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The effect of maceration and hydration on cranial dimensions: A study of *Oryctolagus cuniculus*

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ABSTRACT Attempts are frequently made in craniofacial identification (e.g., craniofacial superimposition and facial approximation) to relate the anatomy of *dry* skulls to the *antemortem* appearance of individuals. However, drying processes and environmental conditions may affect the morphology and dimensions of skulls relative to their living state. This may have ramifications for the accuracy of craniofacial identification methods. In this study, the effects of maceration, drying and re-hydration on the dimensions of rabbit skulls were examined (*Oryctolagus cuniculus*). A sample of rabbits was chosen as a pilot study to humans since *O. cuniculus* have relatively fine nasal bones that may be prone to changes under changing environmental conditions as previously commented upon in the literature. Twelve rabbit skulls were macerated and exposed to various humidity conditions. At each step, 10 craniometric dimensions were measured using sliding callipers and the skulls were visually inspected. Results indicated that in the macerated condition skull dimensions both increased and decreased whilst measurements generally became smaller upon drying, and increased with hydration. Degree of change was, however, found to vary with measurement site under every condition, indicating that skulls did not simply scale in size. Upon hydration, skull dimensions did not obtain values as high as those in the green state, although they approximated their magnitude. Metric changes between the green state and the dry condition were small and in the vicinity of two to three percent of the original measurements. Large differences in bone morphology were visually evident from the green to the dry state, particularly in the nasal region, even though current metrics were not generally sensitive to them (except in one case). These findings indicate that drying processes may be of practical significance for craniofacial identification using human skulls and that further investigations should be pursued.

KEY WORDS osteology, skulls, craniofacial identification, facial approximation, facial reconstruction, superimposition

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Introduction

In craniofacial identification methods such as facial approximation and superimposition, *dry* skulls are used to predict or compare the *antemortem* appearances of decedents [GERASIMOV 1971; AULSEBROOK *et al.* 1995; TYRRELL *et al.* 1997; TAYLOR and BROWN 1998; JAYAPRAKASH *et al.* 2001; TAYLOR 2001; DE GREEF and WILLEMS 2005]**.** However, it is well known that factors such as maceration, hydration and drying affect the dimensions of many bones of the skeleton [WELCKER 1862; ROLLET 1888; PEARSON 1899; INGALLS 1927; TODD and PYLE 1928], including the skull [TODD 1923, 1925, 1926; ALBRECHT 1983; UTERMOHLE *et al.* 1983; LINDSTEN 2002]. Thus, to ensure accuracy is maintained, it seems necessary to consider the affects of drying on skull morphology and dimensions in case changes are induced relative to the living condition. In other areas of anthropological investigation dimensional stability of bones is regarded to be significant, for example, in stature prediction [MANOUVRIER 1893; STEWART 1979; KROGMAN and ISCAN 1986]. However, in the craniofacial identification literature this aspect has received little comment. KROGMAN and ISCAN [1986: 376] briefly point to its importance under the generalized topic of "Factors of Individualization" in their classic text "The Human Skeleton in Forensic Medicine". Here they state that "It may be necessary to consider these changes when conducting an osteometric evaluation of the skeleton", yet, no further mention is made within their craniofacial identification chapter nor in other major craniofacial texts [see e.g., GERASIMOV 1971; PRAG and NEAVE 1997; CLEMENT and RANSON 1998; TAYLOR and BROWN 1998; WILKINSON 2004; CLEMENT and MARKS 2005].

Dimensional stability of bones has been studied at least since 1862 when WELCKER [1862] found that dry femora, when soaked in water, increased in length by 1.2 mm. These results were essentially repeated by BROCA [cited in PEARSON 1899] who found an increase of 1-1.5mm on another femoral sample, and then also by PEARSON [1899] who found dry long bones when soaked in water for 120 hours (hrs) increased in length by 1-2 mm and shortened by 1-2 mm in length upon redrying. It seems that changes from fresh to dry states were not investigated until 1888. ROLLET [1888] found long bones of the upper and lower limbs to lose 2 mm in length from the "fresh" to the dry state. In the 1920's, INGALLS [1927] came to conclusions similar to those of ROLLET [1888] and PEARSON [1899] finding that fresh femora shrink by 1.55 mm or 0.33% upon drying and regained most of their length upon re-hydration. Additionally, INGALLS [1927] found that scapulae, and ribs follow similar patterns (scapulae: shortening of 0.5 mm or 0.27% with drying; ribs: 1.08 mm or 0.58%). A year later, TODD and PYLE [1928] investigated dimensional stability of vertebrae from the fresh to the dry state and found that ventral body height shrunk by 1.5% upon drying and the dorsal body height and the mid-centrum diameter shrunk by 2.5% upon drying. The cumulative average vertebral column shrinkage was approximately 12mm or 0.27% [TODD and PYLE 1928].

The first in-depth systematic studies specifically quantifying change in linear dimensions of the skull were conducted by

TODD in the 1920's. Earlier studies had been performed but did not specifically examine change in linear dimensions, rather they concerned themselves with qualitative measures of skull change [see e.g., WELCKER 1862] or examined volumetric effects [see e.g., BROCA 1874]. In 1923, TODD found that macerated skulls (n = 24) shrank by about 1% on average when dried, although there was considerable variation between specimens. Most of this change occurred within the first week and appeared to be symmetrical without bone warping (half skulls could be fitted back together) [TODD 1923]. This is contrary to earlier findings of WELCKER [1862] who suggested that dried half skulls would not fit back together. In further investigations of skulls using larger sample sizes (n ≈ 50), TODD [1925, 1926] again found shrinkage from green to dry states to be about 1%. He also found that hydrated dry skulls almost attained the dimensions of the green state [TODD 1925, 1926] and postulated that smaller thinner bones undergo a higher degree of change than larger thicker bones [TODD 1926].

More recently, UTERMOHLE and colleagues [1983] have conducted humidity experiments using two dry human skulls and found, like TODD [1925, 1926], that linear dimensions increase with increasing humidity. In addition, nonhuman studies have been conducted and produced similar results. ALBRECHT [1983] used ten dry macaque skulls and found that increased humidity increased the greatest length of the skull by 0.57% and that the skulls returned to their original dimensions after 1-2 days at decreased humidity. LINDSTEN [2002] studied pig crania from green to (following maceration) dry conditions and found measurements to decrease by between 0.3 and 1.9%. An advantage of these more recent studies is that they include a greater number of skull dimensions than those originally studied by TODD [1923, 1925, 1926] even though their sample sizes are smaller.

All of the above results and conclusions indicate that drying and humidity represent a real though relatively small source for difference in skull measurements [TODD 1923, 1925, 1926; ALBRECHT 1983; UTERMOHLE *et al.* 1983; LINDSTEN 2002]. Despite the small magnitude of such changes (i.e., ~1%) they appear to be of practical significance for craniofacial identification techniques where features of the face need to be assessed/predicted from the skull with precision. For example, shrinkage of one or two percent across the orbits could translate into an error of almost 2 mm in the representation of the distance between the ectocanthions and thus may be significant, particularly for superimposition. In addition, small changes to the nasal bones, which are relatively fine and thus maybe easily influenced [see TODD 1926], may result in nose prediction problems for facial approximation [see Taylor cited in STEPHAN *et al.* 2003]. Furthermore, since sequential errors in facial approximation often occur as a result of standardized methods and accumulate to produce large deviations from actual antemortem face morphology, it seems important to examine all aspects of methods where error is potentially introduced to ensure appropriate measures are used to counter it and increase accuracy. The aim of this project is, therefore, to determine the effects of maceration, drying and hydration on skulls relative to their green state, with special attention given to the nasal bones.

Materials and methods

Twelve male domestic rabbit skulls (*Oryctolagus cuniculus* – eleven Californian and one Angora) were used in this study. Rabbits were selected because they have relatively fine nasal bones which were expected to be prone to changes under changing environmental conditions thus making changes easier to detect if any occur.

Skulls were obtained from rabbits immediately after death, and were prepared for measurement in the green state by removing soft tissue from the superior splanchnocranium, calotte and zygomatic arches. Ten measurements were taken across various craniometric landmarks (Fig. 1). All measurements were taken using dialMax® Swiss Precision® sliding callipers readable to one tenth of a millimetre. The skulls were immediately measured after soft tissue flensing and the specimens were bagged and frozen for 48 hrs before maceration. The skulls were macerated (from frozen) over 72 hrs using hot to warm, but not boiling, water. Immediately following maceration, the skulls (still dripping wet) were measured and set to dry at

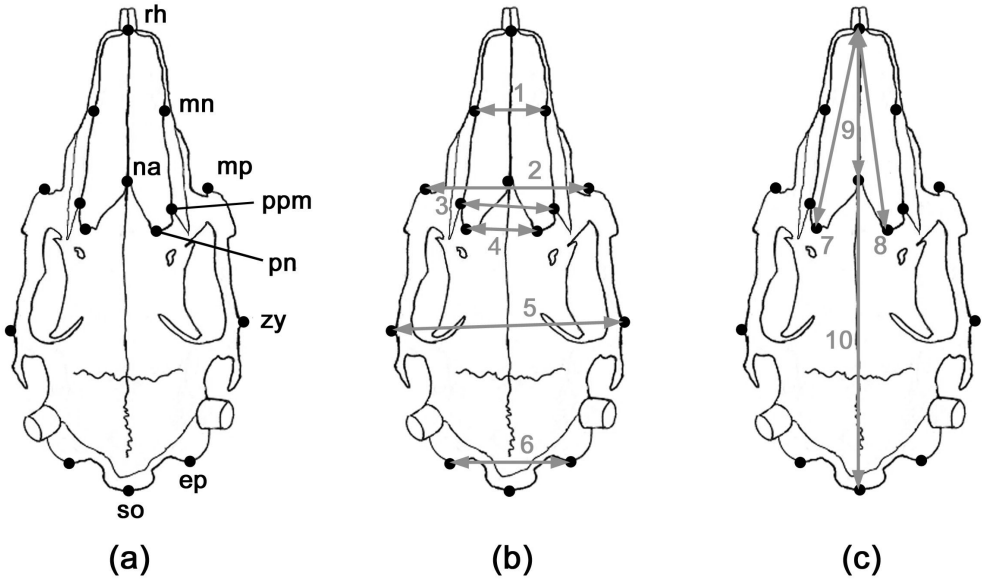

Fig. 1. Landmarks and measurements taken on each skull. **(a)** median landmarks: *rh* – rhinion, *na* – nasion, *so* – supraoccipital point; bilateral landmarks: *mn* – midnasal, *mp* – maxillary point, *pn* – posterior nasal, *ppm* – most posterior junction of nasal bones with premaxillary process, *zy* – zygion, *ep* – exoccipital point. **(b)** transverse measurements: 1 – midnasal width (*mn-mn*), 2 – width between maxillary points (*mp-mp*), 3 – width of nasal bones at most posterior junction with the premaxilla process (*ppm-ppm*), 4 – width between posterior aspects of nasal bones (*pn-pn*), 5 – bizygomatic diameter (*zy-zy*), 6 – width between exocciptal points (*ep-ep*). **(c)** longitudinal/oblique measurements: 7 and 8 – length of nasal bones (*rh-pn* [R&L]), 9 – length of internasal suture (*rh-na*), 10 – skull length (*rh-so*).

room temperature. Measurements of the drying skulls were taken at 24-hour intervals until it was evident that the dimensions were stabilising (found to be five days).

The skulls were also exposed to three levels of humidity: (i) 55-65%; (ii) 65-75%; and (iii) 90-100%. The "55-65%" condition represented room humidity while the higher humidity conditions were created using sealed pots (water added in the base) placed over a warming plate. Skulls were also remeasured after a ten month interval at room conditions following the maximum humidity condition. Humidity was measured using a hygrometer, placed, in the case of the low and high humidity conditions, inside the sealed containers with the skulls (both the skull and the hygrometer were placed on an elevated platform). The skulls were separated into three equally sized sub-samples (n = 4) and rotated through the different humidity levels, allowing time to dry out between each rotation (~36 hrs). This was done to ensure all skulls could be placed in the humidity apparatus in a regular fashion as there were a larger number of skulls than humidity apparatus. The skulls were exposed to each condition for no less than 36 hrs before being measured.

Changes in cranial dimensions were recorded in absolute terms (mm) and as a percentage relative to the green state. Measurement error was determined by remeasuring ten dry skulls after two weeks had elapsed and calculating the coefficient of variation of the error (CVE). The CVE was computed by taking the sum of the squared differences between test and retest divided by two times the number of remeasured specimens. The square root of the result was taken and divided by the mean of the test/retest result of the first specimen. Coefficients of variation of the error for each measurement were found to be low, being less than 4% (Technical error of measurement [TEM] 0.5 mm; Table 1). Data from the main investigations were statistically compared using two-tailed paired *t*-tests, with significance initially set at 0.05 but adjusted using Bonferroni's correction for 10 tests (i.e., a p value of <0.005 was used since each skull had 10 measurement sites). Data were analysed using Microsoft® Excel® 2000.

Table 1. Intra-observer measurement error

	CVE [%]	TEM [mm]
1 (*mn-mn*)	2.7	0.4
2 (*mp-mp*)	1.1	0.4
3 (*ppm-ppm*)	1.1	0.2
4 (*pn-pn*)	3.7	0.5
5 (*zy-zy*)	0.3	0.1
6 (*ep-ep*)	2.2	0.4
7 (*rh-pn*)	0.4	0.2
8 (*rh-pn*)	0.5	0.2
9 (*rh-na*)	1.0	0.0
10 (*rh-so*)	0.2	0.2

Results

Differences between the left and right sides for nasal bone length (measurements 7 and 8) were found to be negligible and so data for these bilateral measurements were combined. In general, the dimensions of the skulls changed in all treatment conditions in comparison to the green state (Fig. 2). However, there were large variations in the degree of change and the direction of

Table 2. Mean measurements of skulls (n = 12; mm) under the four main test conditions

Measurement	Green		Macerated			120 Hours Dry			90-100% Humidity			10 Months		
	Mean	*s*	Mean	*s*	*t*-test	Mean	*s*	*t*-test	Mean	*s*	*t*-test	Mean	*s*	*t*-test
1 (*mn-mn*)	13.9	1.0	14.5	1.1	0.086	14.0	0.8	0.839	14.2	0.9	0.219	13.5	0.8	0.223
2 (*mp-mp*)	32.6	1.1	32.8	1.2	0.573	32.7	1.1	0.854	33.2	1.1	0.029	32.5	1.3	0.871
3 (*ppm-ppm*)	17.6	1.5	17.9	1.8	0.270	17.0	1.6	0.026	17.4	1.7	0.248	17.05	1.9	0.045
4 (*pn-pn*)	13.1	1.1	12.9	1.3	0.675	12.5	1.4	0.196	12.8	1.3	0.458	12.2	1.2	0.048
5 (*zy-zy*)	42.4	1.7	43.0	1.8	0.041	43.0	1.7	0.130	43.3	1.7	0.028	42.6	1.7	0.591
6 (*ep-ep*)	19.6	1.3	18.3	0.7	0.009	18.3	0.5	**0.003**	18.7	0.6	0.024	18.3	0.8	0.009
7/8 (*rh-pn*)	43.1	2.2	42.9	1.9	0.668	42.5	1.9	0.668	42.7	2.4	0.344	42.2	2.3	0.023
9 (*rh-na*)	34.3	2.4	33.0	1.9	**0.004**	32.1	2.0	**0.000**	32.4	2.3	**0.000**	32.3	2.2	**0.000**
10 (*rh-so*)	89.8	3.0	89.7	2.8	0.523	88.8	3.2	**0.000**	89.5	3.1	0.113	88.3	3.1	**0.000**

Bold type represents statistical significance ($p < 0.005$, see methods).

change for some skulls. The maximum amount of change occurred after 120 hrs of drying, and for re-hydration conditions at 90-100% humidity (highest levels of exposure in each condition) (Fig. 2). Therefore, data from green, macerated, 120 hrs drying, 90-100% humidity, and 10 months drying form the initial basis for statistical tests and closer examination.

All mean measurements of macerated skulls changed from the green state. Some measurements became smaller and others larger (Table 2; Fig. 2), but only one of nine measurements (*rh-na*) was statistically significant (Table 2). Skulls dried for 120 hrs also showed mixed results. Six measurements displayed smaller values than the green skulls and three of these were statistically significant (*ep-ep*, *rh-na*, *rh-so*; Table 2). Of the three measurements that were larger, none were statistically significant (see Table 2). Skulls subjected to 90-100% humidity again showed mixed results. Six measurements were smaller than in the green state, but only one was statistically significant (*rh-na*; Table 2). Three measurements were found to be larger than in the green state (*mn-mn*, *mp-mp*, *zy-zy*) although none of these were statistically significant (Table 2). After ten months drying following the 90-100% humidity environment, all nine measurements decreased as expected, most returning to levels representative of the 120 hr dry condition. However, some measurements were even less (see e.g., *mn-mn*, *zy-zy*). Measurement 9 (*rh-na*) was also an exception – this measurement did not reduce to values representative of the 120 hr dry condition, but remained close to the values obtained for the 90-100% humidity state.

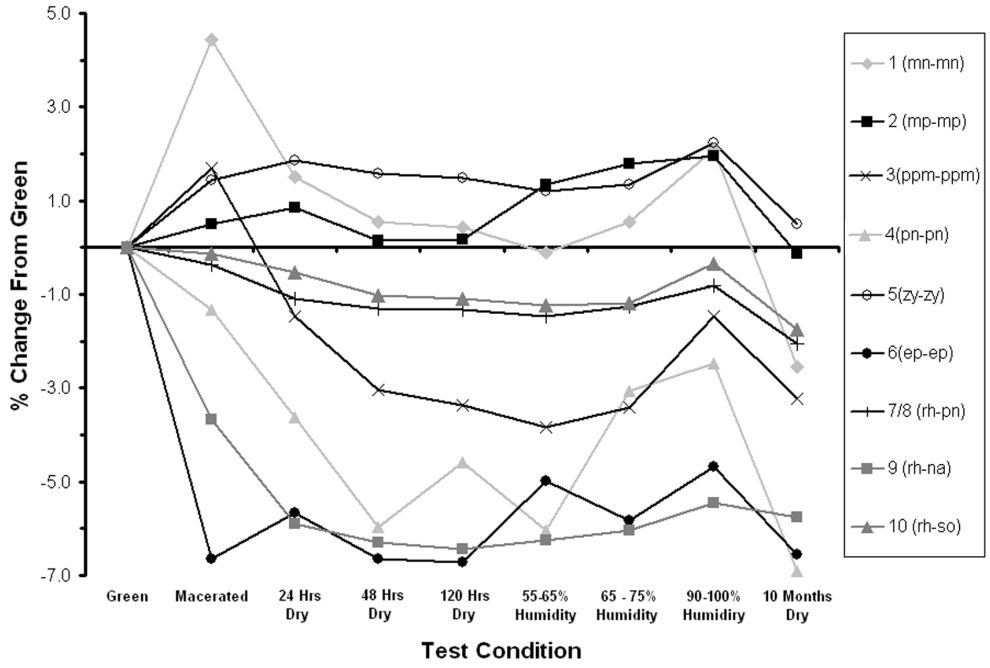

Fig. 2. Percentage change of skull measurements from the green state over each treatment condition.

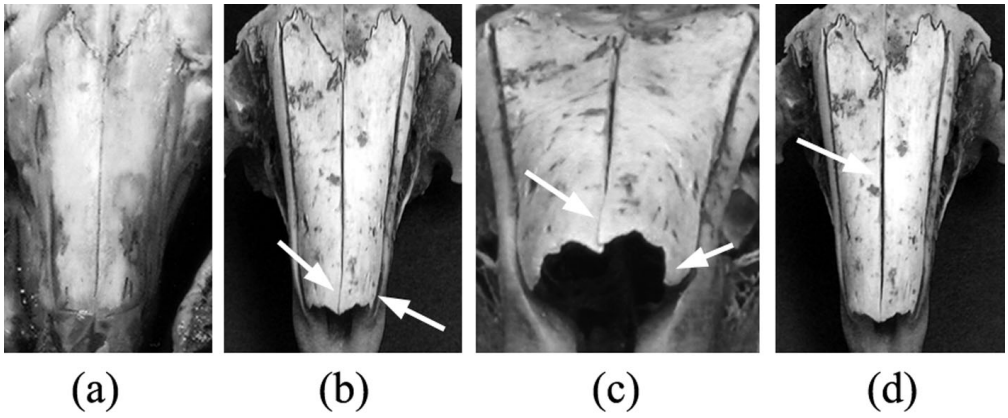

Fig. 3. Example of morphological changes in the nasal bones of a rabbit skull between green and dry conditions. **(a)** Morphology of nasal bones immediately after death. **(b,c,d)** Morphology of the nasal bones following maceration and 120 hrs of drying; **(b)** Warping and curling of (unmanipulated) nasal bones as observed after 120 hrs of drying (see white arrows); **(c)** Anterior view of (b); **(d)** View of nasal bones after being manipulated so that ends align (Note: the widening and lengthening of internasal suture space indicated by the white arrow).

Whilst all measurements of the skulls showed some metrical changes, those in the nasal region appeared to be consistent and relatively large (Table 2; Fig. 2). This is particularly evident from measurements of the internasal suture (*rh-na*), which showed statistical significance at each treatment condition. After both maceration and drying, macroscopic changes were also observed in nasal bone shape. After 120 hrs of drying, the internasal suture had opened to some extent in all specimens (see e.g., Fig. 3). Particularly marked was medial curling of the lateral sides of the nasal bones (Fig. 3c).

Discussion

This study is significant because it is the first to longitudinally measure skulls across all research conditions so far investigated, i.e., green, macerated, drying and increasing humidity. The longitudinal nature of this project not only enabled changes from the green state with drying to be observed, but, also, whether re-hydration was able to compensate for these effects. Data reported in this study are consistent with findings by TODD [1923, 1925, 1926] that measurements of skulls, dried after maceration, reduce in magnitude. Results are also consistent with findings of other investigators [ALBRECHT 1983; UTERMOHLE *et al.* 1983] that "re-hydration" of dry skulls using increasing humidity levels causes increases in skull dimensions, and confirm observations by TODD [1923] that re-hydrated skulls do not in general obtain original green dimensions although they come close to doing so. Furthermore, our data agree with observations by TODD [1923, 1925, 1926] that most changes, with drying, occur within the first 24 hrs after maceration with the majority of changes completed within one week, although small changes may occur thereafter. In contrast to studies that have consistently found a decrease in wet but recently macerated skulls [TODD 1925, 1926; LINDSTEN 2002], this study finds measurements reacting in both directions: some measurements shrink while others display marked increase (Fig. 2). Thus, while water immersion in macerating techniques might be expected to have similar consequences to 90-100% humidity conditions, the more extreme conditions of maceration (high temperature etc.) and the non-dry state of the skull probably produce different responses. In general, most measurements showed an average of about 2% change from the green state for any treatment condition. For measurements in the nasal region, the average change was approximately 3% from the green state. The largest degree of change observed in this study was -7% for measurement 6 (*ep-ep*), in the 120 hr drying condition and the smallest amount of change was 0% for measurement 1 (*mn-mn*), in the 55-65% humidity condition and measurement 10 (*rh-so*), after maceration. This degree of change is consistent with that reported in other studies on other species [ALBRECHT 1983; LINDSTEN 2002], including humans [TODD 1923, 1925, 1926], and thus suggests that dry skulls may not be optimal for use in craniofacial identification methods. Furthermore, if nasal bone contours change in humans upon drying, as is clearly evident for rabbits, prediction/assessment of *pronasale* position may be incorrect if dry skulls are used. Similarly, prediction of

any other facial features using guidelines derived from living individuals may contain error if applied to dry skulls.

As with investigations conducted by TODD [1923, 1925, 1926], this study indicates that it may be possible, through re-hydration of skulls in water, to approximate the dimensions representative of the green state. Thus, casts of water-saturated skulls may be advantageous for traditional facial approximation methods, while hydrated original skulls (subjected to real-time wetting techniques) may be best for video superimposition. Before such conclusions are acted upon, however, research investigations on drying processes of human bones following soft tissue decomposition must be conducted (and are currently underway). Since direction and size changes to various linear distances measured across the skull do not appear to be consistent, investigations that quantify shape change also appear to be useful. Once the global patterns of skull change from the green to the dry state have been documented, computer graphic methods may also be employed to warp electronic representations of dry skulls to shapes and sizes representative of the living condition. This feature could then be added to computer based methods of craniofacial identification where it would assist in increasing the accuracy and speed of results.

Conclusions

Changes in the cranial dimensions of green rabbit skulls were observed after maceration, drying and hydration. Skull dimensions did not show consistent directions of change immediately after maceration, yet, upon drying, all measurements became smaller and increased with exposure to more humid conditions. The only statistically significant change in measurements which was consistently observed was that for the length of the internasal suture. Despite this, all measurements changed on average by about 2-3% from the original green dimensions in any treatment condition. Shape changes for the nasal bones were also clear upon visual inspection. If drying processes in human skulls affect the individual bones as they appeared to do in this experiment, then this change may be significant for craniofacial identification techniques. However, further investigations using human material are required.

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Streszczenie

Często w celach identyfikacyjnych (np. w metodzie superprojekcji) próbuje się odnosić anatomiczne cechy suchych czaszek do przyżyciowego wyglądu osobnika. Jednak proces wysychania i warunki otoczenia mogą wpływać na morfologię i rozmiary czaszki. Może to ograniczać dokładność metod identyfikacji kraniofacjalnej.

W niniejszej pracy przedstawiono wpływ maceracji, suszenia i wtórnego nawilgocenia czaszek królików (*Oryctolagus cuniculus*). Do badań wybrano króliki ze względu na delikatną budowę ich kości nosowych, które – jak zakładano – mogą być wrażliwe na zmiany warunków otoczenia i zmieniać wymiary w zauważalny sposób. Czaszki dwunastu królików

poddano 72-godzinnej maceracji, bezpośrednio po której zmierzono je, a następnie pozostawiono do wyschnięcia w temperaturze pokojowej. W odstępach 24-godzinnych wykonywano pomiary (10 kraniometrycznych pomiarów cyrklem suwakowym), aż do czasu kiedy przestały się zmieniać. Następnie podzielono czaszki na 3 grupy i umieszczono je w warunkach zróżnicowanej wilgotności (55-65%, 65-75% i 90-100%) i ponownie – po ok. 36 godzinach ekspozycji w danym zakresie wilgotności – wykonano pomiary.

Wykazano, że wymiary macerowanej czaszki ulegają zmianom, przy czym – generalnie – maleją w trakcie wysychania, a wzrastają, gdy wzrasta wilgotność. Intensywność tych zmian jest jednak różna w różnych częściach czaszki. Duże różnice w morfologii kości pomiędzy czaszką w stanie świeżym a wysuszoną dotyczą głównie okolicy nosowej. Wyniki pracy sugerują, że skutki procesu wysychania kości mogą mieć praktyczne znaczenie również w badaniach identyfikacji kraniofacjalnej ludzkiej czaszki.