



Effect of seasons and fishing ban period on umami-related and functional components of greeneye from Japanese coast

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1 **Title**

2 Effect of seasons and fishing ban period on umami-related and functional components of
3 greeneye (*Chlorophthalmus albatrossis*) from Japanese coast

4

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18 **Abstract**

19 Greeneye (*Chlorophthalmus albatrossis*) is a small fish with a mild white flesh and texture that
20 can be eaten with bones. In this study, we aimed to clarify the effect of season and fishing ban
21 period of greeneyes in terms of umami-related and functional component values by analyzing
22 their chemical components. Greeneyes from the Japanese coast were analyzed between March
23 2023 and February 2024, excluding the fishing ban period on June 2023. Free amino acids and
24 nucleic acid-related substances were analyzed as components associated with umami-related
25 components. The free amino acid level was the highest (3619.13 ± 719.54 $\mu\text{g/g}$ wet sample) in the
26 spring before the fishing ban, while the inosine monophosphate level in autumn was the highest
27 (3884.28 ± 666.56 $\mu\text{g/g}$ wet sample). Therefore, the umami taste was higher in spring and autumn.
28 The levels of functional components (lipid content, fatty acids, vitamin E, and imidazole
29 dipeptides) increased following the implementation of the fishing ban. Calcium levels were
30 approximately 5 mg/g sample, with no significant differences observed in all seasons; the level
31 was comparable to other fish consumed with bones or alternative high-calcium food sources.
32 Regardless of the season, the cooking method of consuming entire greeneyes, including bones,
33 allows for a stable year-round intake of calcium. In conclusion, greeneyes can be considered a
34 stable source of calcium and fish throughout the year, with varying umami-related and functional
35 components depending on the season.

36

37 **Keywords:** greeneye; fishing ban; umami; Free amino acids; inosine monophosphate; calcium

38 1. Introduction

39 Greeneye is a demersal fish belonging to the *Chlorophthalmidae* family, with the
40 standard Japanese name “Mehicali” *Chlorophthalmidae albatrossis*. Its distribution around Japan
41 extends from Honshu to the Kyushu Pacific coast and from Japan to the Palau Trench (Sakaji et
42 al., 2006). The greeneye species are demersal fish distributed across the continental shelf and
43 upper slope in tropical to temperate waters of all major oceans (Kimura et al., 2024). This group
44 currently comprises 20 valid species and several others that remain undescribed, and substantial
45 amounts of species are caught by bottom trawling and are important fishery resources (Kimura et
46 al., 2024). The Japanese species is *C. albatrossis*, while *C. agassizi* is distributed in the
47 Mediterranean and Atlantic Oceans (Anastasopoulou et al., 2006), *C. brasiliensis* is distributed in
48 Mexico (CHI-ESPÍNOLA et al., 2023), *C. nigromarginatus* is distributed in the Indo-West Pacific
49 (Zhang et al., 2016), and *C. proridens* is distributed in the central North Pacific (Fujiwara et al.,
50 2019). These species are monoecious and exhibit epipelagic development. Adult specimens
51 demonstrate schooling behavior and sedentary habits, predominantly inhabiting muddy substrates,
52 where they can occur at significant densities (D’Onghia et al., 2006). Although the spawning area
53 remains unknown, it is believed that there is a spawning ground in the southern sea areas of Japan
54 and that larvae are transported near Japan by the Kuroshio Current (Sakaji et al., 2006). After
55 settling, they grow and migrate to deeper waters. Between August and February, individuals with
56 body lengths of 4-5 cm settle on the seabed at depths of 150-200 m. By May and June, they grow
57 to 7.5-8.0 cm and move to depths of 200-300 m. After one year of settling, they grow to 12.5-
58 13.0 cm and move to depths of 300-350 m (Sakaji et al., 2006). It is believed that they mature
59 afterward, although no records of mature individuals have been collected.

60 In Japan, greeneyes are mainly caught in the Aichi, Fukushima, and Miyazaki
61 Prefectures. In Aichi Prefecture, the catch remained stable between 300 and 500 tons from 2011

62 to 2020. The Gamagori Fisheries Promotion Council is applying to register "Gamagori
63 Greeneyes" as a regional collective trademark (Aichi Prefecture, 2022). The regional collective
64 trademark promotes to strengthen bonds of a whole region or members, and improve a brand
65 consciousness, advertising image of foods, and services for the local fishing industry.
66 Furthermore, the regional collective trademark also promotes environmental conservation and
67 planned catch to secure fish resources. In Fukushima Prefecture, the catch from 2011 to 2014 was
68 low, and in recent years, it has remained under 100 tons (Fukushima Prefecture, 2023). In Iwaki
69 City, Fukushima, greeneye was designated the "City Fish" in 2001 (Iwaki City, 2016). In
70 Miyazaki Prefecture, the catch was approximately 200 tons from 2010 to 2015 but increased to
71 over 300 tons in 2016, reaching 250 tons by 2022. The resources of greeneye are stable, and the
72 increase in catches since 2016 is not due to an increase in resources, but to an increase in demand
73 in the Japanese market. This is the same trend as in the Aichi and Fukushima prefectures
74 mentioned above (Miyazaki Prefecture, 2023). Historically, in the Miyazaki area, greeneyes
75 caught with deep-sea shrimp bottom trawls were considered miscellaneous fish and used as food
76 for fishermen or as feed for aquaculture. However, their mild white flesh and soft texture have
77 attracted considerable attention. Throughout the year, greeneyes are mainly caught by bottom
78 trawling along the coast of Miyazaki. However, fishing is banned from May to June to protect
79 juvenile yellowtail from spawning and bycatch. They land almost year-round, with the peak
80 season occurring in July and August (Miyazaki Prefecture, 2023). Locally, they are popular in
81 homes and restaurants, and there is a growing movement to recognize greeneye as a special
82 fishery product in Miyazaki Prefecture. It primarily lands in Nobeoka City and Miyazaki City in
83 Miyazaki Prefecture. According to data from 2023, the market price of greeneye is approximately
84 650–850 yen per kg. This price tends to be higher before and after the fishing ban period and
85 summer season (Nobeoka city, 2023).

86 As previously mentioned, while there are studies on the ecology of greeneye and its
87 economic evaluation as a food ingredient, there is little research regarding its nutritional and
88 functional components in the context of food science. Mizuno et al., (2009) reported that free
89 amino acid, fatty acid, and mineral components of greeneyes from various regions in Japan on
90 September and October. Vijayan et al. (2016) also reported that free amino acid and mineral
91 components of greeneyes from India. However, these reports have not yielded results indicating
92 seasonal changes in these components. Along the Miyazaki coast, greeneyes are predominantly
93 captured through bottom trawling operations throughout the year; however, fishing activities are
94 prohibited from May to June to safeguard juvenile yellowtail during spawning and mitigate
95 bycatch. Therefore, it is possible that the umami related and functional components of greeneyes
96 changed before and after the fishing ban period. If fishermen comprehend these seasonal
97 variations, they can modify harvest timing to optimize fish quality, potentially facilitating more
98 sustainable fishing practices. For consumers, this information could be used to inform their dietary
99 choices, enabling them to consume greeneye fish. However, there are no reports indicating
100 seasonal changes in the umami-related and functional components of greeneyes in Miyazaki or
101 other regions and countries.

102 This study aimed to analyze the umami related and functional components of greeneyes
103 from Miyazaki over the course of one year. In fish products, the components that contribute to
104 umami taste are free amino acids and nucleic acid-related compounds. Furthermore, the functional
105 components are fatty acids such as EPA and DHA, and vitamin E, which has antioxidant
106 properties. Imidazole dipeptides, which have anti-fatigue effects, are also attracting attention.
107 Greeneyes are typically prepared by removing only the head and viscera, with subsequent
108 consumption of the entire body, including bones. Consequently, greeneyes may have provided a
109 higher calcium content than other fish species or alternative food sources. By clarifying the

110 relationship between the umami-taste related components (free amino acids and nucleic acid-
111 related compounds) and functional components (fatty acids, vitamin E, imidazole dipeptides, and
112 calcium) and seasonal variations with spawning periods, we intend to further highlight the umami-
113 related and functional component value of greeneyes to promote their sales.

114

115 **2. Materials and Methods**

116

117 *2.1. Chemicals*

118

119 In this experiment, chloroform, methanol, hexane, acetone, acetonitrile, diethyl ether,
120 sodium chloride, pyrogallol, sodium hydroxide, potassium hydroxide, ethyl acetate, 60%
121 perchloric acid, phosphoric acid, ammonium acetate, acetic acid, ammonia water, 1 mol/L
122 hydrochloric acid (all manufactured by FUJIFILM Wako Pure Chemical Corporation, Japan,
123 Osaka), 14% BF₃-methanol solution (FUJIFILM Wako Pure Chemical Corporation), EDTA 2NA
124 (Dojindo Laboratories, Japan, Osaka), AccQ-Tag chemistry package (Waters Corporation, United
125 States, Milford), 8-quinolinol, phthalein complexone (both manufactured by Tokyo Chemical
126 Industry Co., Ltd. , Japan, Tokyo), 2,2'-iminodiethanol (special reagent grade, FUJIFILM Wako
127 Pure Chemical Corporation), polyvinylpyrrolidone K25 (special grade, FUJIFILM Wako Pure
128 Chemical Corporation), diethylamine (special grade, FUJIFILM Wako Pure Chemical
129 Corporation), heptadecanoic acid, and distilled water (Takasugi Pharmaceutical Co., Ltd. , Japan,
130 Osaka) were used.

131

132 *2.2. Sample*

133

134 Greeneyes used in this analysis were caught by commercial vessels off shore of Kitaura,
135 Miyazaki Pref. between March 2023 and February 2024 and provided by the Kitaura Fisheries
136 Cooperative Association in Miyazaki Prefecture. The fish were collected monthly; however, no
137 samples were available for June 2023 because of the fishing ban in Miyazaki Prefecture. Ten to
138 fifteen samples were provided, and five samples were randomly selected for analysis. The sample
139 sizes collected were of medium size (10 to 15 cm body length), which is the predominant size on
140 the market. After measuring the body weight (g) and total length (cm) of the selected samples,
141 only the head and internal organs were removed, considering the commercial form. Then, the
142 condition factor ($1,000 \times (\text{body weight} / \text{body length}^3)$) was calculated. Subsequently, the samples
143 were finely and uniformly processed using an Extreme Mill (Waring, United States, Torrington)
144 and stored at $-30\text{ }^{\circ}\text{C}$ until analysis.

145

146 *2.3. Analysis of lipid-soluble component*

147

148 *2.3.1. Lipid extraction*

149 Lipid extraction was performed according to the method described by Folch et al.,
150 (1957). Three grams processed each sample was precisely weighed into a 50 mL centrifuge tube.
151 A mixture of chloroform/methanol (2:1, v/v) 30 mL was added, followed by homogenization
152 using a homogenizer (PT-DA12/2EC-D148, Kinematica, Switzerland, Lucerne). Subsequently, 4
153 mL of water was added, and the mixture was stirred using a multi-shaker (MMS-12OH, Tokyo
154 Rikakikai, Japan, Tokyo) before being centrifuged at $1,200 \times g$ for 10 min (KN-70 centrifuge,
155 KUBOTA, Japan, Osaka). The upper layer was removed using an aspirator and the lower layer
156 was transferred to a stoppered flask through cotton wool filtration. The lower layer was washed
157 with Chloroform/Methanol (2:1, v/v). The collected solution was concentrated using an

158 evaporator (N-110, Tokyo Rikakikai Co., Ltd) to approximately 5 mL. The solution was then
159 transferred to a 10 mL volumetric flask and adjusted with Chloroform/Methanol (2:1, v/v). This
160 solution was transferred to a brown bottle and stored in a freezer (-30°C) to obtain a lipid solution.
161 Additionally, a portion of this lipid solution was used to determine the lipid content.

162

163 2.3.2. Fatty acid content

164 The fatty acid levels were determined according to the method described by Tanaka et
165 al., (2014). Briefly, lipid solutions containing 3 mg of lipid and 1 mg of heptadecanoic acid (as an
166 international standard) were hydrolyzed with 750 μ L of 0.5 mol/L potassium hydroxide in
167 methanol in a screw-cap tube at 100°C for 9 min using a block heater. The reaction mixture was
168 then added to 1 mL 14% Boron Trifluoride in methanol and incubated at 100°C for 7 min. Next,
169 3 mL of n-hexane and 2.5 mL of saturated sodium chloride solution were added to the mixture,
170 which was vortexed and centrifuged for 10 min at 2500 \times g. The upper n-hexane layer, which
171 mostly contained fatty acid methyl esters (FAME), was transferred to a Sep-Pak Silica column
172 630 mg (Waters) that had been prewashed with n-hexane, and the FAME was eluted with 8 mL
173 of n-hexane/diethyl ether (96:4, v/v). The eluted solution was then evaporated to dryness using a
174 centrifugal concentrator, dissolved in 50 μ L of dichloromethane, and 2 μ L of the solution was
175 injected for gas-liquid chromatography (GLC) analysis. The GLC system included a gas
176 chromatograph (GC-14A; Shimadzu Co., Kyoto, Japan) equipped with a flame ionization detector
177 and capillary column (TC-70, 60 m \times 0.25 mm i.d., GL Science, Tokyo, Japan). The column
178 temperature was programmed for a linear increase of 1°C /min from 180 to 230°C, and the
179 temperature of the injection and detector ports was 250°C. The GLC response was integrated by
180 the HPLC integrator system CDS-Lite (LA soft Ltd, Chiba, Japan). FAME were identified on the
181 chromatogram by conventional methods using the retention time of the standards (Supelco 37

182 Component FAME Mix, Merck. Tokyo, Japan). The FAME content was determined using the
183 internal standard method with a known quantity of heptadecanoic acid.

184

185 2.3.3. *α-tocopherol content*

186 *α*-Tocopherol levels were analyzed according to the method described by Ishimaru et
187 al., (2016). Briefly, an extracted lipid sample (3 mg) was placed in a glass screw-cap tube and 50
188 μ L of 1% sodium chloride, 1 mL of 3% pyrogallol in ethanol, and 100 μ L of 60% potassium
189 hydroxide were added. The mixture was incubated at 70°C for 30 min using a block heater. After
190 heating, 2.25 mL of sodium chloride solution and 3 mL of n-hexane/ethyl acetate (90:10, v/v)
191 were added and the mixture was stirred and centrifuged at $2500 \times g$ for 10 min. The upper layer
192 was transferred to another tube and evaporated to dryness using a centrifugal concentrator. For
193 HPLC analysis, the residue was dissolved in 200 μ L n-hexane/ethyl acetate (80:20 v/v).

194 The HPLC analytical instruments used included a PU-2080 Plus pump, 630-CO column
195 oven (JASCO Corporation, Japan, Kyoto), 7725i injector (RHEODYNE Corporation, United
196 States, California), and 821-FP fluorescence detector (JASCO Corporation). Data processing was
197 performed using the CDS-Lite ver. 5.0 (LA Soft Corporation). The detector was programmed for
198 excitation wavelength (Ex) at 298 nm and emission wavelength (Em) at 325 nm from 0 to 17.0
199 min for the detection of *α*-tocopherol, and Ex at 342 nm and Em at 476 nm from 17.0 to 22.0 min
200 for the detection of retinol. An Inertsil NH₂ 5 μ m (4.6 mm \times 250 mm; GL Science) column was
201 used, with an n-hexane/ethyl acetate (85:15, v/v) mobile phase and a flow rate of 1.0 mL/min.
202 The fluorescence response was integrated using an HPLC integrator system, CDS-Lite. *α*-
203 Tocopherol and retinol levels were quantified using a calibration curve of authentic *α*-tocopherol
204 levels. The calibration curve parameters, including the regression equation, correlation coefficient,
205 and linear range, are presented in the supplementary material (Appendix A Table S1).

206

207 *2.4. Analysis of water-soluble component*

208

209 *2.4.1. Water-soluble component extraction*

210 Water-soluble components were extracted according to the method described by
211 Ishimaru et al., (2016). The water-soluble components comprise low-molecular-weight chemical
212 substances that are soluble in water and do not include high-molecular-weight components such
213 as proteins and polysaccharides or lipid-soluble chemical substances. Approximately 3 g of each
214 greeneye sample was accurately weighed into a 50 mL plastic centrifuge tube (62.548.004,
215 Sarstedt AG & Co. KG, Germany, Niedersachsen), and 20 mL of 1 mol/L perchloric acid solution
216 was added. The mixture was homogenized and centrifuged at $3,000 \times g$ for 10 min. The
217 supernatant was transferred to another 50 mL plastic centrifuge tube, and 11 mL of 2 mol/L
218 Potassium Hydroxide solution was added. The tubes were tightly sealed and vortexed. After
219 incubating in a refrigerator (0°C) for 30 min, it was centrifuged again at $1,200 \times g$ for 10 min.
220 The supernatant was collected by filtering through a cotton plug into a 50 mL volumetric flask,
221 and the flask was rinsed with water. The volume was adjusted to 50 mL with water, and
222 approximately 5 mL of this solution was filtered again using a $0.8 \mu\text{m}$ membrane filter (DISMIC-
223 25CS ADVANTEC Disposable Membrane Filter Unit, Toyo Roshi Kaisha, Ltd., Japan, Tokyo).
224 The filtered solution was stored in a plastic container (Sarstedt AG & Co. KG) and used as extract.
225 The extracts were stored in a freezer (-30°C).

226

227 *2.4.2. Nucleic acid-related substances content*

228 Nucleic acid-related substances were analyzed using the method described by Ishimaru
229 et al., (2016). The extraction solution (section 2.4.1) was used as the sample. Calibration solutions

230 of various concentrations were analyzed, and quantification was performed using the calibration
231 curve method. The nucleic acid-related substances were analyzed using an HPLC system that
232 included an LC-10AD pump, DGU-14A degasser, SIL-10ADVP autosampler, CTO-10A column
233 oven, SPD-10A UV-VIS detector, and SCL-10A integrator (all manufactured by Shimadzu
234 Corporation). This system was equipped with a CAPCELL PAK UG120 column (250 nm× 4.6
235 mm, 5- μ m particle size, Shiseido, Japan, Tokyo) connected to a guard column containing Nova
236 Pak-C18 (Waters Corporation). The mobile phase consisted of solutions A [water: triethylamine:
237 phosphoric acid, 950:10:5 (v/v); pH 3.8] and B [water: acetonitrile, 90:10 (v/v)]. The gradient
238 employed was 0-20 min, isocratic at 0 % B; 20-35 min, 0 % B to 35 % B; 35-45 min, isocratic at
239 35 % B. The flow rate was 1.0 mL/min. The samples were injected at intervals of 55 min. Nucleic
240 acid-related substances levels were quantified using a calibration curve of authentic nucleic acid-
241 related substances levels. The calibration curve parameters, including the regression equation,
242 correlation coefficient, and linear range, are presented in the supplementary material (Appendix
243 A Table S1).

244

245 2.4.3. Free amino acid content

246 Free amino acid analysis was conducted according to the method of Tanaka et al., (2016)
247 using the AccQ-Tag chemistry package (Waters Corporation). In an Eppendorf tube, 140 μ L of
248 AccQ-FBB solution was added, followed by 20 μ L of the sample or calibration solution, and 40
249 μ L of AccQ-Fluor Reagent solution. The mixture was then vortexed. It was then reacted at 55°C
250 for 10 min in a water bath (SB-13, AS ONE Corporation, Japan, Osaka), after which 800 μ L of
251 water was added to create the amino acid analysis solution, which was analyzed by HPLC.
252 Calibration solutions of various concentrations were analyzed, and quantification was performed
253 using the calibration curve method. The analytical instruments used included an LC-10AD pump,

254 DGU-14A degasser, SIL-10ADVP autosampler, CTO-10A column oven, RF-10XL fluorescence
255 detector, and SCL-10A integrator (all manufactured by Shimadzu Corporation). Data processing
256 was performed using the CDS Lite ver. 5.0 (LASOFT). The analytical conditions were as follows:
257 the column used was a Nova-Pak C18 4 μm 3.9 i.d. \times 300 mm (Waters Corporation), with a flow
258 rate of 1.0 mL/min, column temperature of 40°C, and fluorescence detector set to Ex 250 nm and
259 Em 395 nm. A gradient program was applied for elution, with the mobile phases as follows:
260 Solution A 0.133 mol/L sodium acetate buffer solution (pH 6.32); Solution B, acetonitrile;
261 Solution C: Milli-Q water; and Solution D, 0.133 mol/L sodium acetate buffer solution. The
262 gradient program started with A/B/C/D at 100/0/0/0, changed to 57/3/0/40 at 35 min, 0/5/0/95 at
263 75 min, 0/10/0/90 at 85 min, 0/10/10/80 at 100 min, and 0/15/10/75 at 110 min, and maintained
264 the same ratio until 120 min. At 121 min, the ratio changed to 0/60/40/0, maintained until 130
265 min, and then changed back to 100/0/0/0 at 131 min, and this ratio was maintained until the end
266 of the analysis at 140 min. After the analysis, the system was returned to 100% Solution A
267 stabilized under the initial conditions for 20 min before the next analysis. Free amino acid levels
268 were quantified using a calibration curve of authentic amino acid levels. The calibration curve
269 parameters, including the regression equation, correlation coefficient, and linear range, are
270 presented in the supplementary material (Appendix A Table S1).

271

272 *2.4.4. Imidazole dipeptides content*

273 The imidazole dipeptides were analyzed according to the method described by Mori et
274 al., (2018). Calibration solutions of various concentrations were analyzed, and quantification was
275 performed using the calibration curve method. The extraction solution (section 2.4.1) was used as
276 the sample. The HPLC system used included an LC-10AD pump, DGU-14A degasser, SIL-
277 10ADVP autosampler, CTO-10A column oven, SPD-10A UV-VIS detector, and SCL-10A

278 integrator (all manufactured by Shimadzu Corporation). This system was equipped with a PC
279 HILIC column (250 mm × 4.6 mm, 5 mm particles, Shiseido). The mobile phase consisted of a
280 15 mmol/L ammonium acetate solution (pH 4.25): acetonitrile: methanol (3.5:5.85:0.65 by
281 volume). The flow rate was 1.0 mL/min, and the analysis time was one sample per 40 min. Data
282 processing was performed using the CDS Lite ver. 5.0 (LASOFT). Imidazole dipeptide levels
283 were quantified using a calibration curve of authentic imidazole dipeptide levels. The calibration
284 curve parameters, including the regression equation, correlation coefficient, and linear range, are
285 presented in the supplementary material (Appendix A Table S1).

286

287 *2.5. Calcium content*

288

289 Calcium content was analyzed according to the methods described by Suzuki et al.,
290 (1990) and Nishiguchi et al., (2007). One gram of sample was accurately weighed into a 50 mL
291 plastic centrifuge tube, and 20 mL of 0.1 mol/L hydrochloric acid was added. The mixture was
292 homogenized using a homogenizer (POLYTRON PT-MR2100, Central Scientific Commerce Inc.,
293 Tokyo, Japan), and 10 mL of 0.1 mol/L hydrochloric acid was added. Extraction was performed
294 in a water bath (Shaking Bath SB-13-AXEL, AS ONE Corporation) at 30 °C for 1 h. After
295 extraction, the mixture was centrifuged at 3500 × g for 15 min using a tabletop centrifuge (Model
296 4000; KUBOTA Corporation). The supernatant was filtered through a cotton filter and transferred
297 to a 50 mL volumetric flask, and the volume was made up to 50 mL with 0.1 mol/L hydrochloric
298 acid. This solution was stored in a plastic container and used as the calcium extraction solution.
299 A 50 μL aliquot of the appropriately diluted extraction solution was measured in a 15 mL plastic
300 test tube (Sarstedt K.K). Then, 5 mL of water and 0.5 mL of ortho-cresolphthalein complexone
301 reaction solution (a mixture of 4 mg of phthalein complexone, 25 mL of 2,2'-iminodiethanol, 25

302 mL of diethylamine, 100 mg of polyvinylpyrrolidone K25, and 125 mg of 8-quinolinol) were
303 added. The mixture was thoroughly stirred and incubated in a water bath at 30 °C for 10 min.
304 After the reaction, the solution was transferred to a 4 mL plastic cell (Sarstedt K.K.), and the
305 absorbance was measured at 570 nm using a UV-visible spectrophotometer (UV-1280, Shimadzu
306 Corporation). Calcium content was quantified using a calibration curve of authentic calcium
307 levels. The calibration curve parameters, including the regression equation, correlation coefficient,
308 and linear range, are presented in the supplementary material (Appendix A Table S1).

309

310

311 2.6. Statistical analyses

312 The monthly analytical data obtained were compiled into four seasons and compared
313 using statistical analyses. The spring, summer, autumn, and winter periods were defined as March
314 2023 to May 2023 (n = 15), July 2023 to September 2023 (n=15), October 2023 to November
315 2023 (n=10), and December 2023 to February 2024 (n=15), respectively. All statistical analyses
316 were performed using R Ver 4.3.2 (R Development Core Team, 2024). The distribution of each
317 data was evaluated using the Kolmogorov–Smirnov test, and homogeneity of that was assessed
318 using the Levene test. The results indicated that all data were normally distributed and had
319 homogeneous variance. The analysis was performed using parametric statistical tests. The
320 differences among seasons were evaluated using one-way analysis of variance to assess the
321 seasonal variation in sample composition, and Tukey’s test was applied to detect the differences
322 between the data matrices. Differences were considered statistically significant at $P < 0.05$.

323

324 **3. Results and discussion**

325 *3.1. Changes in body weight, total length, and condition factor*

326

327 The results for body weight, total length, and condition factor are shown in Fig. 1. The
328 total length was lower in spring than in the other seasons, but it increased in summer and showed
329 a significant difference compared with the spring values. Body weight followed a similar trend to
330 total length, being low in spring, with no significant differences observed; however, in summer,
331 high levels were observed. The spawning season of greeneyes is in June, making it a closed
332 fishing season (Ministry of agriculture, Forestry and Fisheries, 2015). Therefore, it is possible
333 that body length and weight increased during this season. The condition factor was high in spring
334 and showed a significant difference compared to the other seasons. In winter, lower values were
335 observed, showing a significant difference compared to the other seasons. It is believed that the
336 high condition factor in spring was due to preparation for the spawning season. A significant
337 amount of energy is consumed during spawning, leading to a lower post-spawning condition
338 factor. There are various reports on body size and reproduction of greeneye. Cabiddu et al., (2010)
339 examined the gonadal structure and growth in *C. agassizi* within the Sardinian Channel. The
340 research, conducted from January 2002 to October 2005, revealed that specimens in this region
341 had body lengths spanning from 4.7 cm to 20.4 cm. However, it did not show any seasonal
342 variation. D'Onghia et al., (2006) investigated population biology and life strategies of *C.*
343 *agassizii* in the Ionian Sea from June 1995 to September 2000, revealed that the body lengths
344 ranged from 3.8 cm to 18.9 cm. This study was conducted 17 times and did not observe seasonal
345 changes in the same year. D'Onghia et al., (2006) concluded that despite the high changes in the
346 individual numbers sampled throughout the surveys, the modes in the size distribution were
347 consistent over the years. Anastasopoulou et al., (2006) investigated the body length of *C.*

348 *agassizii* inhabiting the Ionian Sea off Greece monthly from December 1996 to November 1997,
349 and reported that the body length ranged from 4.5 cm to 20.1 cm. After the reproductive period,
350 from June to November, most were 7.0-8.0 cm, but from December onwards, more mature
351 individuals were observed, and most were 12.0-15.0 mm during the reproductive period from
352 April to June. This trend was also observed in *C. albatrossis*, which also lives in Japan, where it
353 was observed to grow 4.0-5.0 cm from summer to winter after the reproductive period and 7.0-
354 8.0 cm in spring (Sakaji et al., 2006). In the present study, the samples analyzed were of
355 commercial size, as selected by fishermen, and therefore may not reflect true growth in the natural
356 environment. Our research findings indicate that body length in spring was reduced compared to
357 that in other seasons, although the exact cause for this discrepancy remains unclear. D'Onghia et
358 al. (2006) and Anastasopoulou et al. (2006) identified April to May (spring) as the spawning
359 period for *C. agassizii* in the Ionian Sea. This finding suggests that the reproductive season of
360 *Chlorophthalmus* species aligns with that of *C. albatrossis*, a Japanese *Chlorophthalmus* species.

361

362 3.2. Changes in lipid content

363

364 The results for lipid content are shown in Fig. 2. In spring, the values were the lowest,
365 and there were significant differences compared to the other seasons. During summer, the lipid
366 content was the highest, and there were significant differences compared to the other seasons. It
367 decreased in autumn and winter with similar values. This phenomenon was the same as the
368 seasonal changes in body length and weight. One possible reason for this is the increased
369 occurrence of plankton in summer to autumn on the Pacific Ocean off Miyazaki's shores, which
370 is a food source for greeneyes (Shirai et al., 2002). According to Anastasopoulou et al., (2008),
371 *C. agassizi* exhibited varied feeding patterns, consuming prey from a wide range of environments

372 including mesopelagic, benthic, and endobenthic zones. Throughout the year, the diet of the
373 species was predominantly composed of crustaceans and fish. Specifically, *the nutritional intake*
374 *of C. agassizi* mainly comprises organisms from three key groups: crustaceans (such as copepods,
375 decapods, euphausiids, and other types), cephalopods, and fish. Located on the Pacific Ocean
376 coast, Miyazaki contains various marine organisms that serve as food sources for *C. agassizi*.
377 These include copepods (Miyamoto et al., 2024), decapods (Nip et al., 2003), and euphausiids
378 (Matsuno, 2009), which inhabit the deep waters of the Pacific Ocean off the shore of Miyazaki.
379 The sea temperature around Miyazaki showed an optimal range for plankton between summer
380 and autumn, indicating that greeneyes could consume more food during these seasons, resulting
381 in an increase in lipid content (Japan Meteorological Agency, 2023).

382

383 3.3. Changes in fatty acid content

384

385 The fatty acid content results are presented in Table 1. Fatty acids exceeding 1 mg/g
386 included C14:0 (myristic acid), C16:0 (palmitic acid), C16:1 (palmitoleic acid), C18:0 (stearic
387 acid), C18:1 n-9 (oleic acid), C20:1 n-9 (eicosenoic acid), C22:1 n-9 (erucic acid), C20:5 n-3
388 (eicosapentaenoic acid), and C22:6 n-3 (docosahexaenoic acid). Saturated fatty acids showed no
389 significant difference between autumn and winter, and summer showed the highest values. During
390 spring, the contents decreased to approximately 5.7 mg/g. Monounsaturated fatty acid was low in
391 spring and showed a significant difference compared to other seasons, but it increased to
392 approximately 30 mg/g in summer. The polyunsaturated fatty acid content was approximately 5
393 mg/g in spring, which was lower than that in other seasons, but doubled to approximately 10 mg/g
394 in summer, showing a significant difference from the other seasons. The n-3/n-6 ratio was
395 between 6.50 ± 0.49 to 7.02 ± 1.00 in all seasons with no significant changes. As n-3 fatty acids are

396 necessary for maintaining health, greeneyes are attracting attention as a source of n-3 fatty acids.
397 n-3 fatty acids inhibit cancer (Souza et al., 2024) and ischemic stroke by reducing vascular
398 dementia, Alzheimer's disease (Yamagata, 2023), cardiovascular disease (Haris et al., 2019), and
399 depression (Matsuoka et al., 2017). One reason for the low fatty acid content in spring was the
400 effect of the spawning season in May and June. Since energy is consumed to develop the gonad
401 in preparation for spawning, the fatty acid content, and hence the lipid content, decreases. After
402 spawning in July, increased plankton led to higher nutrient accumulation, resulting in increased
403 fatty acid content. This is supported by the similarity between the fatty acid composition of
404 plankton and that of greeneyes in July (Shirai et al., 2002). As the spawning season approaches
405 again, the fatty acid content decreases as energy is consumed for gonad development. *Diaphus*
406 *watasei*, which belongs to the myctophids family, lives in the same sea waters and at the same
407 depth as greeneye and has a high lipid content (Catul et al., 2011). They also feed primarily on
408 zooplankton, such as copepods, euphausiids, and other small pelagic crustaceans. Koizumi et al.,
409 (2014) investigated seasonal changes in fatty acid contents of *Diaphus watasei*. The lipid contents
410 of the fish ranged from 27 to 131 mg/g, the DHA contents from 2.97 to 20.1 mg/g, and the ratio
411 of n-3/n-6 total fatty acids ranged from 4.8 to 6.9. In the report, lipid content in October (131
412 mg/g) was higher than that in other months; however, the average lipid content, except in October,
413 was 64 mg/g. Comparing lipid and fatty acid data of *Diaphus watasei*, that of greeneye in this
414 study was at the same level, proving it to be an important fish oil resource.

415

416 3.4. Changes in vitamin E content

417

418 The results for vitamin E content are shown in Fig. 2. Vitamin E plays many important
419 roles in animal reproduction (Mo et al., 2024) and antioxidant activity (Vui et al., 2025). In

420 particular, α -tocopherol (α -Toc) is the most abundant form in nature and has the highest biological
421 activity. Therefore, in the present study, we focused on the change in α -Toc. The α -Toc content
422 showed significant differences only in spring. The value in spring was the lowest at 0.3 ± 0.2 $\mu\text{g/g}$.
423 The other seasons showed values ranging from 0.13 to 0.16 $\mu\text{g/g}$, with no significant difference.
424 The lower content in spring is thought to be due to α -Toc consumption for gonad development,
425 with increased plankton post-spawning leading to individuals with higher content. Since no other
426 researchers have reported measuring vitamin E content, the analyzed data were compared with
427 the myctophids' family. Baby et al., (2014) reported vitamin E content in three important
428 myctophid species of *Diaphus watasei*, *Diaphus luetkeni*, and *Myctophum obtusirostre*. The
429 vitamin E contents of *D. watasei*, *D. luetkeni*, and *M. obtusirostre* were 0.40, 0.16, and 0.20 $\mu\text{g/g}$,
430 respectively. Comparing vitamin E of myctophid species, that of greeneye in this study was at the
431 same level.

432

433 3.5. Changes in nucleic acid-related substances content

434

435 The results for nucleic acid-related substances are shown in Fig. 3. This study focused
436 on monophosphates, including inosine monophosphate (IMP), guanosine monophosphate (GMP),
437 and adenosine monophosphates (AMP). Monophosphates are involved in umami taste (Servant
438 et al., 2022). IMP had a significantly higher content than the other monophosphates. The umami
439 taste derived from monophosphates largely depends on the IMP content. In autumn, the values
440 (3884.28 ± 666.56 $\mu\text{g/g}$ wet sample) were the highest with significant differences compared to the
441 other seasons. AMP showed no significant difference between spring, summer, and autumn, with
442 a value trend of approximately 30 $\mu\text{g/g}$. Winter showed lower values than the other seasons. GMP
443 showed no significant differences in all seasons, and each season contained more than 20 $\mu\text{g/g}$.

444 Nucleic acid-related compounds, such as AMP and IMP, are known to form when ATP
445 decomposes post-mortem and serve as indicators of freshness. AMP and IMP synergistically
446 enhance umami taste. GMP, formed from RNA decomposition, also synergistically enhances
447 umami taste (Mouritsen et al., 2012). The results of this study showed that the IMP content in
448 autumn was significantly higher than that of the other monophosphates. IMP, which is abundant
449 in fish stocks, has a weak umami taste, but coexists with monosodium glutamate to enhance
450 umami and food palatability. Consequently, they play a role in enhancing the taste of foods (Kido
451 et al., 2023). Li et al., (2009) also reported that the umami taste of monosodium glutamate can be
452 drastically enhanced by IMP, and the synergy is a hallmark of this taste quality. Previous studies
453 have investigated the IMP content of various seafood products: *Seriola lalandi* (8,870 µg/g
454 sample), *Cypselurus agoo* (6,410 µg/g sample), *Engraulis japonicus* (10,103 µg/g sample),
455 *Trachurus japonicus* (5,018µg/g sample), *Katsuwonus pelamis* (4,663 µg/g sample), and *Scomber*
456 *japonicus* (13,444 µg/g sample) (Kido et al., 2023; Ishimaru et al., 2016). Japanese soup stock
457 frequently utilizes these fish species as constituents, owing to their elevated concentrations of
458 IMP. In comparison, greeneye exhibited a marginally lower concentration of IMP, ranging from
459 2,000 to 4,000 µg/g sample, relative to the fish species utilized for soup stock. In contrast to its
460 utilization as a soup stock, greeneye is consumed directly, resulting in a higher IMP content upon
461 ingestion.

462

463 3.6. Changes in free amino acid content

464

465 The results of the free amino acid analysis are presented in Table 2. The total free amino
466 acid content was high in spring (3619.13 ± 719.54 µg/g) and remained low in summer, autumn,
467 and winter. The concentration of the essential amino acid histidine (His) was > 100 µg/g between

468 spring and autumn and decreased to < 100 µg/g in winter. Threonine (Thr) showed no significant
469 differences throughout the year, maintaining approximately 100 µg/g. Valine (Val) was
470 approximately 110 µg/g in spring, and slightly decreased between summer and winter, with no
471 significant differences. Methionine (Met) was highest in spring and showed lower values from
472 summer to winter. Isoleucine (Ile) showed no significant differences, maintaining a concentration
473 of approximately 50 µg/g across all seasons. Leucine (Leu) had high values in spring, was highest
474 at approximately 270 µg/g in March 2023, decreased to < 100 µg/g in summer, and increased to
475 approximately 115 µg/g between autumn and winter. Lysine (Lys) exhibited high values
476 (477.30±114.34 µg/g) in spring compared to summer and winter. The phenylalanine (Phe) content
477 was highest in spring at approximately 100 µg/g, with significant differences compared to the
478 other seasons, which had approximately 60 µg/g. Among the non-essential amino acids, taurine
479 (Tau), alanine (Ala), and arginine (Arg) were particularly abundant. Tau had a high concentration
480 of approximately 900 µg/g in spring, which decreased to above 300 µg/g between summer and
481 winter. The Ala content was highest in spring at approximately 190 µg/g, with no significant
482 differences observed compared to the other seasons. Arg showed particularly high values
483 420.79±131.89 µg/g in spring, decreased to approximately 176 to 91 µg/g between summer and
484 winter. The results of free amino acid analysis were presented in composition ratio, with the
485 predominant free amino acids being listed as follows: Tau (24.4%) >Lys (13.1%) >Arg (11.6%)
486 >His (8.7%) >Leu (6.5%). For comparison with these results, the free amino acid analysis results
487 presented in other research reports are shown below. Mizuno et al., (2009) also analyzed free
488 amino acids of grenneye *C.albatrossis* from Miyazaki and Ibaraki in Japan; Miyazaki: Lys
489 (12.8%) >Ala (9.9%)>Glu (9.7%)>Leu (9.4%)>Tau (7.9%); Ibaragi: Lys (13.5%) >Arg
490 (10.8%)>Leu (8.2%)>Ala (6.9%) >Tau (6.5%). Vijayan et al., (2016) also analyzed free amino
491 acids (not analysis Tau) of grenneye *C. corniger* from the Indian Ocean; Glu (15.8%)>Lys

492 (14.9%) >Asp (10.4%) >Arg (7.2%) >Ala (5.8%). These results indicate that all samples
493 contained high levels of Lys, Japanese greeneye *C.albatrossis* has high level of Leu, and all
494 samples except Miyazaki sample. Mizuno et al., (2009) showed high levels of Arg. Lys is present
495 in high concentrations in fish tissues and is considered an essential amino acid for mammals. Leu
496 is indicative of greater stimulation of protein synthesis (Lise et al., 2021; Etzel et al., 2004). Arg
497 is abundantly present in significant quantities in numerous seafood species, contributing to a
498 favorable overall flavor profile rather than imparting bitterness to these products. (Chen et al.,
499 2007). Arg is not found in large amounts in fish muscle and is characteristic of greeneye (Vijayan
500 et al., 2016). The results indicate that the free amino acid composition ratio of greeneye exhibits
501 variation within Japan and other countries. Considering the role of amino acids in fish biology,
502 amino acids, especially His and Tau, play important roles in physiological functions, such as
503 osmoregulation, buffering capacity, reproduction, and early larval development in aquatic
504 animals, suggesting the importance of adequate levels in fish oocytes. (Abe & Ohmama 1987;
505 Van Waarde 1988). Mejri et al., (2020) suggested that the higher percentages of His and Tau in
506 oocytes might have enhanced spawning quality, with possible improved development and
507 survival for embryos and larvae. In addition, fish consume glucose during the initial days of
508 fasting, followed by glycogen and lipid consumption, and prolonged fasting leads to protein
509 catabolism (Fernández Montserrat et al., 2023). Seiliez et al., (2012) also suggested an important
510 role for amino acids released by muscle proteolysis during the fasting period in regulating the
511 subtle balance between using proteins to provide energy, and conserving muscle through protein
512 sparing. Fasting necessitates substantial energy expenditure derived from protein catabolism.
513 Thus, amino acids are essential for both fasting and reproduction, as both processes require
514 significant energy utilization. Based on these findings and other reports, the elevated free amino
515 acid content observed in spring in this study was attributed to the energy obtained through protein

516 catabolism. Notably, during the spawning period in June, high energy consumption likely results
517 in an energy deficit within the organism, which requires energy acquisition through protein
518 degradation. Consequently, the free amino acid content decreased in the summer following the
519 spawning period in June.

520

521 3.7. Changes in imidazole dipeptides content

522

523 Fig. 4 A and B show the changes in the imidazole dipeptide content. The imidazole
524 dipeptides included carnosine, anserine, and balenine. To date, there have been no reports from
525 other researchers regarding the dipeptide content of greeneyes; thus, these results are the first data
526 on imidazole dipeptide contents in greeneyes. The greeneyes had a trace amount of carnosine in
527 the ranging of 0.74-7.68 $\mu\text{g/g}$ and anserine in the range of 61.5-125.5 $\mu\text{g/g}$. Balenine was not
528 detected. Carnosine in spring, summer, and autumn showed no significance and then decreased
529 to approximately 0.74 $\mu\text{g/g}$ in winter. Anserine was 61.5 $\mu\text{g/g}$ in spring, increasing to
530 approximately 130 $\mu\text{g/g}$ between summer, autumn, and winter. Balenine was analyzed but was
531 hardly detected during the all seasons. In our previous study, we analyzed imidazole dipeptides
532 in several marine organisms (Mori et al., 2018). In the report, the anserine contents in Japanese
533 pilchard, Japanese horse mackerel, flying fish, and Pacific cod were 20, 10, 70, and 350 $\mu\text{g/g}$,
534 respectively. These fish did not contain or had trace amounts of carnosine and balenine. However,
535 migratory fish, such as skipjack tuna and bigeye tuna, had high anserine contents of 1,930 and
536 5,000 $\mu\text{g/g}$, respectively. Kasamatsu et al., (2021) also reported that anserine is abundant in
537 migratory fish such as tuna (*Thunnus*) and skipjack (*Katsuwonus pelamis*). Upon comparison of
538 these results, the dipeptide content of greeneye was found to be lower than that of migratory fish,
539 but comparable to that of other fish species. Mommsen (2004) reported that sockeye salmon retain

540 approximately 9,600 µg/g anserine in muscle during migration, where it functions as a significant
541 intracellular buffer and antioxidant. In a human study, carnosine reduced fatigue and exerted
542 antioxidant effects, whereas anserine lowered uric acid levels and improved athletic performance
543 (Shirotsuki et al., 2017). These compounds may play significant physiological roles in the
544 metabolism of fish organisms, such as migration (Abe, 1983). However, their specific functions
545 have not yet been elucidated. In the present study, anserine was approximately 60 µg/g wet sample
546 in spring and increased between summer and winter, with no significant differences. This
547 phenomenon is hypothesized to result from the utilization of anserine in gonadal development or
548 the neutralization of reactive oxygen species generated during the energy-intensive process of
549 gonadal development.

550

551 3.8. Changes in calcium content

552

553 Fig. 4C shows the results of the calcium content analysis. The calcium levels were
554 approximately 5 mg/g sample, with no significant differences observed in all seasons. Unlike
555 skipjack or mackerel (*Scomber japonicus*), greeneyes are generally consumed with bones.
556 Vijayan et al., (2016) also analyzed calcium levels in *C. corniger* from India sea, the level was
557 2.03 mg/g sample. In comparison to other fish species that are typically consumed whole,
558 including their bones, mola (*Amblypharyngodon mola*) and puty (*Puntius* spp.) exhibit calcium
559 levels of 4.96 and 1.2 mg/g sample, respectively (Saha et al., 2024; Larsen et al., 2000). Muleya
560 et al., (2024) reported that when the calcium content of various foods was analyzed, the calcium
561 content of kale, finger millet, and fortified white bread were equal to or higher than skim milk,
562 which has a high calcium content. The calcium content of kale, finger millet, and fortified white
563 bread were 9.59, 1.61, and 1.32 mg/g sample, respectively. These findings suggest that the

564 calcium content of greeneye is comparable to that of other fish consumed with bones or alternative
565 high-calcium food sources. The recommended daily calcium intake for adults is 700-800 mg for
566 men and 750 mg for women; however, only a few Japanese people meet this target (Shinozaki et
567 al., 2023). Greeneyes are typically prepared by removing only the head and viscera and
568 consuming the entire body, including the bones, and we usually eat approximately ten or more
569 greeneyes at a time. Consequently, the consumption of approximately 200 g of greeneyes
570 (equivalent to 10 fish) provides more than 70 to 80% of the daily calcium requirement, rendering
571 them an excellent source of calcium.

572

573 **4. Conclusion**

574

575 This study examined temporal variations in the organoleptic properties and bioactive
576 constituents of small benthic fish species, an area of research that has received limited scholarly
577 attention. The results indicated that body length, body weight, umami-related components, and
578 functional components exhibited changes before and after the fishing ban period, which reflected
579 the spawning season of the greeneye. Nevertheless, the calcium content remained constant
580 throughout the year and was comparable to other calcium-rich food sources. Free amino acids and
581 nucleic acid-related substances were analyzed as components associated with umami taste. These
582 findings indicate that free amino acid levels were elevated prior to the implementation of the
583 fishing ban, whereas nucleic acid-related substances exhibited higher levels during the autumn
584 period following the fishing ban. Nucleic acid-related substances enhance the umami taste
585 perception of free amino acids, although their gustatory effect is less pronounced than that of free
586 amino acids. In the present study, the free amino acid content was higher in spring, but nucleic
587 acid-related substances were higher in autumn, so the overall umami taste was higher in spring

588 and autumn. The levels of functional components (fatty acids, vitamin E, and imidazole
589 dipeptides) increased following the implementation of the ban. This increase was attributed not
590 only to the direct effects of the ban, but also to a concomitant increase in plankton biomass, which
591 served as a primary food source for the fish population. These results indicate that umami taste is
592 superior in spring and autumn; however, functional components are superior from summer to
593 autumn after the fishing ban. This information is beneficial to fishermen and consumers and will
594 increase the value of greeneye. It was also clear that the fishing ban in June not only serves the
595 purpose of maintaining the stock, but also increases the value of greeneye as a foodstuff.
596 Regardless of the season, the cooking method of consuming entire greeneyes, including bones,
597 allows for a stable year-round intake of calcium. In conclusion, greeneyes can be considered a
598 stable source of calcium and fish throughout the year, with varying umami tastes and functional
599 components depending on the season.

600

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609

610

611 **CRedit authorship contribution statement**

612 **Ryohei Horie:** Data curation, Formal analysis, Investigation, Methodology, Validation,
613 Visualization, Writing – original draft. **Takumi Imagawa:** Formal analysis, Investigation,
614 Methodology, Validation, Visualization. **Katsuhisa Uchida:** Project administration,
615 Conceptualization, Resources, Investigation, Methodology, Validation. **Yosuke Taoka:**
616 Investigation, Methodology, Validation. **Ryusuke Tanaka:** Conceptualization, Supervision,
617 Methodology, Validation, Visualization, Writing – review & editing.

618

619 **Declaration of Competing Interest**

620 The authors declare that they have no known competing financial interests or personal
621 relationships that could have appeared to influence the work reported in this paper.

622

623 **Data availability**

624 Data will be made available on request.

625

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631 **References**

632

633 Abe, H. (1983). Distribution of free L-histidine and its related compounds in marine fishes.
634 Nippon Suisan Gakkaishi, 49(11), 1683-1687. <https://doi.org/10.2331/suisan.49.1683>.

635 Abe, H., & Ohmama, S. (1987). Effect of starvation and sea-water acclimation on the
636 concentration of free L-histidine and related dipeptides in the muscle of eel, rainbow trout
637 and Japanese dace. *Comparative Biochemistry and Physiology. Part B*, 88(2), 507-511.
638 [https://doi.org/10.1016/0305-0491\(87\)90335-X](https://doi.org/10.1016/0305-0491(87)90335-X).

639 Aichi Prefecture Government Official Site (2022). Trends in catch by fish species. Retrieved
640 from: <https://www.pref.aichi.jp/>. Accessed July 2, 2024.

641 Anastasopoulou, A., Yiannopoulos, C., Megalofonou, P., & Papaconstantinou, C. (2006).
642 Distribution and population structure of the *Chlorophthalmus agassizi* (Bonaparte, 1840)
643 on an unexploited fishing ground in the Greek Ionian Sea. *Journal of Applied Ichthyology*,
644 22(6), 521-529. <https://doi.org/10.1111/j.1439-0426.2006.00782.x>

645 Baby, L., Sankar, T. V., & Anandan, R. (2014). Comparison of lipid profile in three species of
646 myctophids from the south west coast of kerala, India. *National Academy Science Letters*,
647 37(1), 33-37. <https://doi.org/10.1007/s40009-013-0185-4>.

648 Cabiddu, S., Follesa, M. C., Porcu, C., & Cau, A. (2010). Gonad development and reproduction
649 in the monoecious species *Chlorophthalmus agassizi* (Actinopterygii: Aulopiformes:
650 Chlorophthalmidae) from the Sardinian waters (central-western Mediterranean). *Acta*
651 *Ichthyologica et Piscatoria*, 40(2), 167-177. <https://doi.org/10.3750/aip2010.40.2.10>.

652 Catul, V., Gauns, M., & Karuppasamy, P. K. (2011). A review on mesopelagic fishes belonging
653 to family Myctophidae. *Reviews in Fish Biology and Fisheries*, 21(3), 339-354.
654 <https://doi.org/10.1007/s11160-010-9176-4>.

655 Chen, D. W., & Zhang, M. (2007). Non-volatile taste active compounds in the meat of Chinese
656 mitten crab (*Eriocheir sinensis*). *Food Chemistry*, 104(3), 1200-1205.
657 <https://doi.org/10.1016/j.foodchem.2007.01.042>.

658 Chi-Espínola, A. A., Vega-Cendejas, M. E., & Hernández De Santillana, J. M. (2023). Length–

659 weight relations of 39 continental-shelf and deep-water fishes (*Actinopterygii*) from
660 northwestern Gulf of México. *Acta Ichthyologica et Piscatoria*. 53, 59-64.
661 <https://doi.org/10.3897/aiep.53.101788>.

662 D'Onghia, G., Sion, L., Maiorano, P., Mytilineou, C., Dalessandro, S., Carlucci, R., & Desantis,
663 S. (2006). Population biology and life strategies of *Chlorophthalmus agassizii* Bonaparte,
664 1840 (Pisces: *Osteichthyes*) in the Mediterranean Sea. *Marine Biology*, 149(3), 435-446.
665 <https://doi.org/10.1007/s00227-005-0231-y>.

666 Etzel, M. R. (2004). Manufacture and use of dairy protein fractions. *The Journal of Nutrition*,
667 134(4), 996S-1002S. <https://doi.org/10.1093/jn/134.4.996S>.

668 Fernández-Muela, M., Bermejo-Poza, R., Cabezas, A., Pérez, C., González de Chavarri, E., Díaz,
669 M. T., Torrent, F., Villarroel, M., & De la Fuente, J. (2023). Effects of fasting on
670 intermediary metabolism enzymes in the liver and muscle of rainbow trout. *Fishes*, 8(1).
671 53. <https://doi.org/10.3390/fishes8010053>.

672 Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and
673 purification of total lipides from animal tissues. *The Journal of biological chemistry*,
674 226(1), 497-509. [https://doi.org/10.1016/S0021-9258\(18\)64849-5](https://doi.org/10.1016/S0021-9258(18)64849-5).

675 Fujiwara, K., Wada, H., & Motomura, H. (2019). A new species of the greeneye genus
676 *Chlorophthalmus* (Teleostei: *Chlorophthalmidae*) from the central North Pacific. *Zootaxa*,
677 4555(3), 396-406. <https://doi.org/10.11646/zootaxa.4555.3.8>.

678 Fukushima prefecture home page (2023). Fukushima prefecture resource evaluation table for
679 aome eso. Retrieved from:
680 <https://www.pref.fukushima.lg.jp/sec/37395c/shigenhyoka.html>. Accessed July 2, 2024.

681 Harris, W. S., Jackson, K. H., Brenna, J. T., Rodriguez, J. C., Tintle, N. L., & Cornish, L. (2019).
682 Survey of the erythrocyte EPA+DHA levels in the heart attack/stroke belt. *Prostaglandins*,

683 Leukotrienes and Essential Fatty Acids, 148, 30-34.
684 <https://doi.org/10.1016/j.plefa.2019.07.010>.

685 Ishimaru, M., Haraoka, M., Hatate, H., & Tanaka, R. (2016). Simultaneous analysis of purine and
686 pyrimidine compounds associated with the freshness and taste of marine foods. Food
687 Analytical Methods, 9(6), 1606-1615. <https://doi.org/10.1007/s12161-015-0341-1>.

688 Iwaki City Home Page (2001). Fish from Iwaki city and greeneyes. Retrieved from:
689 <https://www.city.iwaki.lg.jp/www/contents/1001000000619/index.html>. Accessed July 2,
690 2024.

691 Japan Meteorological Agency. (2023). Daily sea surface temperature. Retrieved from:
692 https://www.data.jma.go.jp/kaiyou/data/db/kaikyo/daily/sst_HQ.html. Accessed July 2,
693 2024.

694 Kasamatsu, S., Komae, S., Matsukura, K., Kakihana, Y., Uchida, K., & Ihara, H. (2021). 2-oxo-
695 imidazole-containing dipeptides play a key role in the antioxidant capacity of imidazole-
696 containing dipeptides. Antioxidants, 10(9). <https://doi.org/10.3390/antiox10091434>.

697 Kido, S., & Tanaka, R. (2023). Umami-enhancing effect of mushroom stocks on Japanese fish
698 stock based on the equivalent umami concentration (EUC) value. International Journal of
699 Gastronomy and Food Science, 34, 100832. <https://doi.org/10.1016/j.ijgfs.2023.100832>.

700 Kimura, K., Misawa, R., & Tashiro, F. (2024). Descriptions of post-settlement juveniles of 3
701 greeneye species, *Chlorophthalmus nigromarginatus*, *C. acutifrons*, and *C. albatrossis*
702 (Teleostei: *Chlorophthalmidae*), from Japan, with notes on their rapid metamorphosis after
703 settlement. Fishery Bulletin, 122(4), 186-202. <https://doi.org/10.7755/FB.122.4.4>.

704 Koizumi, K., Hiratsuka, S., & Saito, H. (2014). Lipid and fatty acids of three edible myctophids,
705 *Diaphus watasei*, *Diaphus suborbitalis*, and *Benthosema pterotum*: High levels of
706 icosapentaenoic and docosahexaenoic acids. Journal of Oleo Science, 63(5), 461-470.

707 <https://doi.org/10.5650/jos.ess13224>.

708 Larsen, T., Thilsted, S. H., Kongsbak, K., & Hansen, M. (2000). Whole small fish as a rich
709 calcium source. *British Journal of Nutrition*, 83(2), 191-196.
710 <https://doi.org/10.1017/S0007114500000246>.

711 Li, P., Mai, K., Trushenski, J., & Wu, G. (2009). New developments in fish amino acid nutrition:
712 towards functional and environmentally oriented aquafeeds. *Amino Acids*, 37(1), 43-53.
713 <https://doi.org/10.1007/s00726-008-0171-1>.

714 Lise, C. C., Marques, C., Bonadimann, F. S., Pereira, E. A., & Mitterer-Daltoé, M. L. (2021).
715 Amino acid profile of food fishes with potential to diversify fish farming activity. *Journal*
716 *of Food Science and Technology*, 58(1), 383-388. [https://doi.org/10.1007/s13197-020-](https://doi.org/10.1007/s13197-020-04747-1)
717 [04747-1](https://doi.org/10.1007/s13197-020-04747-1).

718 Matsuno, K., Kim, H. S., & Yamaguchi, A. (2009). Causes of under- or overestimation of
719 zooplankton biomass using Optical Plankton Counter (OPC): effect of size and taxa.
720 *Plankton and Benthos Research*, 4(4), 154-159. <https://doi.org/10.3800/pbr.4.154>.

721 Matsuoka, Y. J., Sawada, N., Mimura, M., Shikimoto, R., Nozaki, S., Hamazaki, K., Uchitomi,
722 Y., Tsugane, S. (2017). Dietary fish, n-3 polyunsaturated fatty acid consumption, and
723 depression risk in Japan: a population-based prospective cohort study. *Translational*
724 *Psychiatry*, 7(9), e1242-e1242. <https://doi.org/10.1038/tp.2017.206>.

725 Mejri, S., Luck, C., Wills, P. S., Adams, A., Shenker, J., & Ajemian, M. J. (2020). Reproductive
726 physiology of bonefishes (*Albula spp.*) across the Northwest Bahamas. *Fish Physiology*
727 *and Biochemistry*, 46(2), 699-712. <https://doi.org/10.1007/s10695-019-00743-1>.

728 Ministry of Agriculture, Forestry and Fisheries (2015). Our regional cuisines. Retrieved from:
729 https://www.maff.go.jp/e/policies/market/k_ryouri/index.html. Accessed July 2, 2024.

730 Miyamoto, H., Okazaki, Y., Itoh, H., Hidaka, K., & Saito, H. (2024). Community structure of

731 whole pelagic copepods along the Kuroshio: Increase in the abundance and size in the
732 north frontal area of the Kuroshio axis near the Japanese archipelago. Deep-Sea Research
733 Part I: Oceanographic Research Papers, 211. <https://doi.org/10.1016/j.dsr.2024.104346>.
734 Miyazaki Prefectural Fisheries Research Institute (2023). Aome eso species - evaluation of other
735 fish species, 10th Edition (2023) Coastal Resource Assessment of Miyazaki Prefecture.
736 Retrieved from: <https://www.mz-suishi.jp/>. Accessed July 3, 2024.

737 Mizuno, T., & Yamada, K. (2009). Nutritional components of greeneye (*Chlorophthalmus*
738 *albatrossis*). Journal for the integrated study of dietary habits, 20(3), 211-219.
739 <https://doi.org/10.2740/jisdh.20.211>.

740 Mo, H. Y., Shan, C. H., Chen, L. W., Chen, X., Han, C., Wu, D., Tao, F. B., & Gao, H. (2024).
741 Antioxidant vitamins' modification of the adverse health effects induced by phthalate
742 exposure: A scoping review of epidemiological and experimental studies. Ecotoxicology
743 and Environmental Safety, 286. <https://doi.org/10.1016/j.ecoenv.2024.117190>.

744 Mommsen, T. P. (2004). Salmon spawning migration and muscle protein metabolism: the August
745 Krogh principle at work. Comparative Biochemistry and Physiology Part B: Biochemistry
746 and Molecular Biology, 139(3), 383-400. <https://doi.org/10.1016/j.cbpc.2004.09.018>.

747 Mori, A., Hikiyama, R., Ishimaru, M., Hatate, H., & Tanaka, R. (2018). Evaluation of histidine-
748 containing dipeptides in twelve marine organisms and four land animal meats by
749 hydrophilic interaction liquid chromatography with ultraviolet detection. Journal of liquid
750 chromatography & related technologies, 41(13-14), 849-854.
751 <https://doi.org/10.1080/10826076.2018.1526803>.

752 Mouritsen, O. G., & Khandelia, H. (2012). Molecular mechanism of the allosteric enhancement
753 of the umami taste sensation. Federation of European Biochemical Societies journal,
754 279(17), 3112-3120. <https://doi.org/10.1111/j.1742-4658.2012.08690.x>.

755 Muleya, M., F. Bailey, E., & H. Bailey, E. (2024). A comparison of the bioaccessible calcium
756 supplies of various plant-based products relative to bovine milk. *Food Research*
757 *International*, 175, 113795. <https://doi.org/10.1016/j.foodres.2023.113795>.

758 Nip, T. H. M., Ho, W.Y., & Kim Wong, C. (2003). Feeding ecology of larval and juvenile black
759 seabream (*Acanthopagrus schlegeli*) and Japanese seaperch (*Lateolabrax japonicus*) in
760 Tolo Harbour, Hong Kong. *Environmental Biology of Fishes*, 66(2), 197-209.
761 <https://doi.org/10.1023/A:1023611207492>.

762 Nishiguchi, Y., Ohsaka, T., Ando, S., Hayasaka, K., Ikeda, J., Hori, K., Suga, Y., & Fukunaga,
763 A. (2007). Rapid analysis of magnesium, potassium and calcium contents in feedstuffs: 2.
764 Colorimetric and turbidimetric determination of magnesium, potassium and calcium.
765 Kinki-Chugoku-Shikoku Agricultural Research Center Research Report. 6. 141-151.
766 <https://doi.org/10.24514/00001629>.

767 Nobeoka city (2023). Nobeoka City Fisheries Handbook 2023 Edition. Retrieved from:
768 <https://www.city.nobeoka.miyazaki.jp/soshiki/38/29204.html>. Accessed October 25, 2024.

769 R Core Team (2024). R: A language and environment for statistical computing. R Foundation for
770 Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.

771 Saha, M., Ng, H., Odjidja, E. N., Saha, M., Olivier, P., McCaffrey, T. A., & Thilsted, S. H. (2024).
772 Small fish big impact: improving nutrition during pregnancy and lactation, and
773 empowerment for marginalized women. *Nutrients*, 16(12), 1829.
774 <https://doi.org/10.3390/nu16121829>.

775 Sakaji, H., Honda, H., & Nashida, K. (2006). Growth and ontogenetic migration of greeneye
776 *Chlorophthalmus albatrossis* in Tosa Bay, Pacific Coast of South-Western Japan.
777 *Fisheries Science*, 72(6), 1250-1255. <https://doi.org/10.1111/j.1444-2906.2006.01282.x>.

778 Seiliez, I., Gabillard, J. C., Riflade, M., Sadoul, B., Dias, K., Avérous, J., Tesseraud, S., Skiba,

779 S., & Panserat, S. (2012). Amino acids downregulate the expression of several autophagy-
780 related genes in rainbow trout myoblasts. *Autophagy*, 8(3), 364-375.
781 <https://doi.org/10.4161/auto.18863>.

782 Servant, G., & Frerot, E. (2022). Pharmacology of the umami taste receptor. In R. Kyle Palmer
783 & G. Servant (Eds.), *The Pharmacology of Taste* (pp. 109-136). Springer.
784 https://doi.org/10.1007/164_2021_439

785 Shinozaki, N., Murakami, K., Masayasu, S., & Sasaki, S. (2023). Usual nutrient intake
786 distribution and prevalence of nutrient intake inadequacy among Japanese children and
787 adults: a nationwide study based on 8-day dietary records. *Nutrients*, 15(24).
788 <https://doi.org/10.3390/nu15245113>.

789 Shirai, N., Terayama, M., & Takeda, H. (2002). Effect of season on the fatty acid composition
790 and free amino acid content of the sardine *Sardinops melanostictus*. *Comparative*
791 *Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 131(3), 387-
792 393. [https://doi.org/10.1016/S1096-4959\(01\)00507-3](https://doi.org/10.1016/S1096-4959(01)00507-3).

793 Shirotzuki, K., Nonaka, Y., Takano, J., Abe, K., Adachi, S. I., Adachi, S., & Nakao, M. (2017).
794 Brief internet-based cognitive behavior therapy program with a supplement drink
795 improved anxiety and somatic symptoms in Japanese workers. *BioPsychoSocial Medicine*,
796 11(1). <https://doi.org/10.1186/s13030-017-0111-y>.

797 Souza, A. L. A., Martins Trindade, L., Dias Borges, A., Caroline Lacerda Leocadio, P., de
798 Oliveira Silva, J., Salgado Fernandes, R., Isaura Alvarez Leite, J., Dantas Cassali, G.,
799 Carlos dos Reis, D., Uceli Maioli, T., Nascimento Cardoso, V., Townsend, D. M., Luis
800 Branco de Barros, A., & de Vasconcelos Generoso, S. (2024). Omega-3 fatty acid
801 supplementation attenuates intestinal mucositis and tumor growth in a murine model of
802 breast cancer. *Journal of Functional Foods*, 115, 106096.

803 <https://doi.org/10.1016/j.jff.2024.106096>.

804 Suzuki, T., Yasui, A., Koizumi, H., & Tsutsumi, C. (1990). Hydrochloric acid extraction
805 procedure for determination of inorganic elements in foods of animal origin. *Nippon*
806 *Shokuhin Kogyo Gakkaishi*, 37(7), 547-553. https://doi.org/10.3136/nskkk1962.37.7_547.

807 Tanaka, R., Ishimaru, M., Hatate, H., Sugiura, Y., & Matsushita, T. (2016). Relationship between
808 4-hydroxy-2-hexenal contents and commercial grade by organoleptic judgement in
809 Japanese dried laver *Porphyra spp.* *Food Chemistry*, 212, 104-109.
810 <https://doi.org/10.1016/j.foodchem.2016.05.166>.

811 Tanaka, R., Shigeta, K., Sugiura, Y., Hatate, H., & Matsushita, T. (2014). Accumulation of
812 hydroxyl lipids and 4-hydroxy-2-hexenal in live fish infected with fish diseases. *Lipids*,
813 49(4), 385-396. <https://doi.org/10.1007/s11745-013-3875-2>.

814 Van Waarde, A. (1988). Biochemistry of non-protein nitrogenous compounds in fish including
815 the use of amino acids for anaerobic energy production. *Comparative Biochemistry and*
816 *Physiology. Part B*, 91(2), 207-228. [https://doi.org/10.1016/0305-0491\(88\)90136-8](https://doi.org/10.1016/0305-0491(88)90136-8).

817 Vijayan, D. K., Jayarani, R., Singh, D. K., Chatterjee, N. S., Mathew, S., Mohanty, B. P., Sankar,
818 T. V., & Anandan, R. (2016). Comparative studies on nutrient profiling of two deep sea
819 fish (*Neopinnula orientalis* and *Chlorophthalmus corniger*) and brackish water fish
820 (*Scatophagus argus*). *The Journal of Basic & Applied Zoology*, 77, 41-48.
821 <https://doi.org/10.1016/j.jobaz.2016.08.003>.

822 Vui, N. V., Ponchunchoovong, S., Kupittayanant, S., & Kupittayanant, P. (2025). Effects of
823 vitamin E, green tea polyphenols, and *Ocimum gratissimum* leaf essential oil as a
824 supplement to extender on frozen-thawed canine sperm quality. *Veterinary Integrative*
825 *Sciences*, 23(1). doi:10.12982/VIS.2025.016.

826 Yamagata, K. (2023). Docosahexaenoic acid inhibits ischemic stroke to reduce vascular dementia

827 and Alzheimer's disease. *Prostaglandins & Other Lipid Mediators*, 167, 106733.
828 <https://doi.org/10.1016/j.prostaglandins.2023.106733>.

829 Zhang, X., Liu, Q. H., Guo, L. M., & Ding, S. X. (2016). Complete mitochondrial genome of
830 blackedge greeneye *Chlorophthalmus nigromarginatus* (Aulopiformes,
831 *Chlorophthalmidae*). *Mitochondrial DNA*, 27(4), 2572-2573.
832 <https://doi.org/10.3109/19401736.2015.1038809>.

Table 1. Seasonal changes in fatty acids contents of greeneye

	Spring	Summer	Autumn	Winter
13:0	nd	0.04±0.01 ^a	0.02±0.01 ^b	0.02±0.01 ^b
14:0	1.06±0.45 ^b	2.65±0.58 ^a	1.29±0.71 ^b	1.22±0.28 ^b
14:1	0.01±0.02 ^b	0.05±0.03 ^a	0.21±0.51 ^a	0.04±0.01 ^a
15:0	0.11±0.05 ^c	0.26±0.05 ^a	0.17±0.06 ^b	0.15±0.03 ^{bc}
15:1	0.01±0.01 ^c	0.04±0.01 ^a	0.03±0.01 ^b	0.02±0.01 ^{bc}
16:0	3.75±1.20 ^c	11.99±2.77 ^a	7.52±2.87 ^b	6.59±1.20 ^b
16:1	1.14±0.38 ^b	2.97±0.60 ^a	1.67±0.56 ^b	1.48±0.28 ^b
17:1	0.14±0.05	0.66±1.09 ^a	0.26±0.07 ^b	0.22±0.04 ^b
18:0	0.72±0.18 ^c	2.38±0.97 ^a	1.71±0.71 ^{ab}	1.35±0.50 ^b
18:1 n-9(cys)	3.01±1.36 ^c	17.24±5.27 ^a	9.44±4.12 ^b	7.65±2.54 ^b
18:1 n-7	0.50±0.13 ^c	1.54±0.35 ^a	0.93±0.29 ^b	1.34±2.17 ^b
18:2 n-6(trans)	0.02±0.02 ^{ac}	0.06±0.02 ^b	0.03±0.02 ^{ac}	0.08±0.23 ^a
18:2 n-6(cys)	0.22±0.07 ^b	0.56±0.10 ^a	0.33±0.12 ^b	0.27±0.10 ^b
18:3 n-6	0.01±0.01 ^{ac}	0.03±0.02 ^{ab}	0.02±0.01 ^{abc}	0.04±0.10 ^a
18:3 n-3	0.12±0.07 ^b	0.35±0.09 ^a	0.19±0.08 ^b	0.20±0.08 ^b
20:0	0.03±0.03 ^c	0.14±0.03 ^a	0.09±0.03 ^b	0.09±0.07 ^b
18:4 n-3	0.21±0.19 ^c	0.51±0.28 ^b	0.61±0.48 ^{ab}	1.06±0.52 ^a
20:1 n-9	nd	nd	nd	nd
21:0	nd	0.02±0.01 ^a	0.01±0.01 ^a	0.01±0.01 ^a
20:3 n-6	0.02±0.01 ^c	0.06±0.01 ^a	0.04±0.01 ^{bc}	0.06±0.08 ^b
20:4 n-6	0.30±0.05 ^c	0.69±0.12 ^a	0.44±0.10 ^b	0.34±0.10 ^{bc}
20:3 n-3	0.01±0.01 ^b	0.05±0.02 ^a	0.04±0.02 ^a	0.04±0.01 ^a
22:0	0.01±0.01 ^c	0.09±0.03 ^a	0.05±0.02 ^b	0.05±0.01 ^b
22:1 n-9	1.82±1.12 ^b	3.39±1.84 ^a	1.13±1.49 ^{bc}	0.34±0.15 ^c
20:5 n-3	1.40±0.47 ^b	2.98±0.42 ^a	1.56±0.60 ^b	1.34±0.33 ^b
24:0	0.02±0.03 ^c	0.10±0.03 ^a	0.06±0.02 ^b	0.04±0.02 ^b
24:1 n-9	0.13±0.07 ^c	0.51±0.13 ^a	0.28±0.15 ^b	0.24±0.08 ^b
22:5 n-3	0.24±0.05 ^c	0.60±0.11 ^a	0.04±0.14 ^b	0.36±0.24 ^{bc}
22:6 n-3	2.42±0.63 ^c	5.81±1.00 ^a	3.95±1.21 ^b	3.46±0.74 ^b
SFA	5.70±1.81 ^c	17.66±4.07 ^a	10.93±4.15 ^b	9.53±1.72 ^b
MUFA	8.01±3.13 ^c	29.29±6.75 ^a	14.83±6.53 ^b	11.46±2.78 ^b
PUFA	5.00±1.43 ^c	11.80±1.83 ^a	7.64±2.32 ^b	7.31±1.61 ^b
n-3/n-6	7.02±1.00 ^a	6.50±0.49 ^a	6.58±0.76 ^a	6.58±0.89 ^a

Values are mean ± standard deviation (mg/g wet sample). SFAs, saturated fatty acid; MUFAs; monounsaturated fatty acid; PUFAs, poly unsaturated fatty acid. n-3/n-6, the ratio of n-3 total fatty acid content to n-6 total fatty acid content. “nd” indicates not detected. Different letters indicate significant difference between groups ($p<0.05$).

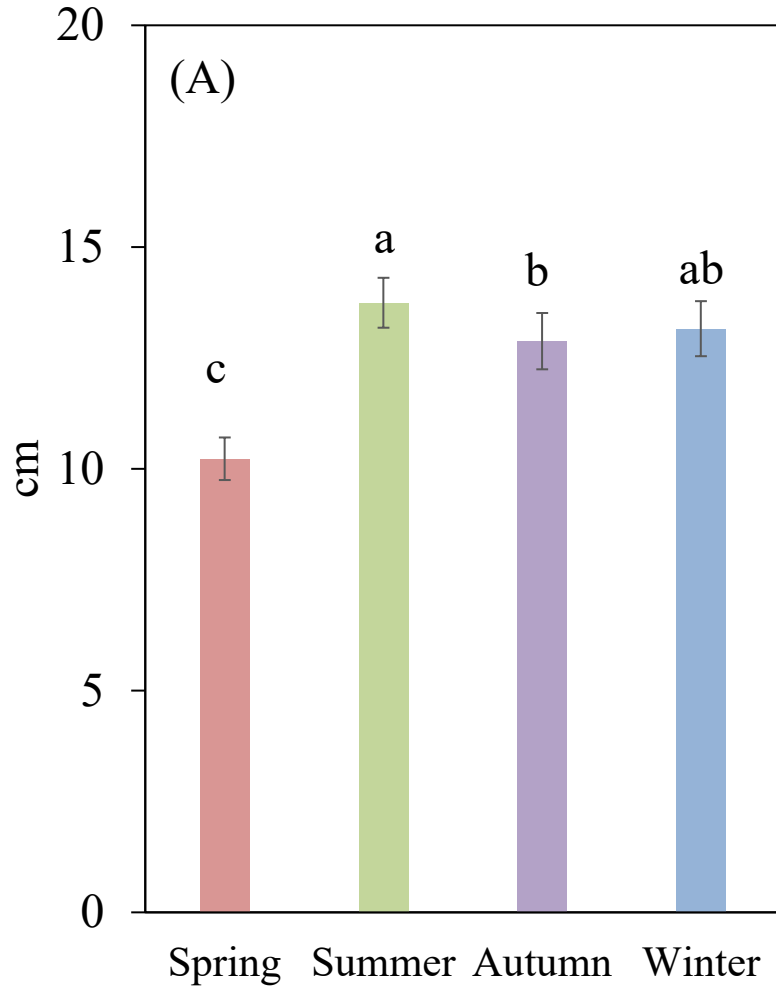
Table. 2 Seasonal changes in free amino acids composition contents of greeneye

	Spring	Summer	Autumn	Winter
Asp	86.63±22.86 ^a	18.69±7.27 ^c	25.31±17.01 ^{bc}	43.75±17.81 ^b
Glu	122.20±24.85 ^a	63.76±14.73 ^b	65.95±24.92 ^b	76.10±25.43 ^b
Ser	72.05±16.26 ^a	30.20±11.55 ^c	42.72±19.95 ^{bc}	54.17±17.10 ^b
Gly	73.43±17.13 ^a	35.48±8.78 ^b	51.15±28.28 ^b	41.76±14.24 ^b
His	318.35±152.14 ^a	130.61±22.46 ^b	194.58±305.13 ^b	69.55±21.17 ^c
Tau	887.30±186.33 ^a	337.43±35.25 ^c	412.81±106.42 ^{bc}	432.72±87.07 ^b
Thr	70.67±65.23 ^a	99.51±35.58 ^a	104.58±55.57 ^a	93.00±41.44 ^a
Ala	190.80±57.95 ^a	93.81±26.01 ^b	114.43±38.64 ^b	108.78±29.99 ^b
Arg	420.79±131.89 ^a	91.28±42.39 ^c	142.5±53.91 ^{bc}	176.76±52.03 ^b
Pro	88.55±57.26 ^{ab}	124.14±46.99 ^a	117.29±107.45 ^{ab}	61.93±49.75 ^b
Tyr	102.77±45.24 ^a	25.92±8.31 ^{bd}	73.32±70.08 ^{ab}	56.84±25.39 ^{bc}
Val	108.96±20.64 ^a	70.62±40.53 ^b	71.70±55.48 ^b	68.98±24.82 ^b
Met	93.12±34.95 ^a	19.69±5.54 ^{bd}	38.63±34.47 ^b	36.51±14.11 ^{bc}
Ile	60.18±19.33 ^b	181.95±575.30 ^a	39.56±32.14 ^{ab}	41.83±18.60 ^{ab}
Leu	237.09±64.15 ^a	66.70±25.81 ^c	105.09±61.77 ^{bc}	126.84±46.82 ^b
Lys	477.30±114.34 ^a	275.62±122.72 ^b	316.72±158.61 ^{ab}	242.04±105.30 ^b
Phe	104.72±37.99 ^a	59.36±79.98 ^c	59.13±35.97 ^{bc}	65.05±25.38 ^b
Total	3619.13±719.54 ^a	2019.79±851.59 ^b	2133.03±866.91 ^b	1933±487.35 ^b

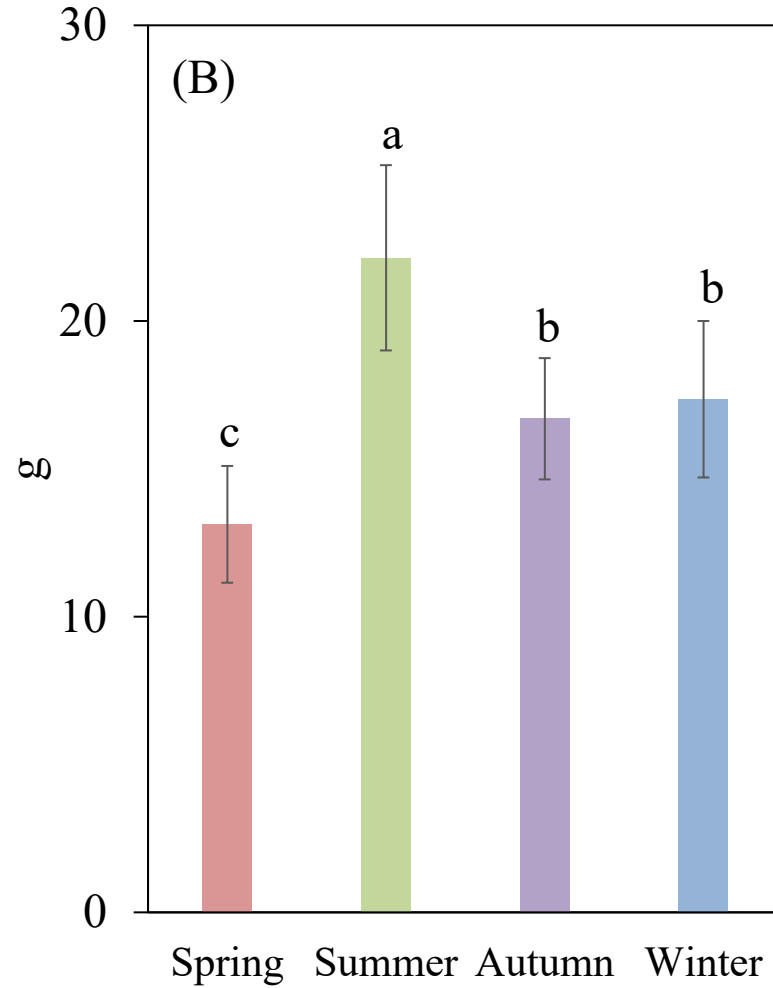
Values are mean ± standard deviation (µg/g wet sample). Different letters indicate significant difference between groups ($p < 0.05$).

Fig. 1

Body length



Body weight



Condition factor

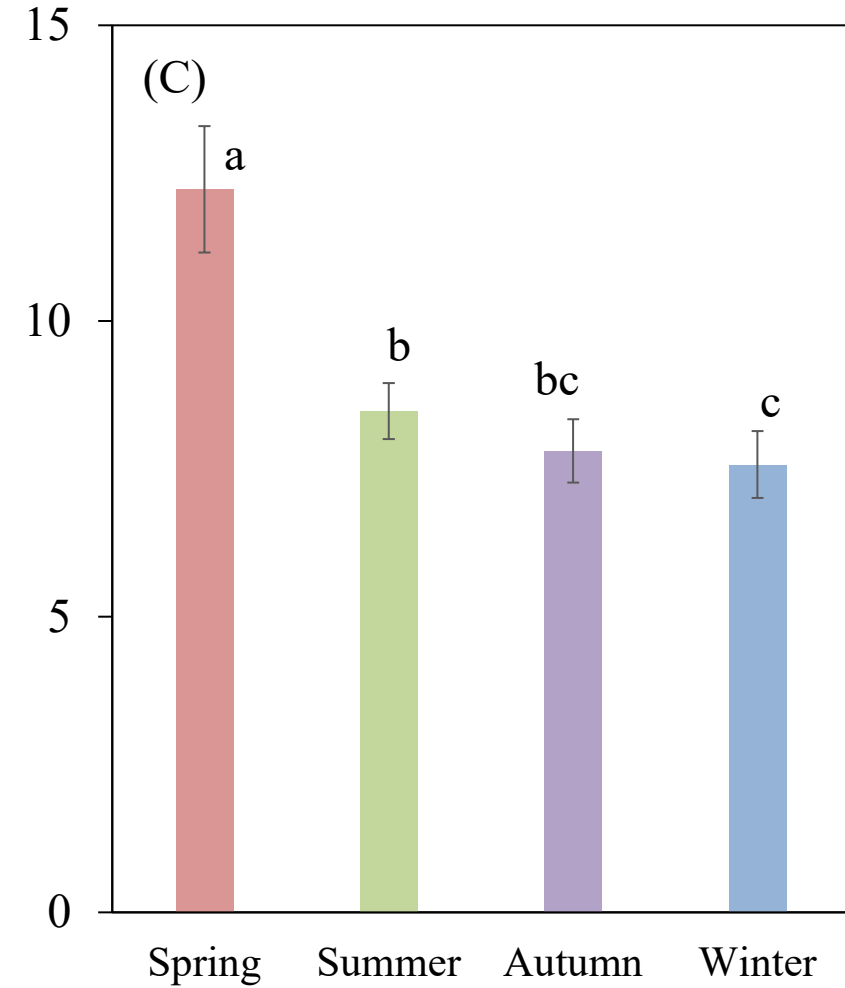


Fig. 2

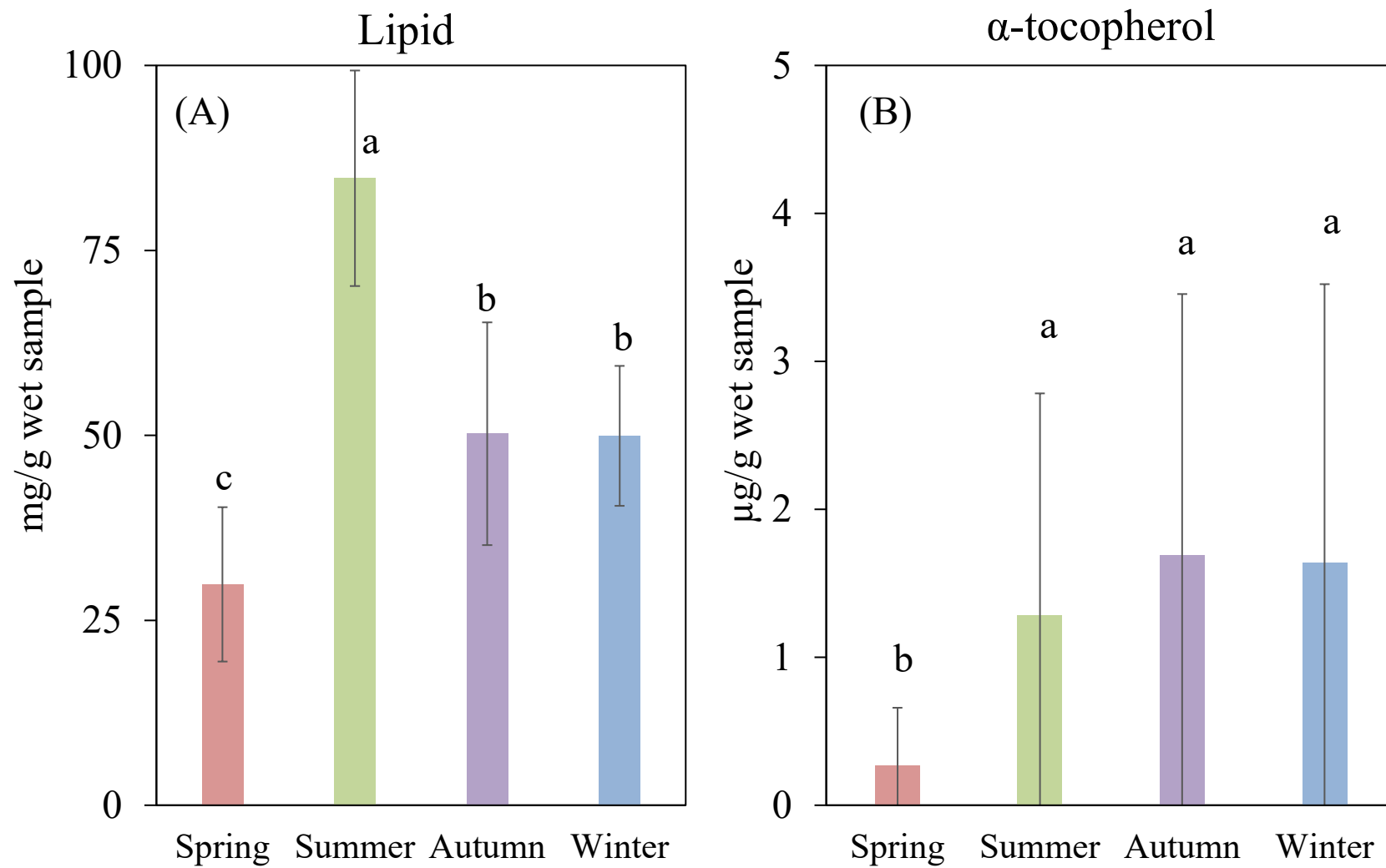


Fig. 3

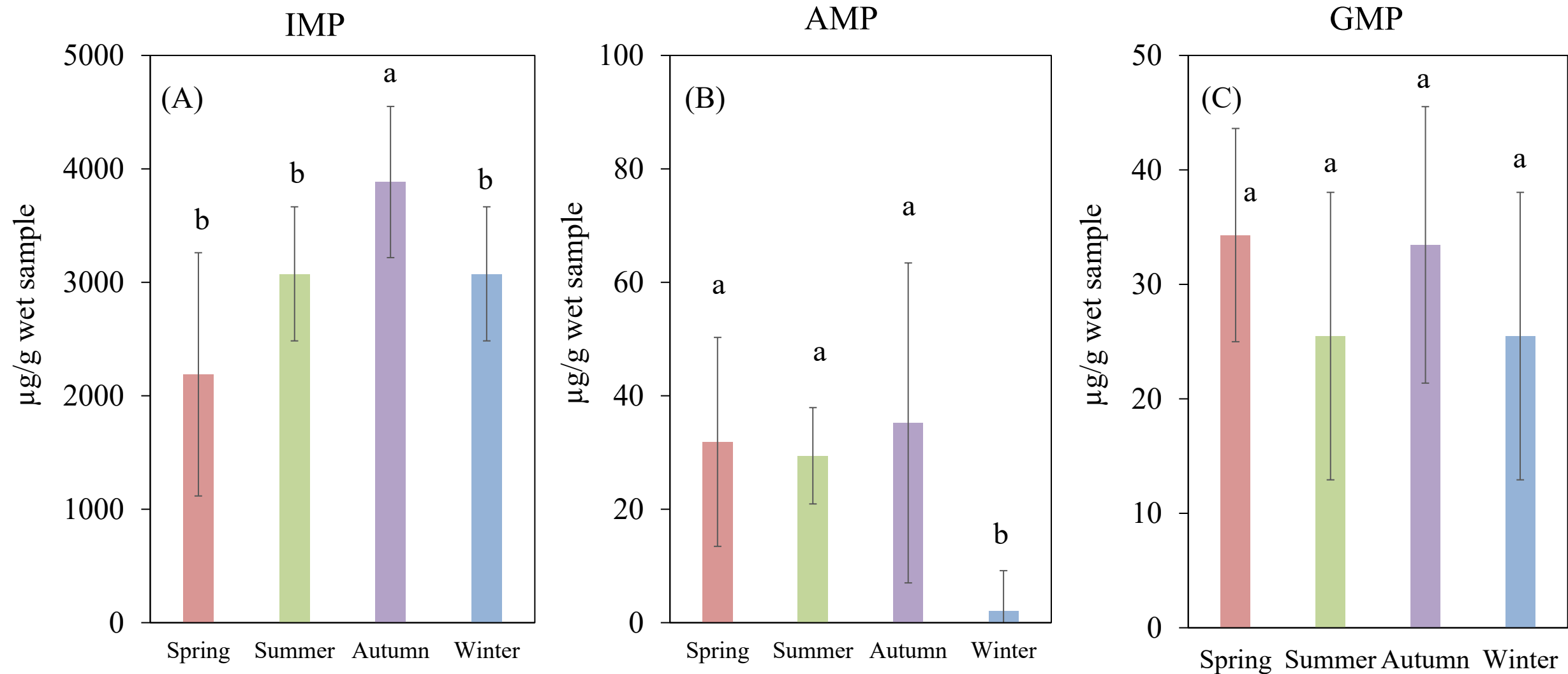


Fig. 4

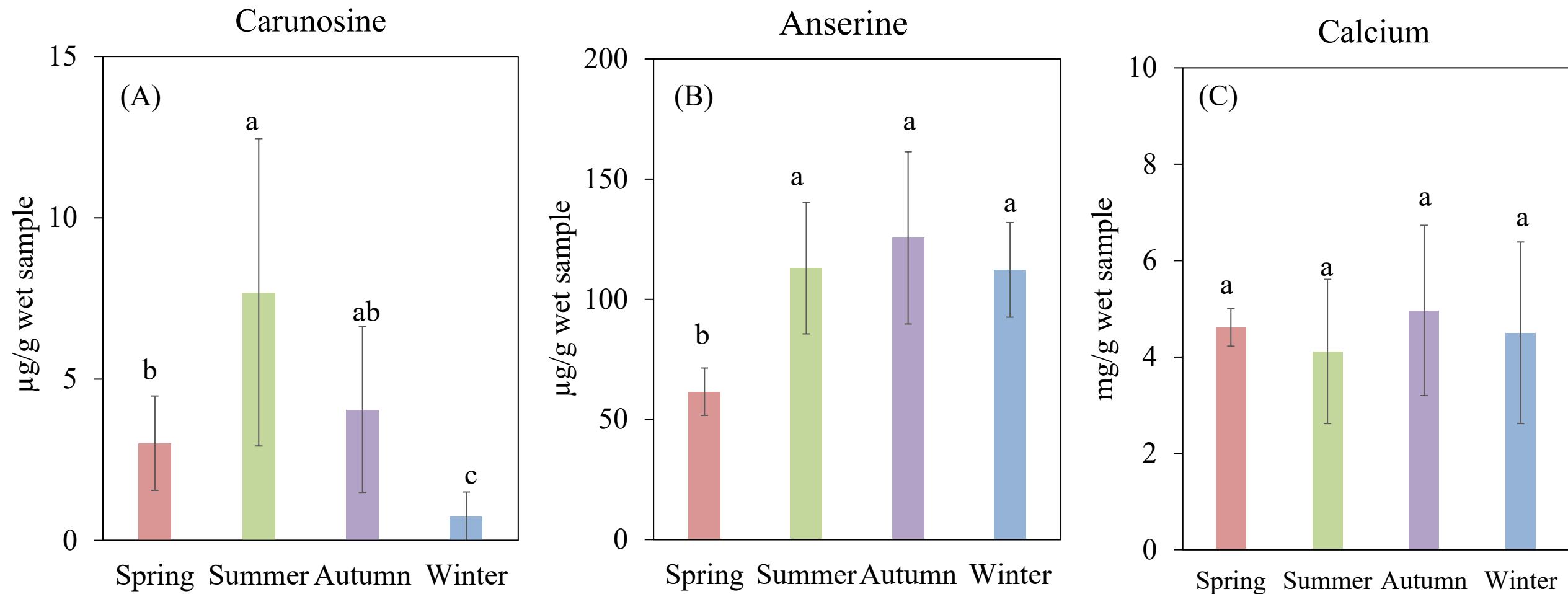


Figure Captions

Fig. 1. Seasonal changes in (a) body length (cm), (b) body weight (g), and (c) condition factor of greeneye over a year. Body length was measured from the snout to the base of the fins. The condition factor was calculated as $1,000 \times (\text{body weight}/\text{total length}^3)$. Values are presented as mean \pm standard deviation. Different letters indicate significant differences between groups ($p < 0.05$).

Fig. 2. Seasonal changes in (a) lipid and (b) α -tocopherol contents of greeneye over a year. Values are presented as mean \pm standard deviation. Different letters indicate significant differences between groups ($p < 0.05$).

Fig. 3. Seasonal changes in (a) inosinic acid (IMP), (b) adenylic acid (AMP), and (c) guanylic acid (GMP) contents of greeneye over a year. Values are presented as mean \pm standard deviation. Different letters indicate significant differences between groups ($p < 0.05$).

Fig. 4. Seasonal changes in (a) carnosine, (b) anserine, and (c) calcium contents of greeneye over a year. Values are presented as mean \pm standard deviation. Different letters indicate significant differences between groups ($p < 0.05$).

Appendix A

Table S1. Chromatographic parameters of regression equation, correlation factor, and linear range in HPLC analysis on the study.

	Regression equation	Correlation factor	Linear range
Vitamin E			
α -Tocopherol	$Y = 8.79 \times 10^{-6} X + 2.43 \times 10^{-1}$	0.9994	0.11-34.80 (nmol/mL)
Inosine monophosphate			
GMP	$Y = 3.25 \times 10^{-5} X + 1.36 \times 10^{-2}$	1.0000	0.55-25.22 (nmol/mL)
IMP	$Y = 8.40 \times 10^{-5} X + 5.13 \times 10^{-1}$	0.9999	1.34-214.41 (nmol/mL)
AMP	$Y = 2.54 \times 10^{-5} X - 2.67 \times 10^{-2}$	0.9999	0.47-60.39 (nmol/mL)
Amino acid			
Asp	$Y = 9.28 \times 10^{-6} X - 2.17$	0.9997	3.56-475.68 (nmol/mL)
Glu	$Y = 6.32 \times 10^{-6} X - 2.25$	0.9997	1.15-476.69 (nmol/mL)
Ser	$Y = 6.52 \times 10^{-6} X - 2.41 \times 10^{-1}$	0.9999	0.52-478.81 (nmol/mL)
Gly	$Y = 6.54 \times 10^{-6} X + 5.40$	0.9976	8.78-475.44 (nmol/mL)
His	$Y = 2.21 \times 10^{-6} X - 9.63$	0.9973	3.63-477.33 (nmol/mL)
Tau	$Y = 5.06 \times 10^{-6} X + 4.21$	0.9987	4.59-473.88 (nmol/mL)
Thr	$Y = 5.30 \times 10^{-6} X + 4.64$	0.9981	4.87-470.65 (nmol/mL)
Ala	$Y = 7.35 \times 10^{-6} X + 6.11$	0.9975	6.43-470.66 (nmol/mL)
Arg	$Y = 4.03 \times 10^{-6} X + 5.94$	0.9974	6.04-468.39 (nmol/mL)
Ans	$Y = 5.31 \times 10^{-6} X + 5.15$	0.9969	5.06-493.29 (nmol/mL)
Pro	$Y = 1.13 \times 10^{-6} X + 2.57 \times 10^{-1}$	0.9999	0.93-479.57 (nmol/mL)
Tyr	$Y = 6.54 \times 10^{-6} X + 4.52 \times 10^{-1}$	0.9999	1.21-478.07 (nmol/mL)
Val	$Y = 4.04 \times 10^{-6} X + 7.97 \times 10^{-1}$	0.9997	0.92-483.43 (nmol/mL)
Met	$Y = 5.02 \times 10^{-6} X + 3.82$	0.9988	3.95-485.81 (nmol/mL)
Ile	$Y = 2.95 \times 10^{-6} X - 2.03$	0.9995	0.97-473.88 (nmol/mL)
Leu	$Y = 2.76 \times 10^{-6} X - 4.77 \times 10^{-1}$	0.9996	0.25-474.14 (nmol/mL)
Lys	$Y = 5.73 \times 10^{-6} X - 1.57$	0.9998	1.79-482.05 (nmol/mL)
Phe	$Y = 2.28 \times 10^{-6} X - 7.77 \times 10^{-2}$	0.9997	0.28-474.95 (nmol/mL)
Imidazole dipeptide			
Carnosine	$Y = 3.20 \times 10^{-4} X + 1.86 \times 10^4$	0.9997	0.92-2212.39 (nmol/mL)
Balenine	$Y = 2.87 \times 10^{-4} X + 2.58 \times 10^4$	0.9999	1.18-2083.33 (nmol/mL)
Anserine	$Y = 2.89 \times 10^{-4} X - 1.65 \times 10^4$	0.9999	1.39-2252.33 (nmol/mL)
Calcium			
Calcium*	$Y = 1.46 \times 10^2 X + 6.71$	0.9603	0.05-8.75 (μ mol/mL)

X = HPLC Peak area; Y = Mass concentration (nmol/mL)

Calcium* X = absorbance at 570 nm; Y = Mass concentration (μ mol/mL)

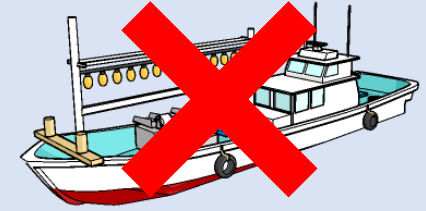


Greeneyes
(Chlorophthalmus albatrossis)

In Japan
Consuming the entire body including
the bones



We can get more calcium



In Japan
Fishing is banned after **Spring** to
protect juvenile greeneye from
spawning and bycatch

Analysis
for 4 seasons

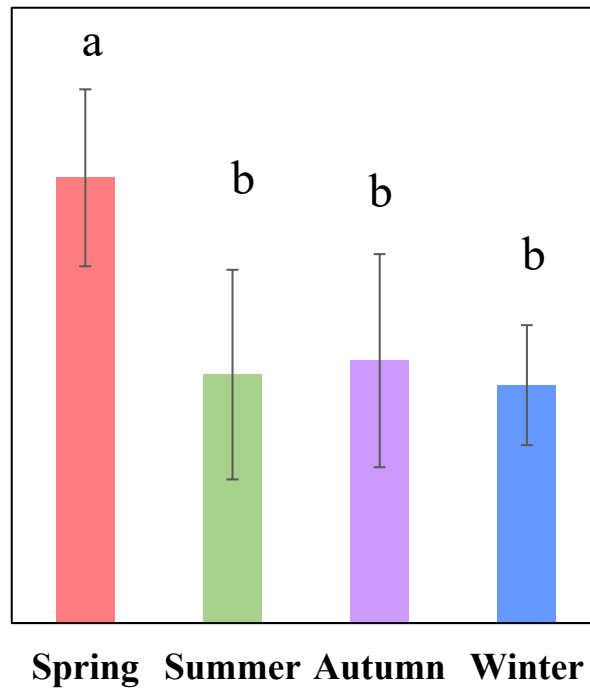
Free amino
acids
Inosinic acid

Lipid
Fatty acids
Vitamin E
Anserine

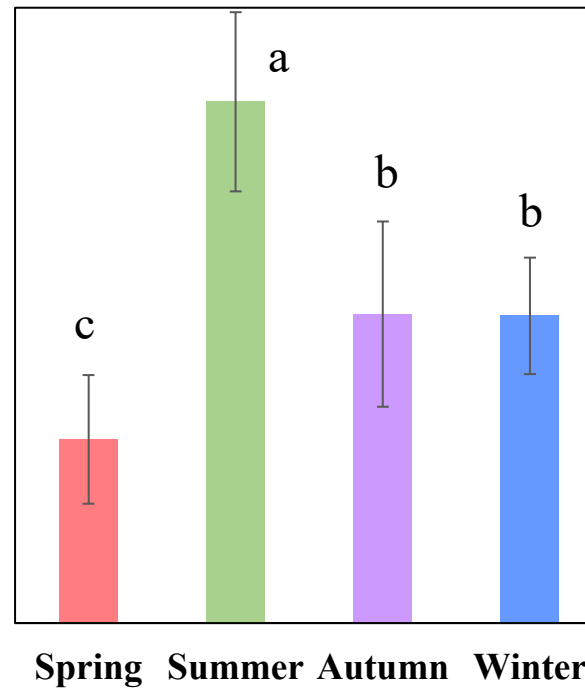
Calcium



Free amino acids



Lipid



Calcium

