ORIGINAL ARTICLE

Biology



Trophic state-dependent distribution of asari clam *Ruditapes philippinarum* in Japanese coastal waters: possible utilization of asari stable isotope ratios as a production indicator

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Received: 24 March 2022 / Accepted: 7 December 2022 / Published online: 9 January 2023 © The Author(s) 2023, corrected publication 2024

Abstract

Adult asari clams *Ruditapes philippinarum* were widely collected from 22 sampling sites including 20 clam fishery grounds in Japan in 2013 and 2014. Their δ^{15} N and δ^{13} C values were suggested to be an effective indicator of environmental trophic state: the stable isotopic signatures showed significant positive correlation with the 5-year (2010–2014)-mean values of total nitrogen (r=0.691, r=0.716) and chlorophyll *a* (r=0.643, r=0.789) calculated from the monitoring data collected by the Ministry of the Environment. Annual asari catch per unit area (CPUA) calculated from fisheries statistics scaled per unit area of each fishery ground showed significant positive correlations with trophic state parameters such as total nitrogen (r=0.721), chlorophyll *a* (r=0.800), δ^{15} N (r=0.732), and δ^{13} C (r=0.742), indicating a close relationship between asari catch and the trophic state of waters. Most clam fishery grounds in the Seto Inland Sea showed low trophic states and CPUA, indicating that recent poor asari production in these areas may be related to poor nutrient supply to the fishery environment.

Keywords Asari clam production $\cdot \delta^{15} N \cdot \delta^{13} C \cdot Stable isotope ratio \cdot$ *Ruditapes philippinarum* $\cdot Trophic state$

Introduction

Asari clam *Ruditapes philippinarum* catch in Japan has shown a drastic decrease from the 1980s, reflecting stock depletion: the annual catch of asari clams was 151,000 metric tons in 1983, 81,000 tons in 1989, 44,000 tons in 1996, and 9,000 tons in 2016 (Ministry of Agriculture Forestry and Fisheries Japan Statistics Department 2017). Many factors

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Yoshitake Takada takada_yoshitake41@fra.go.jp have been suggested to explain asari stock depletion, including overfishing, reclamation of tidal flats, infectious disease, environmental deterioration, and increase of predatory fishes (Kakino 1991; Hamaguchi 2011; Tezuka et al. 2014; Toba 2017; Waki et al. 2018). Among them, various researchers have traditionally nominated overfishing as a significant factor to explain the decrease of asari stock. However, it seems difficult to attribute the decrease of asari stock after

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the 2000s to overfishing. Asari clam is a relatively inexpensive fishery product and fishing activity is easily abandoned if the asari stock level decreases below the break-even point of cost performance. For example, at Nakatsu tidal flat, a former major asari fishery grounds in the Seto Inland Sea, asari fishing activity has been scarcely conducted over the last decade because it is difficult for the fishers to catch five individual adult asari a day (M. Uchida, unpublished observations obtained from a field study conducted at Nakatsu fishery ground during 2002-2017). On the other hand, the number of asari spats observed is not small, even where fishing has been abandoned (M. Uchida, unpublished observations obtained from a field study conducted at Nakatsu fishery ground during 2002-2017). Nevertheless, restoration of the asari stock has not been observed at all (Kimura 2012; Ministry of Agriculture Forestry and Fisheries Japan Statistics Department 2017). Another commonly nominated possible cause is reclamation of tidal flats; however, most reclamation projects had finished by the year 2000, after which the decline persisted in Japan (The Association for the Environmental Conservation of the Seto Inland Sea 2019). After comprehensive analysis of individual factors that could cause a negative impact on asari stock, an increasing number of asari researchers are wondering whether change of environmental conditions in the fishery grounds such as global warming, decrease of food supply, and increase of predator fishes are possible significant causes of the asari stock depletion (Toba 2017; Fujiwara et al. 2020; Kamohara et al. 2021).

The present study focuses on the possible involvement of the deterioration of water trophic state in asari stock depletion. Eutrophication induced by anthropogenic activities was widespread in Japanese coastal waters in the period 1960-1980 (The Association for the Environmental Conservation of the Seto Inland Sea 2019). Subsequently, nutrient load from land to coastal waters has been regulated in areas adversely affected by eutrophicated waters, such as places where red tides have caused serious damage to fish aquaculture (The Association for the Environmental Conservation of the Seto Inland Sea 2019). Regulation of chemical oxygen demand (COD) level began in 1979, followed by regulation of nutrient load of nitrogen and phosphate from 2001. As a result, nutrient levels have decreased in coastal waters such as the Seto Inland Sea (Tada et al. 2014), Mikawa Bay (Kamohara et al. 2021) and Tokyo Bay (Kubo et al. 2019). For example, total nitrogen (T-N) and total phosphate (T-P) levels have decreased by 27% and 38%, respectively, with a 58% decrease in chlorophyll a (Chla) level compared with the levels found 20 years ago in Mikawa Bay (Kamohara et al. 2021). However, the decrease of nutrients has led to a decrease in primary production, which was suggested to be causing a reduction in coastal fisheries production (Yamamoto 2003). As for asari clams, the decrease of primary

production means a decrease of available food such as phytoplankton (Yokoyama et al. 2005b), and this is expected to result in a negative impact on asari production (Flye-Sainte-Marie et al. 2007). Fujiwara et al. (2020) reported that soft body weight and the condition index of asari clams were strongly correlated with the mean T-N level of the water. Kamohara et al. (2021) observed that Chla level from June to November had a large influence on asari soft body weight, and suggested that Chla level in 2007-2017 in Mikawa Bay was not sufficient for sustaining the reproduction of asari clams. Information on time-course nutrient decrease and the resulting negative impact on asari production has been accumulating recently in individual local coastal waters. However, there is no study, to the best of our knowledge, investigating the relationship between the eutrophic state of the water and asari production, based on the data collected nationwide in Japan.

The objective of the present study was to investigate the possible relationship between the trophic state of the water and asari production, based on data over a large geographic extent. The unique approach of the present study is to use information on stable isotope ratios (SI) of asari clams as possible indicators of the water trophic state such as T-N and Chla: it is known that nitrogen and carbon SI (δ^{15} N and δ^{13} C) of organisms reflect the feeding log of the organisms and their SI reflect those of the food (Kasai et al. 2016). Therefore, SI analysis is a useful tool for investigating the food web system (Yokoyama et al. 2005b; Komorita et al. 2014). The δ^{15} N value of asari clams is influenced by nitrogen sources of terrestrial origin such as agricultural fertilizer and denitrification-processed sewage (Macko and Ostrom 1994). It was observed that the $\delta^{15}N$ value of asari clams had a positive correlation with dissolved inorganic nitrogen (DIN) of coastal waters, suggesting that asari clam $\delta^{15}N$ could be a trophic state indicator of fishery a ground under the influence of anthropogenic nutrient load (Watanabe et al. 2009b). It is known that eutrophic water elevates primary production (Fujiwara et al. 2020), and a higher growth rate of phytoplankton results in an increase of δ^{13} C of the phytoplankton by shortage of aqueous CO₂ (Finlay 2004). An increase in the δ^{13} C value of particulate organic matter has been confirmed during phytoplankton blooms under experimental conditions (Nakatsuka et al. 1992). Takahashi et al. (1992) also observed an increase in the δ^{13} C content of particulate organic matter during a red tide in eutrophic Mikawa Bay. These observations suggest that the δ^{13} C levels of phytoplankton and asari clams could be a possible indicator of the trophic state of the water. On the other hand, it is known that the δ^{13} C value of clams is influenced by various other factors such as feeding on terrestrial origin organic materials (Kasai et al. 2016) and the salinity of the water (Sato et al. 2006). Furthermore, the δ^{13} C value of asari clams is influenced by type of microalgae they feed on: benthic microalgae have a higher δ^{13} C value than planktonic microalgae (Yokoyama et al. 2005b). It is also known that feeding contents of asari clam change depending on growth size, and affects asari SI values (Watanabe et al. 2009a). Therefore, for SI comparison, asari clam samples should be collected in a standardized manner in terms of sampling location, season, and shell size.

In the present study, asari clams were widely collected from Japanese coastal waters, and the relationship between the stable isotope ratios $\delta^{15}N$ and $\delta^{13}C$ of the clams and the water trophic state (T-N and Chla) were statistically analyzed. Correlation of asari SI values with total phosphate (T-P) was not investigated in this study because T-P is reported to show high correlation with T-N (Fujiwara et al. 2020). As the $\delta^{15}N$ and $\delta^{13}C$ values of the asari clams showed positive correlations both with T-N and Chla of water, respectively, correlations between tentative trophic state indicators of $\delta^{15}N$ and $\delta^{13}C$, as well as indicators of T-N and Chla, and the asari production level obtained from government statistical data, were analyzed.

Materials and Methods

Sampling Site Information

Asari clams were collected at 22 sites including 20 clam fishery grounds in Japanese coastal waters (Fig. 1, Table 1). Mean levels of T-N and Chla of waters at these sampling sites were estimated based on water-monitoring data reported by the Ministry of the Environment (https://



Fig. 1 Sampling sites (Nos.1–22) of asari clams for stable isotope ratio analysis. The asari clams were collected in summer (June to early September) of 2013 (Sites 4, 6, 9, 12, 18) and 2014 (1–3, 5–11, 13–17, 19–22)

www.env.go.jp/water/suiiki/index.html, May 1, 2021). There are two types of monitoring data sets provided by the Ministry of the Environment: the data set collected at T-N monitoring sites (named Koukyouyousuiiki) and the data set collected at T-N and Chla monitoring sites (named Kouikisougou). Approximate distances from the asari sampling sites were 2.6 km on average, ranging from 0.2 km to 7.1 km for each T-N monitoring site [Online resource 1(a)], and 7.2 km on average, ranging from 2.3 km to 20.3 km for each T-N and Chla monitoring site [Online resource 1(b)]. Because there are more T-N monitoring sites than T-N and Chla monitoring sites, T-N data at the T-N monitoring sites closest to the asari sampling sites were used to calculate the mean T-N levels for each asari sampling site. As for Chla level, Chla data in the T-N and Chla monitoring sites closest to the asari sampling sites were used to calculate the mean Chla level at each asari sampling site. There were no monitoring sites for Chla at Akkeshi, Miyako, Hamamatsu, Miyazu, Isahaya, Unzen, or Kumamoto, so the Chla data of these areas were not used for the correlation analysis in the present study. Monitoring data from spring 2010 to winter 2014 were used to calculate the mean values of the time scales 0.25 years, 0.5 years, 1 year, 2013-2014, 2012–2014, and 2010–2014: for example, for the cases of asari samples collected in June 2013, parameters of the mean trophic state (T-N, Chla) were calculated from data sets of April-June (2013), January-June (2013), July (2012)-June (2013), March (2012)-February (2014), and March (2010)-February (2014) for time scales of 0.25 years, 0.5 years, 1 year, 2012-2014, and 2010-2014, respectively [see Online resources 1(a) and 1(b) for individual data used for the calculation].

Asari Clam Collection

Five individual asari clams were collected from the tidal flat area of each clam fishery ground in the summer season from June to early September of 2013 (Sites 4, 6, 9, 12, 18, Fig. 1, Table 1) and 2014 (Sites 1–3, 5–11, 13–17, 19–22, Fig. 1, Table 1). Adult individuals (shell length > 25 mm) were collected in all sites except for 3 cases (Kisarazu 18.0, 19.8, 19.8, 19.8, 20.8 mm, Asaguchi 15.2, 24.5 mm, and Kumamoto 17.6, 20.1, 20.7 mm) where only small-sized individuals were collected. The collected clams were transported cool or frozen to our laboratory, Hatsukaichi Station. The foot muscle (ca. 0.1 g wet weight) of clams was cut out, rinsed twice with 10 ml of distilled water, lyophilized, and analyzed for stable isotope ratios. The sample was not defatted because fat content in the muscle part was negligible.

Site No.	Sampling of asari clam (Fig. 1)		The nearest water monitoring sites (Figs. 3, 4, 5, 6 and 7 and		Tidal flats/fishery grounds used for cal-	Clam fishery statistics used for calculating CPUA at
	City (site)	Date (years– months)	T–N ^a	Chla ^b	culating CPUA at each sampling site (Fig. 8) ^c	each sampling site (Fig. 8) ^d
1	Akkeshi (Akkeshi)	2014–6	ST-1	(No site)	Akkeshi (No. 77–82)	Akkeshi-cho
2	Miyako (Tsugaruishi- kawa)	2014–6	S-7	(No site)	Iwate Pref. total	Iwate Pref. (Other bivalves)
3	Kisarazu (Banzu)	2014-8	Chibaken30	Chibaken30	Futtsu-shi (Futtsukokita, Futtsukouen)	Futtsu
4	Yokohama (Uminokouen)	2013-8	Hirakatawannai	Kanagawa41	Uminokouen	Uminokouen
5	Hamamatsu (Washizu)	2014–7	Washizu	(No site)	Shizuoka Pref. total	Shizuoka Pref
6(1)	Nishio (Isshiki) 2013	2013-9	K-7	Aichiken52	Aichi Pref. total	Aichi Pref
6 (2)	Nishio (Isshiki) 2014	2014–7	K-7	Aichiken52	Aichi Pref. total	Aichi Pref
7	Nishio (Yahagi-river)	2014–7	K-7	Aichiken52	Aichi Pref. total	Aichi Pref
8	Miyazu (Monju)	2014-6	Monjujisaki	(No site)	Kyoto Pref. total	Kyoto Pref
9 (1)	Ako (Karafune) 2013	2013-8	Akositoubuoki	Hyogoken168	Karafune	Ako
9 (2)	Ako (Karafune) 2014	2014–7	Akositoubuoki	Hyogoken168	Karafune	Ako
10	Asaguchi (Yorishima)	2014–6	Yorishimaoki	Okayamaken248	Yorishima-cho (No. 32–34)	Asaguchi-shi
11	Onomichi (Momoshima)	2014–6	Hiroshimaken312	Hiroshimaken312	Onomichi-shi (No. 53–82)	Onomichi
12	Hatsukaichi (Maegata)	2013–7	Hiroshimawan18	Hiroshimaken411	Hatsukaichi (Hatsukai- chimaegata, Tatara)	Hatsukaichi-shi
13	Iwakuni (Monzen)	2014-7	ED-101	Hiroshimaken423	Iwakuni	Iwakuni
14	Kumage (Hirao)	2014-7	AD-4	Yamaguchiken498	Suounadahigashi	Yamaguchi, Hofu, Shunan
15	Shunan (Toda)	2014–6	TD-16	Yamaguchiken550	Suounadahigashi	Yamaguchi, Hofu, Shunan
16	Hofu (Mukoushima)	2014–7	WD-7	yamaguchiken562	Suounadahigashi	Yamaguchi, Hofu, Shunan
17	Sanyoonoda (Habu)	2014–6	UD-6	Yamaguchiken613	Suounadanishi	Ube-shi, Onoda, Sanyo, Shimonoseki
18	Nakatsu (Nakatsu)	2013-7	SuSt-4	Fukuoka617	Nakatsu	Nakatsu
19	Isahaya (Nagato)	2014–7	Konagaiko	(No site)	Isahaya-shi (No. 28–47, 56–63, 146)	Isahaya-shi
20	Isahaya (Kama)	2014–7	Konagaiko	(No site)	Isahaya-shi (No. 28–47, 56–63, 146)	Isahaya-shi
21	Unzen (Mizuho)	2014-8	Tabirako	(No site)	Isahaya-shi (No. 28–47, 56–63, 146)	Isahaya-shi
22	Kumamoto (Midorikawa)	2014–9	St-9	(No site)	Kumamotoariake (18 tidal flas)	Kumamotoariake

Table 1 Asari sampling sites, the nearest water monitoring sites, corresponding tidal flats/fishery ground, and clam fishery statistics used for calculating asari catch per unit area (CPUA)

^aTotal nitrogen data (T-N) were collected from the nearest monitoring sites of Kouikisougou-chousa by the Ministry of the Environment.

^bChlorophyl *a* data (Chla) were collected from the nearest monitoring sites of Koukyoyousuiiki-chousa by the Ministry of the Environment.

^cTidal flat areas corresponding to each asari sampling site are cited from a government report (Nature Conservation Bureau, Environmental Agency and Marine Park Center of Japan 1994) and used for calculating CPUA. The fishery ground area was used instead of tidal flat area in the case of Hamamatsu

^dAsari clam catch data corresponding to each asari sampling site are cited from fisheries statistics (Ministry of Agriculture, Forestry and Fisheries Japan Statistics Department 2017) and used for calculating the CPUA

Stable Isotope Ratio Analyses

The ¹⁵ N and ¹³C compositions of the samples were determined using a mass spectrometer (Thermo Fisher Scientific DELTA V coupled with Flash EA1112). Results are expressed in the standard δ unit notation as $\delta X = [(R \text{ samples/R reference}) - 1] \times 10^3$, where X is ¹³C or ¹⁵ N and $R = {}^{13}C/{}^{12}C$ for carbon and ¹⁵ N/ 14 N for nitrogen (Yokoyama and Fujiwara 2019). The standard reference materials were IAEA sucrose ANU for carbon and IAEA N1 for nitrogen.

Estimation of Annual Asari Clam Catch per Unit Area (CPUA)

Annual asari clam catch per unit area (CPUA) was calculated using the data from fisheries statistics of asari clam catch (Ministry of Agriculture, Forestry and Fisheries Japan Statistics Department 2017) after being scaled by dividing by the tidal flat area (Nature Conservation Bureau, Environmental Agency and Marine Park Center of Japan 1994) for each asari sampling site. Individual data used for the calculation are shown in Online resource 2. There were some exceptional cases for obtaining more accurate data as follows. For the case of Kisarazu, the data of the asari clam catch gathered for analysis were suspected to be an extremely low estimate (Online resource 2), based on the survey data by Iseri et al. (2016), and information given by asari researchers of Chiba Prefecture (Y. Kobayashi and M. Toba, personal communications). So, the CPUA of Futtsu area [4844 tons/(km²·years)] was used instead of that of Kisarazu area (197 tons/km²/years) for the statistical analysis. As for the case of Yokohama (Uminokouen), which is an asari clam fishing ground only used for leisure, with no fishery statistics, the annual asari clam catch data is cited from a report by Kanagawa Prefecture (Kanagawa Prefecture, Environmental Agricultural Bureau, Fisheries Division 2011). The asari clam distribution in Yokohama (Uminokouen) was reported only for 2010, so this figure was used to represent the CPUA for all the periods in 2010–2014. For the area data for clam fishery grounds, the area of the whole tidal flat was basically used to calculate the CPUA. In the case of Hamamatsu, because the major clam fishery grounds are located in subtidal areas in Lake Hamana, area data of the actual fishery ground was estimated from the Shizuoka Prefectural Report (Shimomura 2013). Our study mainly used the tidal flat area data, except in the case of Lake Hamana, to calculate the CPUA, as it was difficult to collect area data of individual fishery grounds from the 22 local governments or fishery cooperatives. To check the suitability of the use of the tidal flat area data for calculating the CPUA, the CPUA was also calculated using the actual fishery ground area data for the sites of Nishio (tidal flat area and subtidal zone or subtidal zone of Isshiki), Ako (fishery ground of Karafune), and Kumamoto (total of Kumamoto-Ariake fishery grounds), respectively (Online resource 2). For the case of Nishio, CPUA 2010-2014 calculated from the prefectural total asari clam catch and prefectural total tidal flat area was 707 tons/ (km²·year). The fishery ground area of Isshiki was 26.5 km² (Yamada 2016), comprising 10 km² of tidal flat area (Sasaki 1998) and 16.5 km² of subtidal zone. Asari clam fishing in the Isshiki tidal flat area is mainly for recreational clam digging and is not reflected in the statistics of the Isshiki asari clam catch. The CPUA 2010-2014 calculated from the Isshiki asari clam catch and fishery ground area of 26.5 km²

or 16.5 km² is 487 tons/(km²·years) or 782 tons/(km²·years), respectively. The present study adopted an intermediate value of 707 tons/(km²·years) (CPUA of Aichi Prefectural total) for analysis in Fig. 8 and Fig. 9. For the case of Ako, the CPUA 2010-2014 calculated from the Hyogo Prefectural total values of asari clam catch and tidal flat area unit was 8.7 tons/(km²·years), while that calculated from Karafune's asari clam catch and fishery ground unit of 0.25 km² (Yasunobu H 2007, personal communication) was 1.7 tons/ (km²·years). However, the CPUA 2010–2014 of Karafune's fishery ground unit was calculated based on asari clam catch for only 2012 and 2013 because the fishery was not opened in 2010, 2011, or 2014. Considering the inaccuracy of the mean asari clam catch value of Karafune, the prefectural total CPUA 2010–2014 was used for analysis in Fig. 8 and Fig. 9. For the case of Kumamoto, the CPUA 2010-2014 calculated from Kumamotoariake's asari clam catch for the tidal flat area (38.70 km²), and fishery ground (22.76 km²) (Fisheries Division, Department of Forestry and Fisheries, Kumamoto Prefecture 1991) was 13 tons/(km²·years) and 23 tons/(km²·years), respectively. This difference in CPUA 2010–2014 did not give a statistically different result on the relationship between water trophic state related parameters and the CPUA 2010-2014 (data not shown).

Statistical Analyses

Outlier data of isotopic ratios obtained from asari clams were detected by the quartile deviation method (Excel 2021 version 2206, Microsoft), after calculating the distance of each data point from a linear regression equation, and eliminated before correlation analysis. Pearson's correlation coefficients (*r*) were estimated between trophic state indicators (T-N, Chla), stable isotope ratios (δ^{15} N and δ^{13} C), and asari production level. The statistical significance of the correlations was tested, and coefficients of linear regression were estimated. These statistical analyses were done by Ystat2000 (version 2000, Igakutosho Shuppan).

Results

Trophic States of Asari Clam Fishery Grounds

Mean T-N and Chla values at each asari sampling site were calculated for time scales of 0.25 years, 0.5 years, 1 year, 2013–2014, 2012–2014, and 2010–2014. The mean T-N values of 2010–2014 were high (over 0.3 mg/l) for Hokkaido (Akkeshi), Tokyo Bay (Banzu, Yokohama), Lake Hamana (Hamamatsu), Mikawa Bay (Nishio-Isshiki, Nishio-Yahagi-River), Miyazu Bay (Miyazu), and Ariake Sea (Isahaya, Unzen, Kumamoto), while low (below 0.3 mg/l) for Tohoku (Miyako), and most of the Seto Inland Sea sites

(Ako, Asaguchi, Onomichi, Hatsukaichi, Iwakuni, Kumage, Shunan, Hofu, Sanyoonoda, and Nakatsu). The mean Chla values of 2010–2014 were high (over 5 µg/l) for Tokyo Bay (Kisarazu, Yokohama), Lake Hamana (Hamamatsu), Mikawa Bay (Nishio-Isshiki, Nishio-Yahagi-River) and Seto Inland Sea (Hatsukaichi), while low (below 5 µg/l) for most of the Seto Inland Sea sites (Ako, Asaguchi, Onomichi, Iwakuni, Kumage, Shunan, Hofu, Sanyoonoda, and Nakatsu). The calculated mean values of T-N and Chla were used for the following correlation analysis.

Correlation Between Nutrient Level (T-N) and Primary Production Level (Chla) of Waters

The correlation between water T-N level and Chla level was examined using the data at T-N and Chla monitoring sites nearest to the asari clam fishery grounds. Five-year means 2010–2014 of T-N and Chla showed a significant positive correlation (r=0.758, p < 0.01, Fig. 2a), showing that primary production at each site is closely related to water nutrient level represented by the T-N level. Similar correlation analysis was also conducted between T-N data at the nearest T-N sites and Chla data at the nearest T-N and Chla sites, and a positive correlation was found (r=0.835, p < 0.001, Fig. 2b), suggesting that difference of trophic levels at the T-N site and the T-N and Chla site is limited.



Fig. 3 Correlation between δ^{13} C and δ^{15} N of asari clams. Each plot point shows mean ± SD (n=5) of 24 field samples. There were two outlier points (*1 Asaguchi and *2 Onomichi) which led to a decreased correlation coefficient. It is reported that the δ^{15} N value is comparatively high also for the case of *Ulva* spp. (Kobayashi and Fujiwara 2015) and the influence of a nitrogen source having high δ^{15} N value from the terrestrial zone is suspected. These two points were not used for statistical analyses in Figs. 4, 6, or 9c

Stable Isotope Ratio of Asari Clams

SI (δ^{15} N and δ^{13} C) of the asari clams were analyzed and plotted on a graph (Fig. 3). The mean values of δ^{15} N ranged from 5.7% (Nakatsu) to 15.5% (Asaguchi). The mean values of δ^{13} C ranged from -22.2% (Nakatsu) to





Fig. 2 Relationship between the two water trophic state parameters, mean total nitrogen (T-N) and mean chlorophyll a (Chla) in 2010–2014. The T-N data were obtained from (**a**) T-N and Chla monitoring sites, or (**b**) T-N monitoring sites located nearest to the asari sampling sites, individually. For both cases, the Chla data were obtained from

the Chla monitoring sites. The number of T-N and Chla monitoring sites were more limited than the T-N monitoring sites. The T-N and Chla data are cited from the online database of the Ministry of the Environment, Government of Japan

-15.0% (Uminokouen). The values of individual δ^{15} N and δ^{13} C showed a significant positive correlation (r = 0.489, p < 0.05). There were two outlier data from Asaguchi (δ^{15} N 15.5%, δ^{13} C -18.8%) and Onomichi (δ^{15} N 14.0%, δ^{13} C

Fig. 4 Relationship between water trophic state (T-N) and $\delta^{15}N$ of asari clams. Each $\delta^{15}N$ data point shows mean \pm SD (n=5) of 22 field samples. The asari clam samples were collected in 2013 and 2014. The T-N data are a means of (a) 3 months (0.25 years), (**b**) 6 months (0.5 years), (**c**) 12 months (1 year) before the collection day of asari clams, or means of (d) 2013–2014, (e) 2012-2014, and (f) 2010-2014. The T-N data are cited from the online database of the Ministry of the Environment, Government of Japan, measured at the nearest T-N monitoring sites

Fig. 5 Relationship between water trophic state (T-N) and δ^{13} C of asari clams. Each δ^{13} C data point shows mean ± SD (n=5) of 24 field samples. Methods for collecting the asari samples and calculating the mean T-N level are the same as in Fig. 4



-18.5%), which reduced the correlation coefficient, and these two data were excluded in the following correlation analyses.

Correlation Between Nutrient Level (T-N) of Waters and Stable Isotope Ratio of Asari Clams

Results of correlation analyses between nutrient level (T-N of waters) and SI of asari clams are shown in Fig. 4 (δ^{15} N, n=22) and Fig. 5 (δ^{13} C, n=24). The correlation was significant between the mean T-N level (0.5 years, 1 year, 2013–2014, 2012–2014, 2010–2014) of waters and δ^{15} N of clams (Fig. 4). The correlation coefficient was higher for the mean T-N values calculated based on longer time period data: means of 2010–2014 (r=0.691, p<0.001), 2012–2014 (r=0.620, p<0.01), 2013-2014 (r=0.588, p<0.01), 1 year (r=0.538, p<0.01), and 0.5 years (r=0.511, p<0.05). The positive correlation was also significant between the mean T-N level of waters and δ^{13} C of clams (Fig. 5). The mean T-N level had a tendency to have a high correlation coefficient for δ^{13} C of asari clams when calculated based on longer time period data: means of 2010–2014 (r = 0.716, p < 0.001), 2012–2014 (r = 0.664, p < 0.001), 2013–2014 (r=0.650, p<0.001), 0.5 years (r=0.582, p<0.01), and 0.25 years (r=0.561, p<0.01), except in the case of 1-year mean of T-N (r = 0.542, p < 0.01).

Primary Production Level (Chla) of Waters and Stable Isotope Ratio of Asari Clams

Results of correlation analyses between primary production level (Chla) of waters and SI of asari clams are shown in Fig. 6 (δ^{15} N, n = 14) and Fig. 7 (δ^{13} C, n = 16). The mean Chla level of waters and $\delta^{15}N$ of clams showed significant correlation for five cases of 0.25 years, 0.5 years. 2013-2014, 2012-2014, 2010-2014, out of six cases (Fig. 6). The correlation coefficients of mean Chla levels for δ^{15} N of asari clams were: r = 0.643 for 0.25 years (p < 0.05), r = 0.546 for 0.5 years (p < 0.05), r = 0.562 for 2013–2014 (p < 0.05), r = 0.729 for 2012–214 (p < 0.01), and r = 0.656for 2010–2014 (p < 0.05). The mean Chla level of waters and δ^{13} C of clams showed significant correlation for four cases of 0.25 years, 2013-2014, 2012-2014, 2010-2014, out of six cases (Fig. 7). The mean Chla level had a tendency to have a high correlation coefficient for $\delta^{13}C$ of asari clams when calculated based on longer term data: means of 2012-2014 (r=0.805, p < 0.001), 2010-2014 (r=0.789, p < 0.001), and 2013–2014 (r = 0.581, p < 0.05), although the 0.25-years mean of Chla also showed a significant correlation (r = 0.575, p < 0.05).

Calculation of CPUA for Fisheries Grounds in Japan

Annual asari clam catch per unit area of tidal flat or fisheries ground (CPUA) was calculated and is shown in Fig. 8. The CPUA was high [>100 metric tons/(km²·year)] at Akkeshi [195 tons/(km²·year)], Kisarazu [3273 tons/(km²·year)], Yokohama [4470 tons/(km²·year)], Hamamatsu [3228 tons/ (km²·year)], Nishio [754 tons/(km²·year)], and Hatsukaichi [318 tons/(km²·year)], and low [<100 metric tons/ (km²·year)] at Miyako, most of the Seto Inland Sea sites (Ako, Asaguchi, Onomichi, Iwakuni, Kumage, Shunan,

Fig. 6 Relationship between water trophic state (Chla) and $\delta^{15}N$ of asari clam. Each $\delta^{15}N$ data point shows mean \pm SD (n=5) of 14 field samples. The Chla data are cited from the online database of the Ministry of the Environment, Government of Japan, measured at the nearest T-N and Chla monitoring sites. Methods for collecting the asari samples and calculating the mean Chla level are the same as in Fig. 4



Fig. 7 Relationship between water trophic state (Chla) and δ^{13} C of asari clams. Each δ^{13} C data point shows mean \pm SD (n=5) of 16 field samples. Methods for collecting the asari samples and calculating the mean Chla level are the same as in Fig. 6



Fig. 8 Comparison of asari catch per unit area (CPUA) among the major clam fishing grounds in Japan. The CPUA was calculated from the annual asari catch statistics of 2010-2014 after being divided by the tidal flat area size, except for the case of Hamamatsu. For the case of Hamamatsu, the major fishery ground is Lake Hamana and the clam fishery ground area data were used instead of the tidal flat area data for calculation. As for the case of Yokohama, the CPUA

Hofu, Sanyoonoda, Nakatsu), and the Ariake Sea sites (Isahaya, Kumamoto).

Correlation Between Trophic State Indicators of Waters and CPUA

Besides the T-N and Chla levels of waters, $\delta^{15}N$ and $\delta^{13}C$ values of asari clams were also tentatively regarded as

of 2010 is shown because asari catch data is available only for 2010 (Kanagawa Prefecture, Environmental Agricultural Bureau, Fisheries Division 2011). The tidal flat area data were cited from a governmental report (Nature Conservation Bureau, Environmental Agency and Marine Park Center of Japan 1994). The CPUA is shown on a logarithmic scale

trophic state indicators of waters. So, the mean value of these four parameters (T-N 2010-2014, Chla 2010-2014, δ^{15} N, δ^{13} C) and the mean asari catch of CPUA 2010–2014 was examined (Fig. 9a-d). The four indicators showed significant correlation with the CPUA 2010-2014: T-N 2010-2014 (r=0.721, p < 0.001, n = 19), Chla 2010-2014 $(r=0.800, p<0.01, n=13), \delta^{15}N (r=0.732, p<0.001, n=13)$ n = 21), and δ^{13} C (r = 0.742, p < 0.0001, n = 23). Ten sites

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Fig. 9 Relationship between asari catch per unit area [CPUA, tons/ (km²·year)] and water trophic state related indicators (**a**) T-N (n=19), (**b**) Chla (n=13), (**c**) δ^{13} C (n=23), and (**d**) δ^{15} N (n=21) of asari clams. Plots are fishery grounds of Seto Inland Sea (\bigcirc) and others (\bigcirc). The CPUA are calculated from the mean asari clam catch in 2010–2014 after being divided by the tidal flat area for each asari fishing ground area. The CPUA is plotted on a logarithmic scale

located in the Seto Inland Sea and connecting Nakatsu site (shown as open circles) are distributed in lower positions in Fig. 9a–d, suggesting sites of the Seto Inland Sea, including the connected area, have a low trophic state and poor CPUA, except the Hatsukaichi site.

Discussion

The major objective of the present study was to examine the possible correlation between the trophic state of waters and the richness of asari clam production. For this objective, an effective indicator representing the water trophic state needs to be developed. The monitoring data sets of T-N and Chla, collected by the Ministry of the Environment, are direct indicators representing the water trophic state. However, these indicators have several problems. First, the monitoring data is a snapshot measured only once in 1 to 3 months. Second, the distribution of water parameter monitoring sites were 2.6 km (T-N monitoring site) or 7.2 km (T-N and Chla monitoring site) away, on average, from the clam sampling sites, possibly reducing the predictability of water conditions in the sampling site. Third, it requires 1 to 2 years before the monitoring data become available to internet users. Accounting for these drawbacks, the present study examined the possibility of using SI of asari clams as an indicator of the water trophic state. It is known that the SI of asari clams reflects that of microalgae on which the asari clam has fed (Kasai et al. 2016). Half-life metabolic turnover values in freshwater bivalves were reported to be 12–23 days for carbon and 7–9 days for nitrogen (Kasai et al. 2016). For asari clams, the half-life turnover values have not been reported; however, it can also be assumed that the SI of asari clams also reflect that of microalgae on which they have fed for the past several weeks (Yokoyama et al. 2005a). Asari clam δ^{15} N has been suggested to be a candidate indicator of anthropogenic eutrophication, showing significant correlation with DIN concentrations in the coastal environment (Watanabe et al. 2009b). In the present study, asari clam δ^{15} N and δ^{13} C showed significant correlation with each other, but the δ^{15} N values at Momoshima and Fukuyama showed remarkable outlier data (Fig. 3). It is reported that the δ^{15} N value of *Ulva* spp. (green macroalgae) also shows elevated values at Momoshima and Fukuyama areas among coastal waters in the Seto Inland Sea (Kobayashi and Fujiwara 2015). Industrial sewage containing ¹⁵ N at a high percentage flowing from the Fukuyama industrial zone maybe the reason for increase of the δ^{15} N value of organisms in these water areas. Thus, the $\delta^{15}N$ values at Momoshima and Fukuyama were eliminated before statistical analysis. The asari clam δ^{15} N was observed to have a significant correlation with T-N and Chla of adjacent waters (Figs. 4 and 6) and can be tentatively regarded as a trophic state indicator. The δ^{13} C of asari clams is theoretically expected to be a suitable indicator for expressing the average values of T-N and Chla level of coastal waters, as mentioned in the Introduction. The present study observed that asari clam δ^{13} C is also a suitable indicator for expressing T-N and Chla levels in coastal waters. Moreover, values of T-N and Chla averaged for a longer period of time had a higher correlation with asari clam δ^{13} C, indicating that asari δ^{13} C can be a stable indicator for expressing the averaged trophic state of fishery grounds. Therefore, asari δ^{13} C may be useful as a tool for finding suitable aquaculture sites for phytoplankton-feeding animals such as bivalves.

The next objective of this study was to examine the possible correlation between water trophic state and asari clam production. The present study observed that trophic state indicators (T-N and Chla of water) and tentative trophic state indicators (asari clam δ^{15} N and δ^{13} C) had a significant correlation wirh CPUA (Fig. 9). These observations indicate that asari clam production richness primarily depends on the richness of the diet (i.e., Chla level), which correlates with the water trophic state. It can be supposed that a shortage in food supply will result in a decrease of nutritional conditions (expressed as the condition index) of asari and therefore results in various negative effects on asari survival from environmental stress, disease infection, and reproduction (Hattori et al. 2021). The present study was not conducted as a time course analysis on the relationship between the water trophic state and asari clam production. However, it is well known that the nutrient load from land to water

has been regulated and the trophic state is in a decreasing trend in Japanese coastal waters (Tada et al. 2014: Kamohara et al. 2021; Kubo et al. 2019). Therefore, it is not difficult to imagine that the decrease in water trophic state has produced a negative impact on asari clam production (Matsuno et al. 2005; Fujiwara et al. 2020; Kamohara et al. 2021). It is noteworthy that many of the studied fishery grounds in the Seto Inland Sea showed both a lower trophic state and poor CPUA among the studied areas (Fig. 9), suggesting that the recent remarkable decreasing trend of asari clam production in the Seto Inland Sea is related to water quality regulation, resulting in the decrease of primary production and hence the diet for asari clams. Among the investigated areas, the asari fishing industry is managed by fishery cooperatives at Akkeshi [195 tons/(km²·year)], Kisarazu [3273 tons/(km²·year)], Hamamatsu [3228 tons/(km²·year)], Nishio [754 tons/(km²·year)], and Hatsukaichi [47 tons/(km²·year)]. Based on this, the minimum resource level necessary for profitable asari fishing industry is tentatively suggested as a 50-ton/(km²·year) level. The trophic state indicators at asari production of 50 tons/(km²·year) were estimated to be 0.34 mg/l (T-N), 5.91 μ g/l (Chla), -17.4% (δ^{15} N) and 10.0% (δ^{13} C). The 50-ton/(km²·year) level is supposed to be an underestimate compared with the actual minimum resource level necessary for a profitable asari fishing industry because this estimate is calculated based on tidal flat area including non-fishery grounds. Future study is necessary to clarify a more accurate value of the minimum asari resource level necessary for profitable fisheries using data from asari fishery ground areas instead of tidal flat areas. As for the positive correlations between CPUA and asari clam δ^{15} N and δ^{13} C, it may also be a result of the asari assimilating benthic algae with higher values of $\delta^{15}N$ and $\delta^{13}C$ compared with planktonic algae. For example, Yokoyama et al. (2005b) observed 8.9% δ^{15} N and -14.7% δ^{13} C for benthic algae and 7.3% $\delta^{15}N$ and -19.0% $\delta^{13}C$ for POM. Therefore, the increase of asari clam $\delta^{15}N$ and $\delta^{13}C$ can be explained by a higher proportion of benthic algae in the diet of asari clams at high CPUA fishery grounds. Contrasting studies show that the contribution of benthic algae to estuary production is large (Kasim and Mukai 2006; Yoshino et al. 2012; Komorita et al. 2014) or small (Yokoyama et al. 2005b). However, the idea that the contribution of benthic algae is larger at high CPUA tidal flats was not supported by the fact that as ari clam $\delta^{15}N$ and $\delta^{13}C$ are rather small at Nakatsu where the fishery ground is a shallow tidal flat and where resuspension of benthic algae is supposedly high, and high at Hamamatsu where the fishery ground is submerged and resuspension of benthic algae is supposedly low. Further study is necessary to identify the contribution level of benthic algae to the diet of asari clam.

In conclusion, this manuscript reported on two topics. First, $\delta^{15}N$ and $\delta^{13}C$ values of asari clams may be used as a

convenient indicator of the coastal water trophic state when sampling of asari clams is conducted in a standardized manner. Second, asari clam production level is closely related to water trophic state represented by T-N and Chla levels, and asari clam δ^{15} N and δ^{13} C values may be used as a proxy for asari production. Based on these results, it seems that management of water nutrient levels is a significant element for sustainable clam fisheries.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12562-022-01663-5.

Acknowledgements We are grateful to Drs. or Mrs. Shigeru Taga, Masahiko Matsuda, Fumiyasu Murayama, Tsuguhito Shimomura, Ryosuke Ohhata, Hiroshi Hirauchi, Yuichiro Fujinami, Atsushi Ito, and some members of prefectural Fisheries Research Institutes and Fishery Cooperative Organizations for kind corporation in collecting asari clam samples. We thank Ms. Kaori Taura for collecting statistical data for asari catch and water quality. We also thank the reviewers for making many critical comments and helpful advice. This research was funded by a research project for the development of a biodiversity index in fishing grounds and their environments from the Fisheries Agency, Japan. Chris Norman improved the English text.

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