface or along the shorelines or riverbanks. If there is presence of some terrestrial insects on the corpse, one can elucidate that the body was on land and then it was sunk to the bottom. Proper protocols are followed by police and investigators while recovering the bodies from the water. Some type or shroud or sheet is placed underneath the body if it is floating or if it is partially in water. This is placed before moving the corpse. The key function of this shroud is to maintain

the integrity of the evidence on the body that might get disturbed. Care must be taken while dealing with this evidence as some insects residing on aquatic substrates would detach themselves even with slight disturbance. For this purpose, sheet or large, finely woven mesh net can be employed so that all the insects can be captured. Most of the insects make use of corpse for shelter as well as for food. The samples or evidences are collected with proper care, they are either preserved if dead or are reared if they are alive. Specific methodology is followed for preserving and rearing of insects.

- Live immature samples should be collected and transported in the jars containing water collected from the environment in which they were residing.
- Some of the immature insects are usually heat sensitive and hence, temperature of the water must not be elevated and one must keep the jars containing the insect samples in shade, covered in wet cloth or make use of chemical ice as such things will help in reducing excess heat during transportation.
- The specimen jars are placed in a Styrofoam or an ice chest.
- All the specimens should be labeled and data labels should also be placed on the vials

as soon as collected and with same label format.

- The wings of the insects play imperative role in the identification of the species hence, they should be collected with immense care.
- Forceps or fingers must be used for picking off the larvae, eggs and pupa from the corpse and the collection of these evidences should be done prior to movement of the body as some aquatic insects crawl off or might get disturbed during the movement and may not be found.
- Aquatic insects possess small size and are inconspicuous, therefore, the investigating officer must notice the insects carefully on and around the corpse. A hand lens is preferred for the same.
- The investigator and his team must have keen knowledge regarding the entomological evidences for species identification and to know the behaviour of such insects.
- It is mandatory to put some aquatic plant material in the preserving jar. This feeding material should be collected from the scene itself as it will aid in rearing and in supplementing with fish food.
- Emerged adult aquatic insects are placed in 80-90% alcohol for preservation.
- The rearing containers work well for both terrestrial as well as aquatic insects and are requisite part of rearing equipment.^{2,3}

Preservation Solutions for Aquatic

Evidences:

Kahle's Solution

This solution is used for preserving adult insects and larvae.

95% ethanol – 30ml

Formaldehyde – 12ml

Glacial Acetic Acid – 4ml

Water – 60ml

Carnoy Fluid

It acts as a killing agent and preservative for most soft-bodied aquatic insects. It is not used much because of restriction for the use of chloroform.

Chloroform (30%) - 30ml

Ethyl alcohol (95%) – 60ml

Glacial acetic acid – 10ml

Pampel's Solution:

Formalin – 10ml

Ethyl alcohol – 30ml

Glacial acetic acid – 7ml

Water – 53ml

Ethyl alcohol (90-95%):

Ethyl alcohol is the most favoured preservative for eggs, larvae and pupae of aquatic insects. The adult form of these insects can be preserved in 75-80% ethanol.²

Decomposition of Corpse

Decomposition study in aquatic habitat is not well understood because there has not been much research on the same. Most of the research conducted in past have been conducted on the individual cases. As soon as the corpse submerge in any aquatic habitat, the decomposition process initiates. The decomposition rate and procedure completely depends upon the environmental factors and the condition of the corpse. The key role of decomposition study is to determine time since death for analyzing decomposition in aquatic habitats, one must have knowledge regarding environmental factors and their effect on the decomposition process. Decomposition characteristics that corresponds with aquatic habitat includes:²

- Bloating
- Lividity
- Skin sloughing
- Flesh decaying
- Exposure of internal organs
- Algae accumulation
- Silting discoloration of bones

Stage of Decomposition:

Decomposition of corpse in aquatic environment usually takes place at approximately half the rate as that of in terrestrial habitat because of the cooler temperature and less insect activity.^{2,3} The stages of decomposition in aquatic habitat are described by several authors and are

discussed below:

Submerged Fresh

It is time period when the corpse is initially submerged and when it starts to bloat and rise to the substrate. This observation is based on the study of pig carcass. The process depends on the geographic location of stagnant or flowing water, microhabitat inside the water body and the season. During this stage, truly aquatic insects like chironomid midges, mayflies and caddis flies were observed by the researchers and investigators.

Early Floating

After death, the humans and animal body releases certain gases which are produced by anaerobic bacterial respiration. This leads to the movement of the carcass or the corpse on the water surface. When the body moves toward the surface. When the body moves toward the surface, the terrestrial insects get attracted towards it. These insects lay their eggs on the carcass where exposure is there. If the carcass is totally submerged, then aquatic insects like aquatic isopods, caddisflies, mayflies, chironomid midges invade the carcass. Researchers have found that there is release of decay odor in this stage along with this, the tissues turned from pinkish to blue-green color, yellow fluids and gases are released from the anus, algal or periphyton growth enhances significantly more on the carcass or corpse. The season when the process initiates plays an imperative role in the determination of duration of each stage of decomposition.

Floating Decay:

Calliphorid maggots feed intensively on the carcass of pig which was floating on the surface and they create many apertures on the exposed skin. Certain beetle species invade on carcass in abundance during this duration, as they find predators and copulate studies have revealed that aquatic micro-invertebrate colonization on the pig carcass varied in both temporarily and spatially between riffle and pool microhabitats.

Bloated determination:

In this stage, the exposed portions of the carcass that float above the surface of water usually

disappear due to constant feeding activity of blow fly maggots. On the flip side, completely colonized by aquatic insects like chironomid, black fly, larvae etc. Researchers have found that the hind limbs get disarticulated, body fluids including the blood leak from the orifices of the carcass, the flesh gets sloughed off in large sections.

Floating remains:

Little maggots were found on the limbs and tissues that were floating on the surface of the water. This happens because of the migration of insects from the carcass, drowning death or the predation from aquatic and other terrestrial insects. Sloughed off flesh, disarticulation of phalagial as well as limb bones were all the factors recorded in completely submerged carcass. Black fly larvae and chironomid midge are the dominant organisms for invasion in this stage. Other vertebrate predators like dace, sunfish etc. were also found feeding on the carcass flesh as well as in the micro-vertebrates that were present on the carcass. Studies have revealed that some fishes and other arthropods also feed on the maggots that invaded the corpse in the pond during floating remains stage.

Sunken remains:

This stage has vast variability in its duration. This stage primarily focuses on the bones and skin pieces that remain on the substrate. Researchers have claimed that the decomposition process is completed by microorganisms like bacteria and fungi during this stage. Benthic organisms like fauna might be recovered within the carcass remain along with other organisms like snails, mayfly, amphipods, annelids.³

Factors Influencing Aquatic Decomposition

Corpse immersed in water would likely to be exposed to variable and changing environmental conditions. The factors affecting the decomposition process are:

Flora and Fauna:

Microbes like bacteria, fungi, algae play an imperative role in the decomposition process of human remains or corpse. These organisms are somewhat present in the body or are injected along with the water during drowning. Their

occurrence inside the body aids in estimation of time since death and in analyzing the cause of death. Many studies have been conducted in past which focused on the use of these microbes but there is no such reliable method for analyzing them.⁴

Forensic limnology is the scientific discipline which focuses on the examination of diatoms recovered from the crime scene or the body of the victim. Diatoms can help in detection of type of drowning whether it is ante-mortem or post-mortem. If a person is alive and falls into the water, these diatoms enter the lungs through the water inhaled by an individual. Further, they are circulated to different organs of the body like the brain, bone marrow, kidneys, cavity fluids etc. If any individual is dead and is thrown into the water body, in that case the diatoms do not get entry inside the body. It is an undeniable fact that absence of diatoms inside the corpse does not exclude drowning as the cause of death. Ante-mortem injuries during the drowning process, post-mortem injuries due to flow of dead body or invasion by aquatic organisms can be a barrier for detecting the cause and manner of death in drowning.^{4,5}

The use of insects in determination of time since death in the aquatic habitat is deficient as compared to those in terrestrial habit. This is due to inadequacy of sarcophagus benthic organisms. Therefore, it is merely impossible to develop an entomological succession for benthic creatures. The interaction the aquatic organisms especially insects with the corpse is an accidental encounter as they do not colonize on the corpse deliberately. If the maggots are present on the submerged body, it indicates that the corpse was exposed to air for longer period of time. Thus, resulting in post mortem drowning. Such maggots are predominantly used for toxicological analysis as compared to the time since death analysis.⁶

Fauna activity on the corpse enhances the decomposition process because these organisms consume the soft tissues and play major role in dismembering the tissues. This action proves to be a complication for identification of

the victim as well as for identification of the wounds. Scavenging activity by fishes and other arthropods and molluscs expedite the process of decomposition and can significantly cause skeletonization within week.⁷ Microbes also play an eminent role in natural decomposition of the body. The microbes invading the cadaver are either endogenic or those which are already present in the environment. Various aquatic communities have remarkable forensic value. Marine bacteria have been proved as a commendable indicator for drowning cases in the seawater. Moreover, they also play crucial role in the decomposition process. These marine bacteria and their succession in the aquatic habitat act as an innovative indicator for estimating the PMSI.⁸ Algae is also considered as a reliable evidence for forensic studies. Algae is also utilised as an aquatic evidence because of its ubiquitous nature and the community composition also varies according to the seasons. The study of algae in forensic scenario is known as forensic phycology.⁹

Applications of Forensic Phycology includes:

- Confirmation of drowning death
- Crime scene identification
- Novel indicator of PMSI

Temperature

Temperature is the paramount environmental factor that persuade the decomposition rate through the colonization time, insect growth and therefore, is the most imperative factor in determining PMI specifically in terrestrial habitat. Aquatic insects also respond to absolute ambient temperature as well as to the summation of the thermal units. Temperature decreases with the increase in depth of water. One needs to have accurate information regarding the surface water temperature as well as the temperature at the depth from which the remains were recovered. These parameters are important for observation of post-mortem changes. Water temperature suppresses the gas formation and the time of reappearance of the body at the water surface.

Some researchers claimed that temperature ranging from 21-45°C is suitable for adipocere formation as this much temperature is also suitable for microbial growth and survival. Others have claimed that this adipocere is formed in cooler temperatures. Coldwater temperature decelerates the process of decomposition, specifically the microbial breakdown. Higher temperature enhances the larval growth rate of benthic organisms by altering the quality and quantity of the organic matter which is related to the corpse.¹⁰

Water Chemistry and Water Flow

Salinity and bacterial content of the aquatic habitat both affect the decomposition process. Freshwater is taken into lungs by the process of osmosis and hence lead to haemolysis due to blood dilution due to high saline content, osmosis will allow to move water from blood to other organs. Specifically lungs and hence, leads to thickening of blood. Because of the modification in bacterial activity, the decomposition process is slower in saline environment as compared to fresh environment. For example; body submerged in water comprising of high organic content will decompose at higher rate as compared to that in which body is submerged in freshwater.

The rate of flow of water is also a determining factor during the investigation of any corpse. The motion of the body has notable effect on the soft tissue and its decay process. Different parameters like interaction with sea bed or river and other environment factors can destroy the tissues and also enhance the decomposition.

^{13,14}

Body Coverings

The decomposition process is affected by the presence of clothes or other coverings of the body. The putrefaction gets delayed if there are presence of bodily coverings as they acts as a barrier. In some cases, these clothes accelerate the decomposition by keeping intact the temperature of the body as well as the conditions favorable for all the microorganisms that play role in decomposition. Moreover, it

also depends on the clothes or the covering that are worn by the victim. For example; heavier clothes have more insulating effect, synthetic clothing materials absorb less water than natural textiles. Bacterial effect gets dwindled when the corpse is wrapped tightly in plastic, there is no source of oxygen in that case leading to the disruption of microbes. Unclothed area of the corpse was subjected to feeding activity of carrion at comparatively higher rate.¹⁴

CONCLUSION

From the literature survey being conducted in past, it can be inferred that plenty of study has been conducted on forensic entomology but there is scarce research work in the field of aquatic forensics. Both terrestrial and aquatic forensics play crucial role in estimating time since death or Post mortem interval (PMI). In many cases, the body is discharged into aquatic habitats after the commission of crime in order to mislead the investigators regarding the cause of death, as the investigators may infer that the death is caused by drowning. In such cases, forensic aquatic entomology comes into play. The study on this discipline is quite challenging because of different environmental and allied factors that affect the rate and nature of decomposition that goes on in the aquatic environment on the human corpse. Decomposition is a complex phenomenon involving various variables that influence each other. These aquatic organisms have immense significance in criminal investigations, therefore a reference data comprising of all the forensically imperative aquatic species should be made all over the world which can assist various law enforcement agencies to deal with different cases and investigate the cases with great aid.

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Conflict of Interest:

The authors declare that there is no commercial or financial links that could be construed as conflict of interests.

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