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Establishing reference intervals and a minimally invasive skin-based biomarker profile to assess the health and welfare of free-ranging bottlenose dolphins (*Tursiops truncatus*)

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Understanding the health and welfare of free-ranging bottlenose dolphins is critical for their conservation. This study aimed to establish an analytical profile of biochemical markers in skin samples to assess the health status of dolphin populations, as well as the condition of the marine environment, given their role as apex predators.

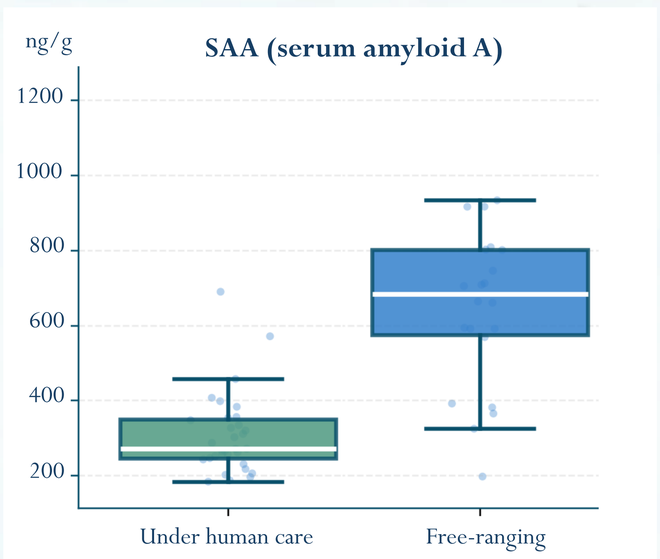
ELISA techniques optimized for minimal sample mass (10–30 mg) were developed and validated using samples from necropsied individuals (n=21).

The analytical panel included biomarkers related to the hypothalamic–pituitary–adrenal (HPA) axis (ACTH, aldosterone, GH, catecholamines –adrenaline, noradrenaline and dopamine–, corticosterone, cortisol and DHEA-S), inflammatory response (fibrinogen, LDH and SAA protein) and metabolic status (albumin, creatinine, urea and ferritin).

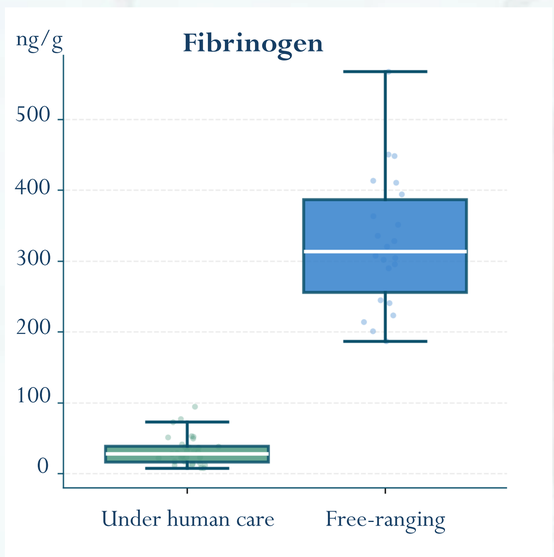
Reference intervals were established from clinically healthy adult dolphins under human care (n=38), following standardized sampling protocols during routine health checks (fasted animals, early morning sampling), and the panel was further explored in free-ranging dolphins (n=22) to assess its applicability for population health monitoring.

The results support the selection of a reduced biomarker panel integrating complementary physiological axes.

Inflammatory markers (SAA protein and fibrinogen) showed the highest consistency and discriminatory potential, suggesting a central role in detecting early population-level pathophysiological variation.

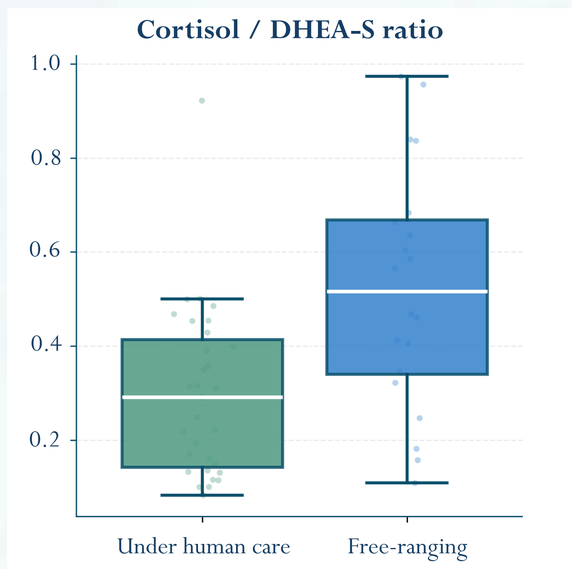


Reference interval (under human care): 184 – 690 ng/g



Reference interval (under human care): 8 – 95 ng/g

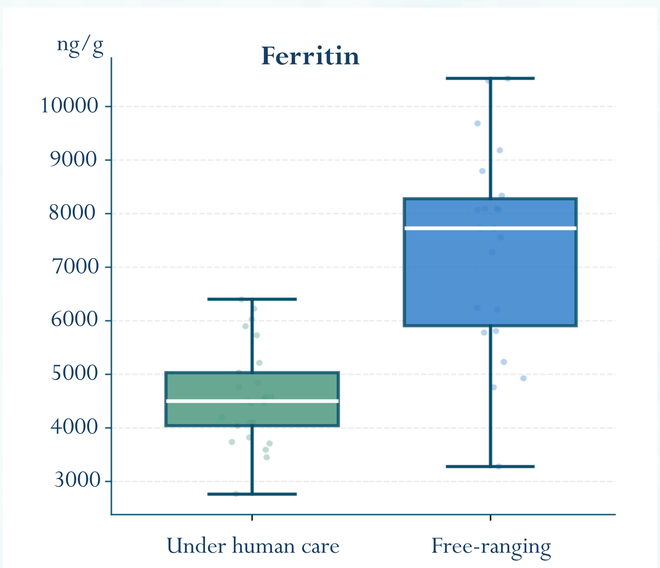
Hormonal markers showed high variability, limiting their standalone interpretability, whereas the cortisol/DHEA-S ratio provided a more stable and meaningful indicator of stress balance.



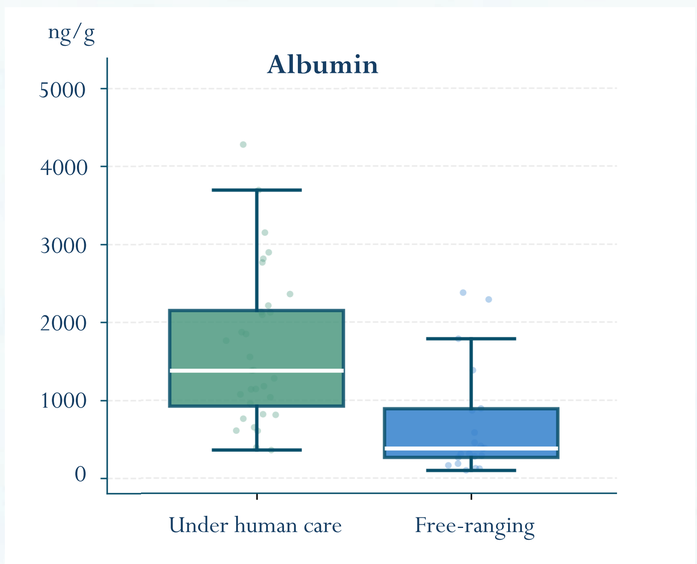
Reference interval (under human care): 0.09 – 0.92

Although ferritin and albumin were initially considered metabolic markers, their distribution patterns, inter-individual variability and responsiveness to inflammatory stimuli indicate a dual functional role. Ferritin behaved as a positive acute phase reactant, while albumin, as a negative acute phase protein, reflected broader shifts in physiological condition. This supports their interpretation as markers linking inflammatory and metabolic processes rather than belonging to a single functional category.

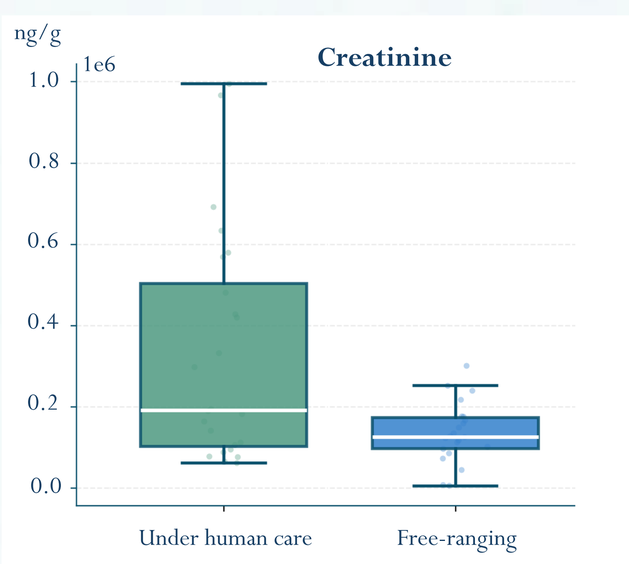
Metabolic condition, represented by creatinine, provided complementary insight into general physiological status, contributing a more stable and less inflammation-driven component within the overall assessment.



Reference interval (under human care): 2773 – 6497 ng/g



Reference interval (under human care): 400 – 4200 ng/g

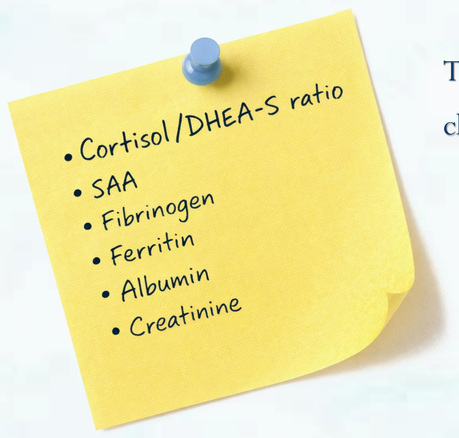


Reference interval (under human care): 62000 – 995000 ng/g

Other biomarkers (e.g., GH, LDH, urea or catecholamines) showed either high variability or limited specificity, reducing their suitability for inclusion in a reduced analytical panel.

A minimally invasive cutaneous biomarker panel is proposed for the assessment of health and welfare in bottlenose dolphin populations.

The panel is not intended for individual diagnosis, but as a population-level tool to detect deviations from a healthy reference baseline.



This approach supports a population-level health assessment framework to detect early pathophysiological changes and guide conservation management priorities at both population and habitat levels.

Further validation with larger sample sizes is recommended to strengthen its application.

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