Length estimation of Atlantic bluefin tuna (*Thunnus thynnus*) using vertebrae: Appendix 2

Guide to identifying *Thunnus* spp. vertebrae rank or type

This guide aims to illustrate morphological features present on *Thunnus* vertebrae which allow for the identification of vertebrae (V) recovered in archaeological assemblages to rank (sequential position in vertebral column) or type (grouping of vertebrae with similar morphologies) level. Spines or articular processes (zygapophyses) are often broken or eroded in archaeological vertebrae. As such, here there is a particular focus on the joining position, and angle of, spines and articular processes in relation to the vertebral body, instead of the height of spines, arch of spines or the length of articular processes. Variation between individuals is likely to produce differences in the appearance of vertebrae, compared to the figures below, although, the characteristics described herein hold true for all 13 reference specimens studied.

In the genus *Thunnus*, the total number of vertebrae is 39, including the first vertebra (V1, Figure S2) which often fuses with the skull (at the basioccipital) in adults (~100+ cm FL), and the ultimate centra (V39) connected to the urostyle.

Prior to rank or type identification, it is advised to identify anterior and posterior centra. All lateral views of vertebrae shown here are arranged from anterior (left) to posterior (right). It is possible to differentiate between anterior and posterior surfaces as parapophyses are pronounced at the anterior in V2-32 (also in V1 for juvenile specimens), because hemal spines are attached at the anterior in V8-23 and because in V33-39 spines are directed towards the posterior.

Vertebrae in this guide are grouped into types (V2-7, V8-18, V19-23, V24-31, V30-32) which can be used when it is difficult to identify rank. Error in size estimations will increase if rank/type is misidentified, therefore care should be taken at this step. It is advisable to make identifications checkable by providing identification criteria and photographic evidence where possible. Note that identifying rank is challenging and ought to take considerable time.

V1 may simply be identified if found disarticulated, or unfused in juvenile specimens, by the centra heavily narrowing anteriorly and in adult specimens as the anterior centrum is absent.

Figure S2. First vertebra in *Thunnus* spp., fused to the skull in adults. In ventral view (left) and latero-posterior view (right).

V2-7 (Figure S3) can be recognised by 1) their shorter length-width relation in comparison with other vertebrae and 2) their lack of a hemal spine. The rank of vertebrae 2-7 can be identified when assessing their posterior and ventral view, but the lateral view is optimal where:

In V2, the neural spine is more separated from its anterior articular process than in V3-5, and its parapophysis is barely developed and more anterior than V3-5. In V3, the neural spine stretches across the whole length of the vertebrae and has a proportionally large parapophysis. In V4 and V5, the neural spine is separated from its posterior articular process, more so in V5 and now parapophyses have a protruding costal articulation (see Figure S4, S5), increasing in size to V6. In V6 and V7, vertebra length is increased compared with V2-5 and the neural spine is more distinct from the posterior portion of the centrum than in V2-5, especially for V7. If well preserved, the neural spine is more rounded in V6 and V7 than V2-5, especially for V7. If also well preserved, the parapophyses are enlarged in V5-7, increasing with rank. Parapophyses have protruding articulations that become compressed in V7, especially V8 and on. The parapophyses are best assessed from the anterior or posterior or ventral view (Figure S4, S5).

In addition, three easily identifiable fossae (depressions) can be used when assessing ventral view to distinguish between vertebrae V1-6 and V7-8. Fossae are formed along the midline and laterally (Figure S5) and decrease in size with rank.

Figure S3. Lateral view of V2-7, as labelled on the neural spine in order from V2 (left) to V7 (right). N.B. Images of vertebrae V6-7 are cropped at the neural spine.

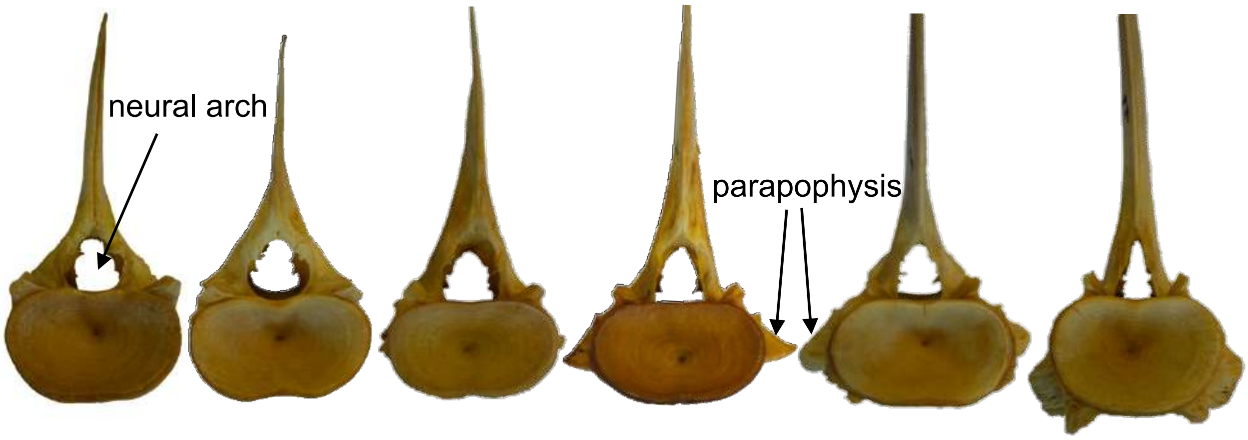

Figure S4. Posterior view of V2-7 in order from V2 (left) to V7 (right). N.B. Images of vertebrae V4-7 are cropped at the neural spines.

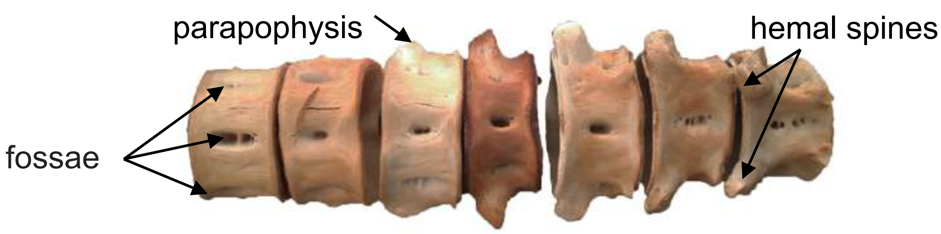

Figure S5. Ventral view of V2-8 in order from V2 (left) to V8 (right). V8 shown to illustrate compressed parapophyses in comparison with V5-7. V8 is the first vertebra to develop anteriorly-projecting hemal spines.

V8-18 are particularly challenging to differentiate. They can principally be recognised by the presence of both neural and hemal spines, which are much broader than in V19 onwards. If well preserved, V8-18 can, if it is sufficiently preserved, be identified according to the angle of the hemal spine in relation to the vertebral body as it is less anteriorly angled with each rank (Figure S6). In V8-13 the hemal spine is orientated anteriorly—particularly so in V8-10 which should make V8-10 more readily identifiable than others of this type. In V14 and V15, the hemal spine is oriented vertically, whereas in V16-18 the hemal spine is posteriorly angled.

V8-18 can also be somewhat identified by the width of their neural spines as these decrease with each rank. Note a particular distinction in neural spine width between V8-13 and V14-18 (Figure S7).

Figure S6. Lateral view of V8-18, as labelled on the neural spine V8 (top left) to V13 (top right), V14 (bottom left) to V18 (bottom right). N.B. Images are cropped at the neural and hemal spines.

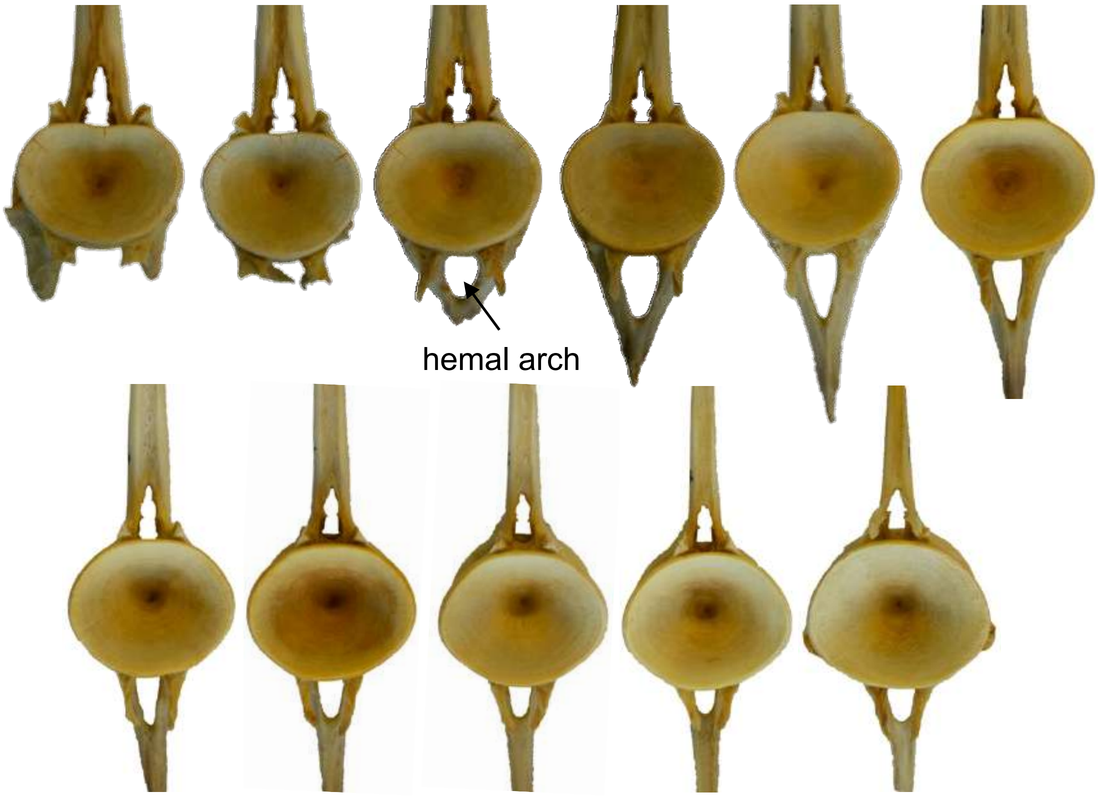

Figure S7. Posterior view of V8-18 in order from V8 (top left) to V13 (top right), V14 (bottom left) to V18 (bottom right). N.B. Images are cropped at the neural and hemal spines.

V19-22/23 were perceived as too morphologically similar to be differentiated. Therefore, their type equation must be applied. V19-22/23 may be recognised by the presence of neural and hemal spines where the neural spine is narrower than in V8-18 (Figure S8, S9). Furthermore, they lack a transverse foramen which is present on V23/24-31 and otherwise appear similar. Variation exists between V22 and V23 because vertebrae V23 sometimes carries a transverse foramen (small hole at the base of the hemal spine). The cause of this variation is unknown.

Figure S8. Lateral view of V19-23, as labelled on the neural spine V19 (left) to V23 (right). N.B. Images are cropped at the neural and hemal spines.

Figure S9. Posterior view of V19-23, in order from V19 (left) to V23 (right). N.B. Images are cropped at the neural and hemal spines.

V23/24-29/31 may be recognised by the presence of a transverse foremen at the base of the hemal spine. Note that a transverse foramen is not always present on V23, V30 and V31. If a transverse foramen is missing, apply the type equation for V19-23 or V30-32, respectively.

V23-31 may be recognised according to the neural and hemal spines, which are more posteriorly positioned, and more posteriorly angled with each rank. Vertebral length can also be used to identify rank since length increases with rank in these vertebrae (Figure S10). The posterior view may be of little use to aid rank identification (Figure S11).

Figure S10. Lateral view of V24-31, as labelled on the neural spine V24 (top left) to V27 (top right), and V28 (bottom left) to V31 (bottom right). N.B. Images are cropped at the neural and hemal spines.

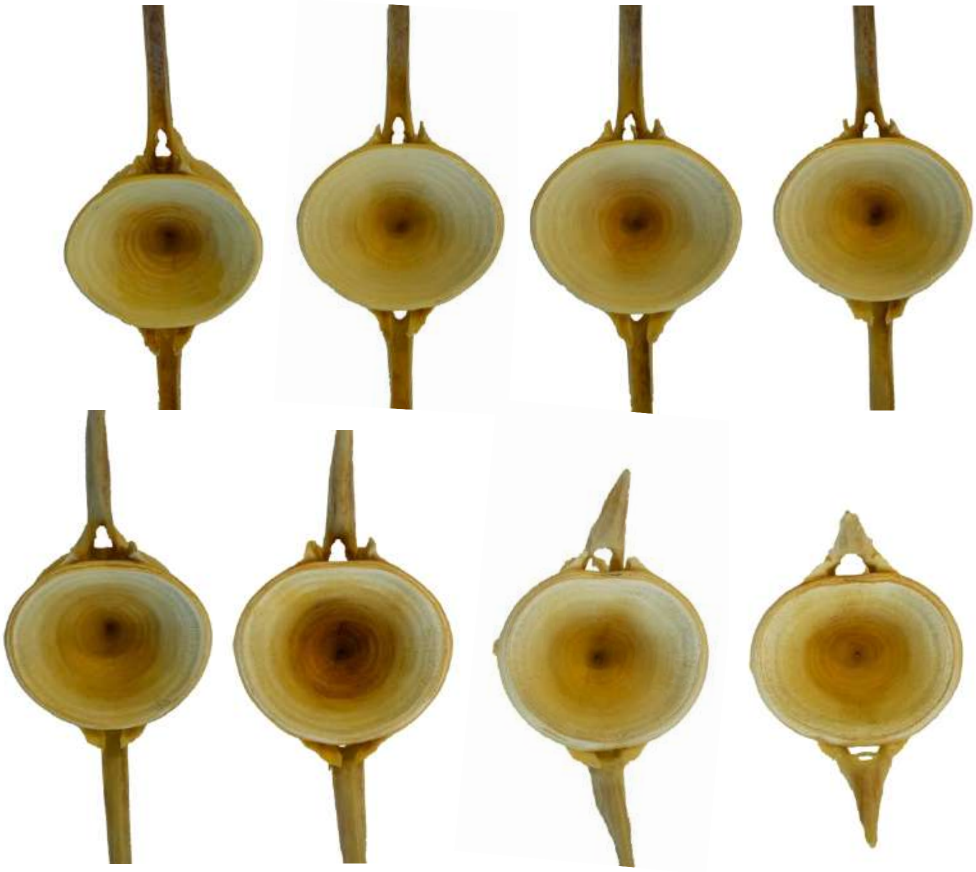

Figure S11. Posterior view of V24-31, in order from V24 (top left) to V27 (top right), and V28 (bottom left) to V31 (bottom right). N.B. Images are cropped at the neural and hemal spines.

V30-32 are challenging to differentiate. They may be recognised due to being elongated, similar to V28 and V29, with posteriorly positioned neural and hemal spines but V30 and V31 may not carry a transverse foramen as is present in the reference specimens shown (Figure S12). If well preserved, each may be identified by the angle of the neural and hemal spines, which is more acute with each subsequent rank (Figure S12, S13).

Figure S12. Lateral view of V30-32, in order from V30 (left) to V31 (right). N.B. the spines of V31 were eroded in this reference specimen.

Figure S13. Posterior view of V30-32, in order from V30 (left) to V31 (right). N.B. the spines of V31 were eroded in this reference specimen.

V33-39 are considered unique and able to be identified to rank, as follows:

V33-35 may be differentiated according to the height of their parapophyses, which are now pronounced in comparison with all other vertebrae, and increase towards the posterior in V33, are at their greatest and more horizontal in V34, and decrease towards the posterior in V35 (Figure S14, S15, S16).

V36 may be differentiated from V33-35 by the obtuse angle of its neural and hemal spines, and because its centra is shortened (Figure S14).

V37 can be differentiated from 38 because vertebral length is notably shortened in V38 (Figure S14).

V39, the final vertebra is attached to the urostyle.

Figure S14. Lateral view of V33-39, in order from V33 (top left) to V35 (top right)., V36 (bottom left) to V39 (bottom right).

Figure S15. Dorsal view of V33-36 in order from V33 (left) to V36 (right).

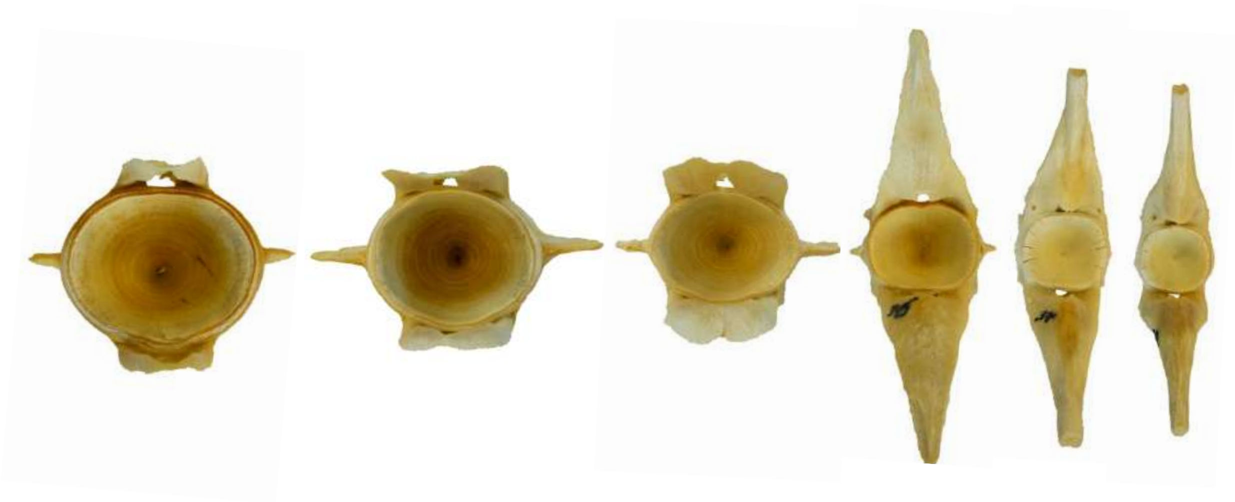

Figure S16. Posterior view of V33-38, in order from V33 (left) to V38 (right).

Vertebra identification and size estimation tips

- First identify anterior and posterior surfaces and orientate vertebrae as per the figures (anterior, left and posterior, right).
- Attempt identification to rank level where possible as variation between vertebrae in types will decrease the accuracy of estimates.
- Once vertebral rank or type has been identified, measure on the posterior surface of centra where possible using the optimal dimension (H, W or L) for the vertebra rank or type in question (as indicated in Table S2 & S3). Measurements should be made as per Figure 1.
- If measurements can only be made on the anterior surface of centra be aware that slight variation will further affect the accuracy of estimates, particularly for V2-3 and V33-36. Likewise, the accuracy of estimates will decrease if using a vertebra dimension that is not the best fitting (as indicated in Table S2 & S3).
- Apply regression equations using the formula ($FL=aX^b$, where X is your appropriate vertebra measurement in mm) and values in Table S2 & S3.
- Alternatively, visit https://tunaarchaeology.org/lengthestimations and input your rank or type and measurement where your estimate will be calculated. Note estimates are straight fork lengths, in cm.
- Consider that measurements require the identification of the edge of the vertebrae centra. Thus, if spines and processes are heavily eroded to make difficult the identification of vertebrae to rank or type level, the centra edge is likely to be damaged too and will lead to underestimated measurements.
- To improve the reliability of size estimations: provide criteria for rank or type identification, evidenced by a photograph and any notes of uncertainty when publishing.
- At any time, the corresponding author will welcome queries on rank or type identification.