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Postmortem Submersion Interval in human bodies recovered from fresh water in an area of Mediterranean climate. Application and comparison of preexisting models.

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Highlights

- Van Daalen's and Heaton et al. aquatic decomposition scores were tested
- Both scores were tested on submerged corpses recovered from Italian rivers
- The PMI was obtained using the PMSI expressed in days or the ADD model
- Both scores showed high correlations when dividing the cases according seasonality
- This study supports the need to establish further regionally-specific models

Abstract

Purpose. The methods developed in recent years for the assessment of the Postmortem Submersion Interval (PMSI) have proven to be promising, but are strictly related to specific geographical areas or climates. The aim of this study is to assess the suitability of two of the most recent total aquatic decomposition scores (TADS) for the determination of the PMSI in bodies recovered from fresh water in an area of Mediterranean climate in the last 15 years. To do this, the correlation coefficient (r^2) between PMSI and Accumulate Degrees Days (ADD) or PMSI and days was studied.

Methods. The correlation between PMSI (expressed in days or in ADD) and the TADS was evaluated considering: (a) bodies recovered during the entire period; (b) bodies recovered during the cold season; (c) bodies recovered during the warm season. A linear regression analysis was set comparing the statistical significance of a model plotting TADS versus ADD, and another model plotting TADS versus PMSI (expressed in days) for both scores.

Results. Scores were scarcely applicable when considering the entire casuistry, as regression models showed low r^2 values, but both scores showed high correlations after dividing the cases into 2 groups. In fact, after performing the seasonal partition, we observed a strong correlation between PMSI and TADS, using either of the scores.

Conclusion. This study helps increase the accuracy, reliability, and validity of PMI estimation in bodies recovered from freshwater in an area of temperate climate, such as Northern Italy, supporting the need to establish regionally-specific equations for estimating PMI in a forensic context

Keywords. Postmortem Submersion Interval; Aquatic decomposition score, Mediterranean area Italian rivers.

Introduction

Postmortem Submersion Interval (PMSI) is defined as the period between entry into the water and recovery of the dead body [1]. Determining PMSI has long been problematic, since factors due to aquatic environments, such as algal growth, adipocere formation and water composition, can affect the rate of post-mortem decay [2-10]. Indeed, cadaveric putrefaction in water is closely related to the characteristics of the submerging fluid, since bodies submerged in freshwater may show more putrefactive signs than bodies submerged in seawater, as salt slows down bacterial activity [11]. However, temperature is the leading factor affecting the rate of decomposition of human bodies, as happens in air [12-14]. As early as 1967, Reh et al. established a statistical correlation between the putrefactive signs on submerged corpses and the aquatic temperature along PMSI [15, 16].

As reported by Madea and Doberentz [17], the water temperatures of the rivers used in the experiments performed by Reh et al. [15, 16] have increased during the last 40 years. For water temperatures over 20°C, Reh's table is unreliable and further investigations were considered necessary, especially for higher water temperatures on the correlation between temperature and progression of putrefaction. Actually, the chart is suitable for lower temperatures. During subsequent years, several methods for the visual determination of decomposition have been developed [17-19]. These methods are based on modifications (such as marbling, wrinkling, and bloating) detectable at external inspection, on several body districts (head and neck, trunk and limbs). These methods also included the evaluation of the accumulated degree-day (ADD). ADD represent the accumulation of thermal energy that is needed for chemical and biological reactions to take place in soft tissue during decomposition and is a combined function of chronological time and temperature [20]. Simmons et al [21] explained this concept and stated that ADD measures the energy that is placed into a system as accumulated temperature over time and when an equal amount of thermal energy (ADD) is placed into a body or carcass, then an equal amount of reaction (decomposition) is expected to take place. As a result, ADD are used to reduce as much as possible the influence of temperature during decomposition.

In 2005, Megyesi et al. [20] developed a method to calculate total body decomposition score (TBS) in order to predict the postmortem interval (PMI) for bodies recovered in a terrestrial environment. TBS was used as an independent variable, and ADD was calculated considering the average daily temperatures along the PMSI (ADD=mean ambient temperature×PMI). ADD and TBS proved to be key instruments for PMI estimation, through regression models developed for specific geographical areas such as UK, the Delaware Region and Canada [1, 19, 22, 23].

The Total Aquatic Decomposition Score (TADS) was developed by Heaton et al. in 2010 [23], on the basis of the TBS model proposed by Megyesi [20]. The TADS considers both internal and external post-mortem modification, and was defined as the sum of facial aquatic decomposition (FAD) points, body aquatic decomposition (BAD) points, and limb aquatic decomposition (LAD) points. It can vary from 3 points, with no visible changes, to 25 points with complete skeletonization. This model (hereinafter referred as Heaton's score), considered both external and internal signs. It was used for bodies recovered from rivers in the United Kingdom, and was unsuitable for estimating PMSI when corpses were submerged in very cold water or for a reduced interval (i.e. for ADD values lower than 10).

In 2017, van Daalen et al. [24] developed another model to calculate the TADS (hereinafter referred as van Daalen's score), and applied it to predict PMSI in human bodies recovered from the North Sea and from freshwaters in the Netherlands. Van Daalen et al. [24] studied the progression of TADS against the PMSI, regardless of ADD, since aquatic temperature in the North Sea was assumed to be relatively constant across different seasons. This score is based exclusively on the external inspection of the corpse and ranges from 3 points (no visible changes) to 18 points (complete skeletonization).

Although quantitative methods for the estimation of the PMSI are proving to be particularly promising, they are strictly related to environmental conditions and ecosystems, which of course are related to specific geographical areas or climates. Therefore, there is a growing need for more systematic studies of human decomposition in aquatic environments from different climatic areas [1, 25].

The aim of this study is to compare:

- the accuracy of two of the most recent aquatic decomposition scores: the Heaton's score and the van Daalen's score, for the determination of the PMSI;
- the prediction model using the PMSI expressed in days or the ADD model;

in order to assess their suitability in bodies recovered from fresh water of an area with Mediterranean climate. To do this, the correlation coefficient (r2) between PMSI and Accumulate Degrees Days (ADD) or PMSI and days was studied.

Materials and methods

A retrospective study was performed on cases of submerged corpses recovered in the last 15-year period (2003-2017) from rivers of Northern Italy.

Inclusion criteria.

Only cases in which the following data were available were included: date of last sighting, date of recovery, external features of the corpse, aquatic temperature and PMSI. Bodies with missing parts were excluded, so that decomposition could be scored for the entire body.

PMSI was estimated considering the date of last sighting and the date of recovery of the corpse. The state of decomposition at the time of recovery was assessed and scored from photos taken during the death scene investigation and detailed taphonomic data provided in forensic reports. Post-mortem changes were evaluated according to Heaton's score and van Daalen's score. TADS was estimated by summing the scores of each of the three anatomical regions. The scores are reported in **Supplementary material 1**.

ADD were measured as follows: ADD = time [days] × temperature of daily aquatic temperature [°C]). Daily aquatic temperature was obtained from the National official database [26] and used for calculating ADD.

Differences in seasonal timeframes

The study of the local hydrological annals, from the national temperature database [26], allowed the identification of a periodical fluctuation of water temperature. The partition according to "seasonality" was carried out considering the highest difference in the aquatic temperature value between two seasonal periods, that is, "cold months" and "warm months". In fact, it was possible to detect a difference in the average aquatic temperature of about 10 Celsius degrees between the average temperatures of two annual periods: the first period included months from October to April (cold season, mean aquatic temperature 9.44 °C) and the second period included months from May to September (warm season, mean aquatic temperature 22.9 °C).

In order to assess if and whether aquatic seasonal differences may influence the applicability of both scores, the correlation between PMSI (expressed in days or ADD) and TADS (calculated with both scores) was evaluated in relation to our casuistry, considering:

- *sample 1*, bodies recovered during the entire period;
- *sample 2*, bodies recovered during the cold season;
- *sample 3*, bodies recovered during the warm season.

The scores are better detailed in **Supplementary material 1**.

Statistical analysis

In order to determine whether ADD or PMSI (expressed in days) better relate to the TADS calculated with both scores, a linear regression analysis was used comparing the statistical significance of a model plotting TADS versus ADD, and another model plotting TADS versus PMSI for both scores.

For each population sample:

- TADS (with both scores alternatively) will be identified as dependent variables;
- PMSI and logADD will be identified as independent variables.

Coefficients of determination, r^2, calculated for each linear regression model, were compared. Calculation of the standardized skewness and kurtosis was performed to determine whether the sample came from a normal distribution. As ADD and PMSI showed a non-parametric distribution, the Spearman Rank correlation coefficient was calculated to determine whether PMSI or ADD correlated with the TADS, using both scoring methods. As adopted in previous studies [20, 23, 27, 28], the ADD was log-transformed (log 10) to fulfil the requirement of a linear relationship and a normal distribution when performing linear regression analysis to investigate the relationship between ADD and TADS, using both scoring methods. Therefore, a regression analysis was conducted in order to produce an equation for each model and determine which better fits the variation in decomposition.

The following analysis were performed for Sample 1 (entire period), 2 (cold season) and 3 (warm season).

- TADS vs PMSI (days) using van Daalen's score;
- TADS vs ADD using van Daalen's score;
- TADS vs PMSI (days) using Heaton's score;
- TADS vs ADD using Heaton's score.

A two-sided $p<0.05$ was considered to indicate statistical significance. Statistically significant correlations were studied. Statistical analysis was conducted using SPSS 10.1.4.

Results

The Study Sample

A total of 238 cases met the study criteria and were selected for the dataset. The mean age was 42.3 years. There were 101 females and 137 males. One-hundred-twenty-seven (n=127) bodies were recovered during the warm months (from May to September), with a peak in July (n=34) (**Table 1**). Almost all bodies recovered during cold months (n=111) wore at least one thin layer of clothes; bodies recovered during warm months were less clothed. The presence of clothes on bodies was not related to outliers in any of the regression plots, justifying their continued inclusion in the dataset.

Mean ADD value was approximately 95.6 (range 0.38-756) during the cold season and 128.8 (range 0.52-600) during the warm season.

ADD and PMSI (days)

Mean ADD value was 113.4 (average 0.38-600). Mean ADD value was approximately 95.6 (range 0.38-756) during cold season and 128.8 (range 0.52-600) during warm season. Mean PMSI value was 8.11 days (range 0.02-90). Highest PMSI value was 30 days, during the warm season, and 90 days, during the cold season. Mean PMSI value was 11.6 days during the cold season and 5.5 days during the warm season. Thirty cases showed PMSI of 1 day (10 in the cold season and 20 in the warm season). Eighty-eight cases (41 in the cold season and 47 in the warm season) showed PMSI values higher than 1 day.

Measuring Decomposition

The distribution of TADS values using Heaton's score ranged from 3 (no visible alteration) to 24 (complete skeletonization). A TADS value of 3 was the most frequent (in 77 cases, 37 cases in cold season and 40 in warm season). Only in the warm season was a TADS over 20 observed (10 cases). The distribution of TADS values using van Daalen et al.'s score ranged from 3 (no visible alteration) to 18 (complete skeletonization). The casuistry showed all TADS values. TADS value of 3 was the most frequent (in 83 cases, 35 cases in cold season and 48 in the warm season). A TADS value of 18 was the least frequent (3 cases). Only in the warm season was a TADS over 15 observed (16 cases). The graphical representations of all regression analysis, as well the values of r^2 referring to scattered plots based on the different analyzed casuistries, are reported in **Figure 1** and better detailed in **Supplementary material 2**.

Discussion

PMSI estimation according to the scores proposed by Heaton et al. [23] and by van Daalen et al. [24] is based on the visual assessment of the state of decomposition. Although these scores were tested in northern Europe, to the best of our knowledge they have never been applied in a Mediterranean climate area, such as Italy. In Northern Italy the average temperature of the hot season is over 22°C, defining the climatic zone as *Cfa*, according to the climate classification of Koppen [29]. In other countries, such as the UK, Netherlands and Norway, the mean temperature of the hottest month is lower than 22°C, with almost 4 months with a temperature over 10°C, thus defined as *Cfb*.

Since temperature is one of the most influencing factors of the decomposition rate, considering both air and water environments, we hypothesized that Heaton's and van Daalen's scores should be adapted to our climatic area. In order to evaluate their efficacy in predicting PMSI, two models were derived for each score, taking into consideration time and temperature. Linear regression analyses were used to determine whether ADD or PMSI better describe the rate of post-mortem decomposition:

- in the entire casuistry;

- in the “cold season casuistry”;
- in the “warm season casuistry”.

When considering the entire casuistry, the scores were scarcely applicable, as regression models showed low r^2 values (**Figure 1a-d)**. The highest correlation coefficient was observed with the application of the Heaton’s score and using PMSI as an independent variable (r^2=0.83). As for the practical application in the forensic casework, if we considered the linear regression model derived from the analysis of the whole casuistry, the estimation of PMSI would lead to a wrong prediction of the time since death or prediction with a low degree of accuracy.

As easily observed in **Figure 1a-d**, the low prediction rate through the analysis of scattered plots is due to a distribution along two main directions, which may be interpreted as the seasonal distribution.

Based on these observations, according to wide climatic variations of the temperate region, the seasonal split of the casuistry was thus necessary to obtain a homogeneous and unequivocal distribution of the points in the scattered plots, with higher r^2 values. The seasonal split was not applied in studies conducted in Northern European countries [23, 24, 27, 28], where climate and temperatures are stable. The study of Heaton et al. [23], in fact, analysed bodies recovered from U.K. waterways, exposed to relatively low aquatic temperature fluctuation. The van Daalen’s score was applied on corpses recovered in the North Sea, with almost constant and very low acquatic temperatures, and therefore not relevant for influencing the post-mortem decomposition rate. Moreover, van Daalen et al.’s casuistry [24] included only bodies recovered in salt water, thus preserving the corpse and delaying the bacterial action and, for example, the early development of adipocere [24].

After performing the seasonal partition, we observed a strong correlation between PMSI and TADS, either using van Daalen’s score and Heaton’s score. The analysis of r^2 values does not show significant differences between the two scores. Moreover, the prediction of the post-mortem interval appears to be more accurate if PMSI is expressed in days rather than in ADD, showing r^2 always greater than 0,94 in both seasons using both scores when considering the PMSI expressed in days. Even if the use of the ADD model is generally more accurate for predicting the PMI than the time expressed in hours/days, in our study the effect of temperature is much better explained by using seasonal partition, since the use of ADD in the prediction of the entire period did not show satisfactory results [21]. This means that after taking into consideration seasonal fluctuations, the scores used to calculate TADS are more useful to predict PMSI expressed in days than ADD. Contrarily to what happens in open air, our data show that fluctuation in water temperature within the same season (both warm than col season), that may influence the calculation of ADD, may act as a confounding factor. Anyway, when using logADD as independent variable, the ADD calculation (time [days] × temperature of daily aquatic temperature [°C]) could be made considering the daily aquatic temperature from the national databases [26], which in our case were measured at a mean depth of 1.5-2 meters. The slope of regression models observed in this study was greater for the "warm season" casuistry (**Figure 1e-h**). Higher temperatures imply in fact a more rapid decomposition, thus explaining a higher degree of decomposition and therefore of TADS, also for minimum variations of the PMSI. In fact, during the hot months, the body is exposed to higher average daily water temperatures, as shown by the average values of ADD for the two "seasons". In our casuistry, during the "cold season", the average PMSI was 11,16 days, with an average value of ADD of 95,57; during the "warm season", although the average PMSI was distinctly lower (5.5 days), the average ADD was 128.84.

Both the scores appear applicable to our casuistry, and the score of Heaton et al. [23] was also suitable to estimate the PMSI in cases with ADD values inferior to 10 (58 cases showed ADD lower than 10.37 in the "cold season" and 19 in the "warm season").

Considering the practical application in the forensic casework, there are some differences between the two scores. Van Daalen's score [24] is based on the findings detectable at the external cadaveric inspection. The description of the single decomposition patterns allows the TADS to be calculated directly during the death scene investigation, helping the forensic pathologist to estimate the time of death in this preliminary phase. On the other hand, Heaton's score [23] provides the evaluation not only of signs detectable to external cadaveric inspection, but also internal signs detectable during autopsy. This is important because it may sometimes be difficult to perform the autopsy immediately after death, and the exposition of body to air accelerates the decomposition processes, thereby misleading PMSI estimation. For the aforementioned reasons, even if the two scores show a similar accuracy, the van Daalen's score [24] seems to be the most readily applicable, allowing a rapid and accurate PMSI estimation at the death scene.

Our study displays some limitations mainly related to the available casuistry.

1. Approximately one third of the cases (88 cases, 37% of the whole casuistry) showed PMSI lower than 1 day, few cases showed PMSI≥15-20 days (12 recovered in the "warm season" and 23 recovered in the "cold season").
2. During the "warm season", we found only 3 cases with PMSI≥20 days, and, more generally, only 12 cases with PMSI≥15 days.

Due to the limited numbers of cases with PMSI greater than 20 days occurred during 2003-2017, further cases with longer PMSI should be collected in order to validate the robustness of the proposed model for longer PMSI. In the future, it would be useful to conduct more specific studies, considering single climate regions and the related peculiarities of ecosystems and average aquatic temperatures, as well as the correlation of these scores with biochemical post-mortem fluctuations [30]. More cases are needed, in particular during the "warm season" in order to highlight the possible presence of a PMSI cut-off, as emerged from our study of the "cold seasonal" sample. Finally, it might be interesting to test both the scoring methods in different aquatic environments, such as lakes or the sea, in order to study the influence of additional environmental factors on the applicability of the two scores.

In conclusion, this study helps increase the accuracy, reliability, and validity of PMI estimation in bodies recovered from freshwater in an area of temperate climate, such as that of Northern Italy, supporting the need to establish regionally-specific equations for estimating PMI in a forensic context [31]. Since the periodical fluctuation of aquatic temperature, PMI estimation seems more accurate if corrected according to two seasonal timeframes. Both scores resulted reliable for PMSI estimation; however, van Daalen's score seems easier to use, since it could be applied immediately after the recovery of the body.

Credit authorship contribution statement

Chiara Palazzo: conceptualization and writing – original draft; Guido Pelletti: writing – original draft; Paolo Fais: Investigation, writing - review & editing; Rafael Boscolo Berto: formal analysis; Federica Fersini: investigation and data curation; Rosa Maria Gaudio: data curation; Susi Pelotti: Supervision.

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Figure legends

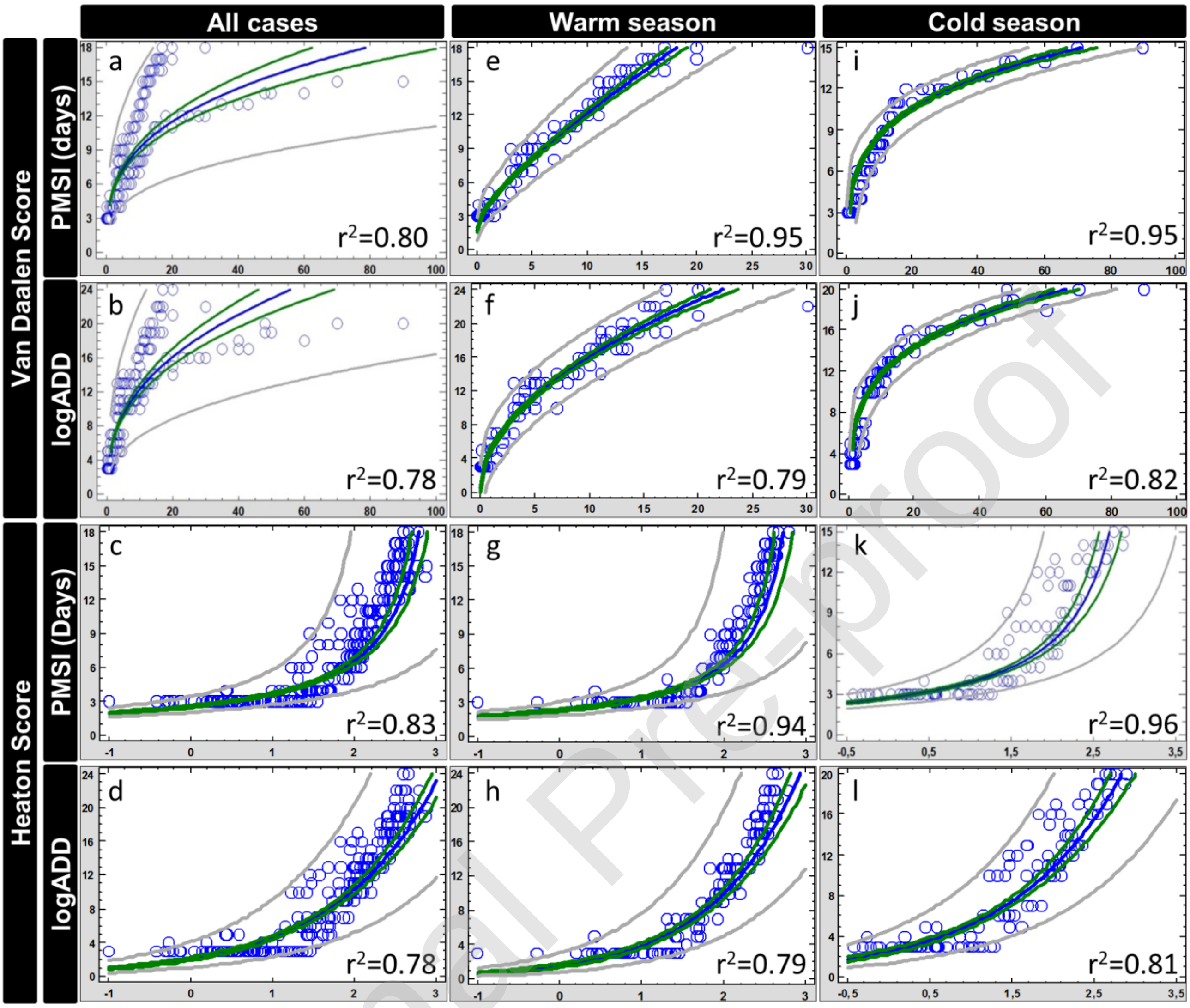

Figure 1. The graphical representations of all regression analysis, as well the values of r^2 referring to scattered plots based on the different analyzed casuistries, are reported.

a. Dependent variable: TADS (van Daalen Score), Independent variable: PMSI (days), Number of observations: 238 (all cases).

b. Dependent variable: TADS (van Daalen Score), Independent variable: logADD, Number of observations: 238 (all cases).

c. Dependent variable: (Heaton Score), Independent variable: PMSI (days), Number of observations: 238 (all cases).

d. Dependent variable: TADS (Heaton Score), Independent variable: logADD, Number of observations: 238 (all cases).

e. Dependent variable: TADS (van Daalen Score), Independent variable: PMSI (days), Number of observations: 127 (warm season).
f. Dependent variable: TADS (van Daalen Score), Independent variable: logADD, Number of observations: 127 (warm season).
g. Dependent variable: TADS (Heaton Score), Independent variable: PMSI (days), Number of observations: 127 (warm season).
h. Dependent variable: TADS (Heaton Score), Independent variable: logADD, Number of observations: 127 (warm season).
i. Dependent variable: TADS (van Daalen Score), Independent variable: PMSI (days), Number of observations: 111 (cold season).
j. Dependent variable: TADS (van Daalen Score), Independent variable: logADD, Number of observations: 111 (cold season).
k. Dependent variable: TADS (Heaton Score), Independent variable: PMSI (days), Number of observations: 111 (cold season).
l. Dependent variable: TADS (Heaton Score), Independent variable: lodADD, Number of observations: 111 (cold season).

Table 1. Monthly distribution of all cases.

Month	Number of cases
January	14
February	14
March	19
April	22
May	23
June	24
July	34
August	25
September	21
October	11
November	13
December	18
	Total=237