The story of invasive algae, arginine, and turtle tumors does not make sense

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We are presenting a rebuttal letter to the following article that appeared recently on PeerJ: Van Houtan KS, Smith CM, Dailer ML, and Kawachi M. 2014. Eutrophication and the dietary promotion of sea turtle tumors. PeerJ 2:e602. This article is available at the following URL: https://peerj.com/articles/602/. We argue that the article lacks an inferential framework to answer the complex question regarding the drivers of the turtle tumor disease fibropapillomatosis in Hawaii. The article also contains procedural flaws and does not provide any compelling evidence of a link between algae, arginine, and turtle tumors.

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The story of invasive algae, arginine, and turtle tumors does not make sense

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In a recent paper in PeerJ (Van Houtan et al. 2014), the authors propose that eutrophication of marine ecosystems promotes growth of non-native algae that sequester excess nitrogen as arginine. The authors further propose that by consuming these algae, green turtles are developing herpes-induced tumors due to increased availability of arginine that is necessary for herpesvirus replication. This is a very appealing story that resonates with a broad audience (e.g. pollution is bad for the environment and is harming wildlife). However, scratch the surface, and the component parts of the story do not add up.

For example, the authors find that glycine, proline, and to a lesser degree, arginine, in tumors are elevated compared to skeletal muscle. They argue that "glycine..... is a building block for nucleic acids and is required in large amounts by rapidly proliferating cancer cells," that elevated proline "…may reflect the importance of proline for herpesvirus in counteracting cell defenses," and that turtles' ingestion of invasive algae is "...activating latent herpes infections and promoting tumors by foraging on arginine-enriched macroalgae." A more straightforward explanation for the difference in amino acid signatures is that fibropapilloma tumors comprise mainly connective tissues (collagen and fibroblasts) (Work et al. 2004), a tissue type highly enriched in glycine and proline relative to skeletal muscle (Nguyen & Zarkadas 1989). It is no surprise that connective tissue of skin tumors and skeletal muscle from the same animal have different amino acid profiles, and this difference likely has nothing to do with herpesviral replication or ingested arginine. In fact, replicating herpesviruses are extremely rare in tumors of turtles with fibropapillomatosis (FP) (Herbst et al. 1999) where the virus is mostly in a latent state (Work et al. 2009).

There is also no formal inferential framework to help disentangle what is clearly a complex issue. The authors argue that increasing eutrophication of coastlines has promoted persistence of FP by encouraging growth of arginine-laden invasive algae. True, some coastal areas of Hawaii are eutrophic, and indeed, invasive algae continue to be an ongoing ecological issue (Dailer et al. 2010). However, the authors fail to acknowledge that the prevalence of FP in green turtles in Hawaii has been on a secular decline since the mid-1990s (Chaloupka et al.

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2009), a trend that is confirmed by a decline in the percentage of turtles stranding with FP over time. The paper correlates arginine to the nitrogen footprint calculated by land use maps (Van Houtan et al. 2010), but does not correlate arginine with FP rates. The analyses in the paper do not take into account that amino acid and nitrogen levels in macroalgae can vary temporally (McDermid et al. 2007). Finally, the focus on arginine to the exclusion of other potential factors in disease pathogenesis seems imprudent. For instance, the invasive non-native alga *Hypnea musciformis*, suspected by the paper (Van Houtan et al. 2014) of playing an important role in tumors of sea turtles, contains abundant glutamic acid, an amino acid documented to play a role in tumor suppression (Li et al. 1998). One might speculate an equally plausible scenario in which ingestion of glutamic acid-laden invasive algae is somehow leading to the decline of FP in green turtles in Hawaii through the tumor-suppressing effects of glutamic acid.

It is likely that FP in green turtles, like all other wildlife diseases, has an environmental component (Herbst & Klein 1995). Understanding the pathogenesis of herpesvirus infection in turtles and how the environment influences development of fibropapillomas is important, and could help us potentially to devise ways to manage this disease. Unfortunately, this paper (Van Houtan et al. 2014) does not provide compelling evidence that algae, arginine, and tumors in sea turtles are causally linked.

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