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Comment on Watanabe et al. (2009)

In a recent paper (MPB 58:1447–1453), Watanabe et al. (2009) described the use of stable isotope (SI) ratios in soft tissues and shell from the manila clam to detect and trace anthropogenic wastewater. This manuscript is the 4th published work to describe measurement of stable isotope ratios in the organic portion of bivalve shell (Table 1), but only the 2nd to test the ability of bivalve shell material to detect and trace anthropogenic wastewater sources.

The new and timely nature of organic-based SI studies on bivalve shell demands careful consideration of past work to inform the reader and advance the science of method development and application that is still largely untested. Watanabe et al. (2009) missed an opportunity to make significant groundbreaking comparisons between their findings and others by not presenting or discussing their data in the context of past studies. Hence, the contribution of their work to the next generation of users is somewhat limited. We attempt to make some of the suitable comparisons here (Table 1), but most importantly, point out the needed background information and methodological details prerequisite to meaningful comparisons among studies.

Previous work (O'Donnell et al., 2003; Carmichael et al., 2008) provided evidence that SI techniques can detect shifts in anthropogenic N sources and trophic linkages recorded in bivalve shells. Important questions remain, however, regarding species- and location-specific variation in SI ratios in bivalve shell as well as the relationships between SI values in shell and soft tissue. These data are essential to apply SI values in shell as a proxy for soft tissues.

Based on initial studies, two basic types of information are needed. First, clear and thorough descriptions of shell processing methods and controls are necessary to determine the accuracy of reported SI values. Such information should include reporting the quantity of N analyzed by the IRMS and the methods used to determine whether reported SI values are absolutely rather than relatively accurate. A description of controls for potential handling effects and well-defined isotopic endpoints of comparison would allow readers to better evaluate accuracy among studies. Isotopic endpoints should be directly measured rather than assumed and may include comparison to different types of soft tissues, distinctly different food sources, or defined anthropogenic nutrient sources (such as wastewater compared to estuarine waters).

Second, data on species-specific variation are needed to determine biological relevance of reported values. For each new species studied it is beneficial to present aspects of structural and biochemical composition of the shell, physiology, and

ontogeny that may affect analyses. Differences in shell structure are typically associated with differences in amino acid composition in the organic portion of shell (Galtsoff, 1964; Fritz, 2001; Kobayashi and Samata, 2006), and the type of shell structure formed in bivalves can vary seasonally, with age, and between sites (Fritz, 2001). All of these factors can, in turn, alter SI ratios in target tissues (Michener and Lajtha, 2007; Lorrain et al., 2002).

Watanabe et al. (2009) reportedly analyzed N and C SI ratios in conchiolin from shell of the manila clam. Conchiolin is only one organic component of the shell matrix in many species (Kobayashi and Samata, 2006). Depending on shell structure and methods of organic extraction (typically acidification of whole shell powder), the organic portion analyzed is likely a mixture of organics, including conchiolin (Kobayashi and Samata, 2006). From the information presented by Watanabe et al. (2009) it is not possible to corroborate whether the organic fraction recovered was purely conchiolin. Such species-specific data are essential to understand differences in SI ratios among bivalves and determine their biological meaning.

Another important benefit to reviewing past research is to consider merits of past analyses. For example, it is well known that the SI ratios in different tissues within the same animal are correlated (Tieszen et al., 1983; Lorrain et al., 2002; Piola et al., 2006), including between shell and soft tissues (LeBlanc, 1989; O'Donnell et al., 2003; Carmichael et al., 2008). As yet, soft tissues are not known to determine SI values in shell. Hence, application of regression analysis, which defines a causal relationship between variables, can be misleading if applied to compare shell and soft tissue SI values. Watanabe et al. (2009) used regression analysis to compare SI values in shell and soft tissues of manila clams and concluded that a y-intercept at 0.17, although significantly different from 0, was negligible because instrument precision is typically 0.2. The actual mean difference between SI values in tissue and shell in their study, however, was $1.1 \pm 0.4\%$. This difference is approximately half of a trophic step, which could have important implications for future studies and should not be ignored. This mean difference is highly consistent with previous reports (Table 1), a corroborating point that was not captured in the analysis by Watanabe et al. (2009).

To make reliable comparisons of SI ratios among species and across studies, it is crucial that authors take care to thoroughly describe their methods, studies species, and targeted tissues. This information is particularly important for technical methods that are in their infancy and have implications for broader ecological interpretations. These data can then be aligned across studies to better inform the composite understanding of the method, application, and results. We hope that other authors will consider these points.

Table 1

Summary of studies that measured $\delta^{15}\text{N}$ values in the organic portion of bivalve shell, including the mean difference between $\delta^{15}\text{N}$ values (\pm standard error) in soft tissue and shell, type of soft tissue used for comparison, age class of bivalves sampled, and method of shell processing for each study. Positive $\delta^{15}\text{N}$ values reported here represent lighter $\delta^{15}\text{N}$ values in shell compared to soft tissues.

Species	$\delta^{15}\text{N}_{\text{tissue-shell}}$	Soft tissue	Age class	CaCO ₃ removal method	Source
<i>Ruditapes philippinarum</i>	1.1 \pm 0.4**	Whole tissues	NR	Acidification	Watanabe et al. (2009)
<i>Mercenaria mercenaria</i>	2.4 \pm 0.3	Whole tissues	Juveniles, adults	Acidification	Carmichael et al. (2008)
<i>Mercenaria mercenaria</i>	1.0 \pm 0.8**	Adductor muscle	Juveniles, adults	Acidification	O'Donnell et al. (2003)
<i>Mercenaria mercenaria</i>	0.7 \pm 0.8**	Foot	Juveniles, adults	Acidification	O'Donnell et al. (2003)
<i>Mercenaria mercenaria</i>	0.2 \pm 0.7**	Mantle	Juveniles, adults	Acidification	O'Donnell et al. (2003)
<i>Artica islandica</i>	2.7*	Whole tissues	NR	Dialysis, acidification	LeBlanc (1989)
<i>Mytilus edulis</i>	-0.1 \pm 0.2	Whole tissues	NR	Dialysis, acidification	LeBlanc (1989)

* Single value.

** Calculated from reported means; for O'Donnell et al. (2003), values represent the mean of data combined from juveniles and adults.

References

- Carmichael, R.H., Hattenrath, T., Valiela, I., Michener, R.H., 2008. Nitrogen stable isotopes in the shell of *Mercenaria mercenaria* trace wastewater inputs from watersheds to estuarine ecosystems. *Aquat. Biol.* 4, 99–111.
- Fritz, L.W., 2001. Shell structure and age determination. In: Kraeuter, J., Castanga, N. (Eds.), *Biology of the Hard Clam*. Elsevier, New York.
- Galtsoff, P.S., 1964. The American oyster *Crassostrea virginica*. *Fish. Bull.* 64, 1–480.
- Kobayashi, I., Samata, T., 2006. Bivalve shell structure and organic matrix. *Mater. Sci. Eng. B* 26, 692–698.
- LeBlanc, C., 1989. Terrestrial input to estuarine bivalves as measured by multiple stable isotope tracers, Ph.D. Thesis, McMaster University, Ontario.
- Lorrain, A., Paulet, Y., Chauvaud, L., Savoye, N., Donval, A., Saout, C., 2002. Differential $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures among scallop tissues: implications for ecology and physiology. *J. Exp. Mar. Biol. Ecol.* 275, 47–61.
- Michener, R., Lajtha, K., 2007. *Stable Isotopes in Ecology and Environmental Science*, second ed. Wiley-Blackwell, Cambridge, Massachusetts.
- O'Donnell, T., Macko, S., Chou, J., Davis-Hartten, K., Wehmiller, J., 2003. Analysis of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$ in organic matter from the biominerals of modern and fossil *Mercenaria* spp. *Org. Geochem.* 34, 165–183.
- Piola, R., Moore, S., Suthers, I., 2006. Carbon and nitrogen stable isotope analysis of three types of oyster tissue in an impacted estuary. *Estuar. Coast. Shelf Sci.* 66, 225–266.
- Tieszen, L., Boutton, T., Tesdahl, K., Slade, N., 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: Implications for $\delta^{13}\text{C}$ analysis of diet. *Oecologia* 57, 32–37.
- Watanabe, S., Kodama, M., Fukuda, M., 2009. Nitrogen stable isotope ratio in the manila clam, *Ruditapes philippinarum*, reflects eutrophication levels in tidal flats. *Mar. Pollut. Bull.* 58, 1447–1453.

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