

# Distinguishing between farmed and wild-caught black tiger Prawns, *Penaeus monodon*, using stable isotopes

## Abstract

Determining farmed from wild-caught seafood is important to alleviate public health concerns associated with food safety as well as combatting food fraud. This study applied stable isotope analysis (SIA) to determine provenance of farmed and wild-caught black tiger prawns, *P. monodon*. Isotopic analysis showed that wild *P. monodon* were significantly enriched in  $\delta^{13}\text{C}$  ( $p < 0.0001$ ) compared to those that were farmed. The results of this case study suggest SIA can be used effectively to differentiate between farmed and wild-caught black tiger prawns, and potentially to determine the production method for other seafood commodities.

**Keywords:** aquaculture, prawns, stable isotopes, provenance

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Karthik Gopi,<sup>1</sup> Debashish Mazumder,<sup>2</sup> Neil Saintilan,<sup>3</sup> Jesmond Sammut<sup>1</sup>

<sup>1</sup>Centre for Ecosystem Science, The University of New South Wales, Australia

<sup>2</sup>Australian Nuclear Science and Technology Organisation, Australia

<sup>3</sup>Department of Environmental Sciences, Macquarie University, Australia

**Correspondence:** Debashish Mazumder, Australian Nuclear Science and Technology Organisation, Locked Bag 2001, Kirrawee DC, Australia, Email debashish.mazumder@ansto.gov.au

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## Introduction

The United Nations predicted the global population to reach between 8.1 billion and 10.6 billion by 2050.<sup>1</sup> With the increase in global population, there will be a commensurate increase in food consumption. Seafood has been recommended as a component of a nutritional diet by many authorities because it is considered an essential source of omega-3 fatty acids, protein, vitamins and iodine.<sup>2,3</sup> The importance of seafood is highlighted by its high market value, which is around \$94 billion USD annually. With seafood consumption increasing globally, and with 44% of the world's fisheries being fully to heavily exploited, the only sustainable solution is the use of aquaculture to meet demand.<sup>4</sup> Black tiger prawn (*Penaeus monodon*) is a commercially-valuable species farmed widely in Asian-Pacific countries because global demand is high, and the commodity, thus, lucrative.<sup>5</sup> However, intensification of prawn aquaculture has raised concerns over food safety due to presence of residual antibiotics, heavy metals, hormones and pathogens that present a risk to human health.<sup>6</sup> Banned substances, like antibiotics, have been found in farmed seafood imported into several countries.<sup>7</sup> Over recent years, there have been recurrent reports of substitution of expensive seafood products with cheaper commodities leading to what can be described as food fraud.<sup>8</sup> This illegal practice is of particular concern for the prawn industry because the commodity is often traded across a wide geographic area. Therefore, seafood, such as prawn, is immensely important not only to ensure public health and safety, but also for the sustainability of the industry, whether based on aquaculture or dependent on the wild fishery. Currently, seafood provenance identification relies on several techniques, with DNA most commonly used.<sup>9</sup> However, DNA profiling cannot ascertain production methods because the differences in the DNA of farmed and wild-caught prawns, and other seafood commodities, are unlikely to be significant.<sup>10,11</sup> Stable isotope analysis (SIA) has become an important technique in trophic ecology.<sup>12</sup> The stable carbon isotopes ( $^{13}\text{C}/^{12}\text{C}$ ) indicate the sources of nutrients, while

the nitrogen isotopes ( $^{15}\text{N}/^{14}\text{N}$ ) may indicate the trophic level of an organism in the food web. Because the isotopic values of a consumer are related to the composition of the diet,<sup>13</sup> the differences in such diets, stemming from changes in farming practices, would be reflected in the isotopic profile of consumer muscle. The aim of the case study is to apply stable carbon and nitrogen analysis to distinguish between farmed and wild-caught *P. monodon*.

## Materials and Methods

A total of 14 *P. monodon* (average 12cm), farmed (n=7) and wild-caught (n=7), were collected from subtropical Queensland in Australia. The farmed prawns were collected randomly from different ponds in the same farm. White muscle tissues of the prawns were oven-dried at ~60°C for 48 hours and homogenised to fine powder. Powdered samples were analysed in the isotope ratio mass spectrometer (EA-IRMS; Thermo Fisher Flash 2000 HT EA, Thermo Electron Corporation, U.S.A) at Australian Nuclear Science and Technology Organisation. A two-point calibration was employed to normalise the isotope data, utilising standards that bracket the isotope ratios of the samples being analysed. Both standard and quality control references were included in each run, including replicate analysis of samples. Results were precise (+1 SD) to 1% for both %C and %N and 0.3‰ for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . Values for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were expressed in parts per thousand or per mil (‰) deviations from standard values for the two elements and determined through standard analytical procedures.<sup>14</sup> As lipid content in the muscle tissue affects  $\delta^{13}\text{C}$  values [15], we employed the widely-used mathematical formula from Post, Layman<sup>16</sup> for normalisation adjustment if the C:N ratio was higher than 3.5:

$$\delta^{13}\text{C}_{\text{normalised}} = \delta^{13}\text{C}_{\text{untreated}} - 3.32 + 0.99 \times \text{C} : \text{N}$$

The results were then plotted and validated using an Analysis of variance (ANOVA). The results were analysed using R Studio version 1.0.143.<sup>17</sup>

## Results and Discussion

Stable carbon isotope value for wild *P. monodon* was enriched substantially (about 4.36‰) from farmed *P. monodon*, while the enrichment for  $\delta^{15}\text{N}$  was opposite to wild prawn, farmed prawn was 0.74‰ higher than wild-caught prawn (Table 1). The bi-plot (Figure 1) and ANOVA revealed that the farmed *P. Monodon* were significantly different from wild-caught specimens in terms of  $\delta^{13}\text{C}$  ( $p < 0.0001$ ) and  $\delta^{15}\text{N}$  ( $p < 0.00034$ ).

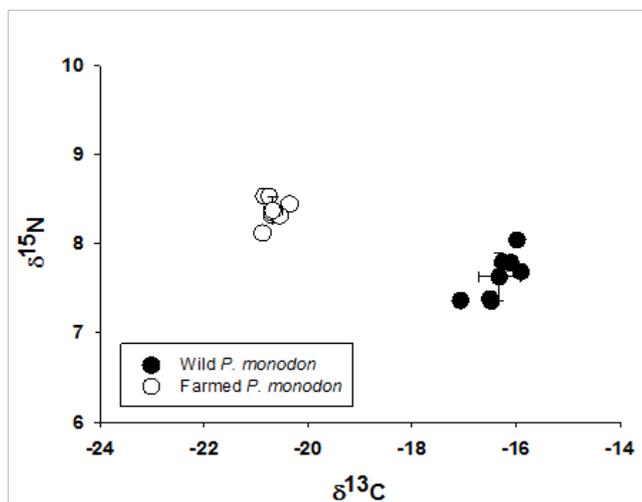
The results of this study clearly determined farmed from wild-caught prawns on the basis of stable isotopes. Other studies, which have focused on the use of SIA to discriminate between the aquaculture from the wild-caught prawns, have had similar results. For instance, Gamboa-Delgado, Molina-Poveda<sup>18</sup> found that wild-caught Pacific white shrimp had significantly enriched  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values when

compared to the farmed samples. Ortea & Gallardo<sup>19</sup> found that SIA was able to distinguish the production methods of seven different shrimp species. The enriched  $\delta^{13}\text{C}$  values of the wild-caught *P. monodon* are linked to their diet comprised of various components available in natural systems, such as benthic diatoms, phytoplankton, macroalgae and seston processed enriched  $\delta^{13}\text{C}$  values.<sup>20-22</sup> The higher  $\delta^{15}\text{N}$  values in the farmed *P. Monodon* can be attributed to the nitrogen enriched animal-based protein feed<sup>23</sup> consistently used under intense farming. The results of this case study suggest stable carbon and nitrogen analysis could prove to be an effective analytical tool to determine farmed from wild-caught prawns, and has potential for other seafood commodities. However, to increase resolution and predictability, SIA should be used in conjunction with other techniques such as elemental analysis, fatty acid profiling and DNA profiling.

**Table 1:** Stable carbon and nitrogen isotope values of farmed and wild-caught *P. monodon* from subtropical Queensland, Australia.

Type	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	Type	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
Farmed <i>P. monodon</i>	-20.69	8.31	Wild-caught <i>P. monodon</i>	-16.25	7.79
Farmed <i>P. monodon</i>	-20.69	8.35	Wild-caught <i>P. monodon</i>	-17.07	7.36
Farmed <i>P. monodon</i>	-20.36	8.44	Wild-caught <i>P. monodon</i>	-15.98	8.04
Farmed <i>P. monodon</i>	-20.85	8.53	Wild-caught <i>P. monodon</i>	-16.1	7.79
Farmed <i>P. monodon</i>	-20.55	8.31	Wild-caught <i>P. monodon</i>	-16.47	7.35
Farmed <i>P. monodon</i>	-20.88	8.12	Wild-caught <i>P. monodon</i>	-16.5	7.38
Farmed <i>P. monodon</i>	-20.75	8.53	Wild-caught <i>P. monodon</i>	-16.9	7.69
Mean ( $\pm$ s.d.)	-20.68(0.18) <sup>a</sup>	8.37(1014) <sup>b</sup>	Mean ( $\pm$ s.d.)	-16.32(0.39) <sup>a</sup>	7.63(0.26) <sup>b</sup>

**Note:** Column values with different letters indicate significant differences at  $P < 0.05$ .



**Figure 1** Stable isotope bi plot indicating individual and mean values of farmed (empty circle) and wild-caught (black circle) *P. Monodon*.

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## Conflicts of Interest

None.

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