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A comparison of food sources of nudibranch mollusks at different depths off the Kuril Islands using fatty acid trophic markers

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Gastropod molluscs such as nudibranchs are important members of deep-sea benthic ecosystems. However, data on the trophic ecology and feeding specialization of these animals are limited to date. The method of fatty acid trophic markers (FATM) was applied to determine the dietary preferences of nudibranchs off the Kuril Islands. Fatty acid (FA) compositions of *Dendronotus* sp., *Tritonia* sp., and *Colga pacifica* collected from deep waters were analyzed and compared with those of *Aeolidia papillosa* and *Coryphella verrucosa* from the offshore zone. The high level of FATM such as 22:5n-6 and C_{20} monounsaturated FAs indicated that *Dendronotus* sp. preys on sea anemones and/or anthoathecates hydroids similarly to the shallow-water *A. papillosa* and *C. verrucosa*. The high percentage of tetracosapolyenoic acids and the ratio 24:6n-3/24:5n-6 indicated that *Tritonia* sp. preys on soft corals such as *Gersemia rubiformis* at a depth of 250 m, but soft corals of the family Primnoidae may be the main item in the diet of *Tritonia* sp. at a depth of 500 m. The high content of Δ7,13-22:2 and 22:6n-3 shows that *C. pacifica* can feed on bryozoans. In *C. pacifica*, 22:5n-6 may be synthesized intrinsically by the mollusks, whereas odd-chain and branched saturated FAs originate from bacteria associated

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Abstract

Gastropod molluscs such as nudibranchs are important members of deep-sea benthic ecosystems. However, data on the trophic ecology and feeding specialization of these animals are limited to date. The method of fatty acid trophic markers (FATM) was applied to determine the dietary preferences of nudibranchs off the Kuril Islands. Fatty acid (FA) compositions of *Dendronotus* sp., *Tritonia* sp., and *Colga pacifica* collected from deep waters were analyzed and compared with those of *Aeolidia papillosa* and *Coryphella verrucosa* from the offshore zone. The high level of FATM such as 22:5n-6 and C_{20} monounsaturated FAs indicated that *Dendronotus* sp. preys on sea anemones and/or anthoathecates hydroids similarly to the shallow-water *A. papillosa* and *C. verrucosa*. The high percentage of tetracosapolyenoic acids and the ratio 24:6n-3/24:5n-6 indicated that *Tritonia* sp. preys on soft corals such as *Gersemia rubiformis* at a depth of 250 m, but soft corals of the family Primnoidae may be the main item in the diet of *Tritonia* sp. at a depth of 500 m. The high content of Δ7,13-22:2 and 22:6n-3 shows that *C. pacifica* can feed on bryozoans. In *C. pacifica*, 22:5n-6 may be synthesized intrinsically by the mollusks, whereas odd-chain and branched saturated FAs originate from bacteria associated.

Subjects Biochemistry, Marine Biology, Aquatic and Marine Chemistry
Keywords Nudibranchia, Fatty acids, Trophic markers, Food sources, Food webs

Introduction

Nudibranchs are a group of marine soft-bodied gastropod mollusks (Gastropoda: Nudibranchia Cuvier, 1817). The greatest diversity of nudibranchs is observed in warm shallow waters, although nudibranchs occur worldwide, from Arctic to Antarctic regions, with some species discovered at a depth near 2500 m (*Ekimova et al., 2015*). Identification of food sources of nudibranchs is important for understanding their ecology and description of trophic interactions in marine benthic ecosystems (*Ekimova et al., 2019*). Nudibranchs are carnivorous, but detritus may comprise some part of their diet (*Ekimova et al., 2019*). Nudibranch can feed on soft corals, reef-building corals, sponges, bryozoans, tunicates, barnacles, sea anemones, jellyfish, ophiuroids, colonial hydroids, and other nudibranchs (*Barnes & Bullough, 1996; McDonald & Nybakken, 1997, 1999; Goodheart et al., 2017*). Many nudibranch species exhibit high dietary specialization (*Hoover et al., 2012; Goodheart et al., 2017; Ekimova et al., 2019; Imbs & Grigorchuk, 2019*). In contrast to shallow-water species, data on feeding of deep-sea nudibranch species still remain limited.

Fatty acids (FAs) have been used as biochemical markers to trace predator–prey relationships in marine ecosystems for more than 40 years (*Kelly & Scheibling, 2012; Braeckman et al., 2015; Calado & Leal, 2015*). The method of FA trophic markers (FATM) was successfully applied to determine possible origins of food in several nudibranch species from tropical shallow waters (*Zhukova, 2014*) and the deep-sea nudibranchs *Tritonia tetraquetra*, *Dendronotus* sp., and *D. robustus* collected in the Kurile Islands region (*Imbs, 2016; Imbs & Chernyshev, 2019; Imbs & Grigorchuk, 2019*). FATM have shown that *Dendronotus* sp. and *T. tetraquetra* prey on different species of cold-water soft corals, while *D. robustus* may consume hydrocorals and bryozoans (*Imbs & Grigorchuk, 2019*). Thus, the difference in the feeding specializations between two species belonging to the same genus (*Dendronotus*) and inhabiting the same waters is confirmed by using FATM.

Waters around the Kuril Islands, with their significant depth differences, are one of the world's most productive marine ecosystem (*Shuntov et al., 2019*). As a continuation of ecological studies on deep-sea mollusks, FA compositions of total lipids of three nudibranch species (*Colga pacifica*, *Dendronotus* sp., and *Tritonia* sp.) collected from deep waters (up to 500 m) were analyzed and compared with those of two nudibranch species (*Aeolidia papillosa* and *Coryphella verrucosa*) from the offshore zone (about 20 m) of the Kurile Islands. Dietary preferences of these five species were studied using the method of FATM. Nudibranchs are a common animal group of this area and, therefore, are important for trophic relationships in the ecosystem studied.

Materials and Methods

Sampling and fatty acid preparation

Sampling was conducted aboard the R/V Akademik Oparin near Simushir Island (Kuril Islands, Sea of Okhotsk, 47°08′ N, 152°14′ E) in July 2019. Nudibranch specimens were collected at a

depth of 20 m by SCUBA and at the depth of 250–500 m by dredging. The samples were represented by two families: Polyceridae (*Colga pacifica* (Bergh, 1894)) and Aeolidiidae (*Aeolidia papillosa* (Linnaeus, 1761), *Coryphella verrucosa* (M. Sars, 1829), *Tritonia* sp., and *Dendronotus* sp.). Lipids were extracted from the specimens as described by *Bligh and Dyer (1959)*. FA methyl ethers (FAME) were prepared using the method of *Carreau and Dubacq (1978)* and were purified by preparative thin-layer chromatography in benzene. The 4,4-dimethyloxazoline (DMOX) derivatives of FA were prepared according to the method of *Svetashev (2011)*.

Fatty acid analysis

A gas chromatography analysis of FAME was conducted with a GC-2010 chromatograph (Shimadzu, Kyoto, Japan) with a flame ionization detector. An Equity-5 (Supelco, Bellefonte, USA) capillary column (30 m × 0.25 mm ID, film thickness 25 µm) was held for 2 min at 170 °C, then heated with a 2 °C · min^{-1} ramp to 240 °C that was held for 5 min. The injector (250 °C) and detector (260 °C) temperatures were constant. Helium was used as the carrier gas at a linear velocity of 30 cm · s^{-1}. Identification of FAs was confirmed by gas chromatography−mass spectrometry (GC−MS) of their methyl esters and DMOX derivatives on a GCMS-2010 Ultra instrument (Shimadzu, Kyoto, Japan) (electron impact at 70 eV) and a MDN-5s (Supelco, Bellefonte, USA) capillary column (30 m × 0.25 mm ID). Carrier gas was He at 30 cm · s^{-1}. The GC−MS analysis of FAME was performed at 160 °C with a 2 °C · min^{-1} ramp to 240 °C that was held for 20 min. The injector and detector temperatures were 250 °C. GC−MS of DMOX derivatives was performed at 210 °C with a 3 °C · min^{-1} ramp to 270 °C that was held for 40 min. The injector and detector temperatures were 270 °C. Spectra were compared with the NIST library and the online FA mass spectra archive website (*Christie, 2021*).

Statistical analysis

Significance of differences in mean contents of FA between the nudibranch species was tested by one-way analysis of variance (ANOVA). Raw data were used after being tested for the homogeneity of variances (Levene's test) and normality of data distribution (Shapiro–Wilk test). Significant differences between levels were examined post hoc with Tukey–Kramer HSD multiple comparisons test. To represent differences between the nudibranch species, the variables (square roots of FA contents) were included in principal components analyses (PCA). All statistical analyses were performed using STATISTICA 5.1 (StatSoft, Inc., USA). A statistical probability of $p < 0.05$ was considered significant. Values are represented as mean ± standard deviation. Cluster analysis was performed using Wada's method (Minimum variance method) and the pvclust( ) function in the pvclust package provides p-values for hierarchical clustering based on multiscale bootstrap resampling (*Suzuki & Shimodaira, 2006*) available in the R-Studio software (R-Tools Technology, Canada).

Results

The full FA composition of total lipids in the five nudibranch species from different depths is summarized in Table S1. The average contents of the major 20 FAs are shown in Table 1. The main saturated FA (SFA) was 16:0, and the major monounsaturated FAs (MUFAs) were 20:1n-9 and 20:1n-7. Lipids of all nudibranchs contained branched and odd-chain SFAs; the highest levels of these acids were detected in some specimens of *A. papillosa* and *C. pacifica* (up to 11 and 17% of total FAs, respectively).

Acids 20:4n-6, 20:5n-3, and 22:6n-3 dominated polyunsaturated FAs (PUFAs) of the nudibranchs studied except for *Tritonia* sp. The lowest level of 22:6n-3 (HSD test, p = 0.0004) and considerable amounts ($F_{4,11} = 22.2735$, $p < 0.0001$) of very-long-chain tetracosapolyenoic acids (TPA), 24:5n-6 and 24:6n-3, were found in *Tritonia* sp. The ratio 24:6n-3/24:5n-6 in the specimens from a depth of 450–516 m (7.0 ± 2.2) was significantly higher ($F_{1,3} = 12.7326$, $p = 0.0376$) than that in the specimens from a depth of 210–247 m (1.3 ± 0.1). The level of 20:5n-3 was significantly lower (HSD test, $p = 0.012$) in the deep-sea *C. pacifica* than that in the shallow-water *C. verrucosa*. Unusually high percentages of 22:5n-6 were detected in two specimens of *C. pacifica* (9.6 and 18.6% of total FAs). *A. papillosa* contained the highest level of 22:5n-3. Several non-methylene-interrupted FAs (NMI FAs) were present in total FAs of all mollusk species. The highest level (HSD test, $p = 0.0007$) of Δ5,11-20:2 in the *Tritonia* sp. specimens distinguished them from other nudibranchs. All species (except for *C. verrucosa*) contained noticeable amounts of Δ7,13-22:2.

Analyses of the FA composition data (Table 1) by ANOVA identified certain FAs that were mainly responsible for the difference between species from deep and shallow waters. Compared to ~~the~~ shallow-water species, ~~the~~ deep-sea ones contained significantly higher ($p < 0.05$) levels of 20:4n-6 and Δ7,15-22:2, but significantly lower ($p < 0.05$) levels of 14:0, 16:1n-7, 20:1n-11, 20:1n-7, 20:5n-3, 22:4n-6, and 22:5n-3. No differences ($p > 0.05$) were found for other FAs listed in Table 1.

Results of a cluster analysis of the FA composition data (Table 1) for the five nudibranch species are shown in Figure 1. All ~~the~~ studied specimens were subdivided into three groups: the first and ~~the~~ second groups consisted ~~by~~ deep-sea specimens of *Tritonia* sp. and *C. pacifica*, respectively, and the third group combined specimens of the deep-sea *Dendronotus* sp. with the shallow-water species *A. papillosa* and *C. verrucosa*.

~~Then,~~ the FAs listed in Table 1 were used as variables for PCA. In this analysis, the first two PCA components explained 50% of the variance of the FA composition data. Figure 2A shows that *Tritonia* sp. is clearly separated from all other nudibranch species along the first PCA component, linking positively with 20:4n-6, 24:5n-6, and 24:6n-3, and negatively with 22:4n-6 and 22:6n-3 (Fig. 2B). The second PCA component separates *C. pacifica* from the group of *Dendronotus* sp., *A. papillosa*, and *C. verrucosa* (Fig. 2A). Figure 2B shows that the level of SFAs (16:0 and 18:0), MUFAs (20:1n-9 and 20:1n-7), and 20:5n-3 vs. the level of 22:5n-6 and NMI FAs is significant for this separation. The level of 22:5n-6 was significantly higher ($F_{1,14} = 6.555$, $p = 0.023$) in the group of *Dendronotus* sp., *A. papillosa*, and *C. verrucosa* than that in *Tritonia* sp. and *C. pacifica*. The PCA results (Fig. 1) agree with the results of cluster analysis

(Fig. 2) and show a significant difference in FA profiles between ~~the~~ deep-sea *Tritonia* sp., *C. pacifica*, and the two shallow-water species. Both statistical methods confirm that the FA profiles of the deep-sea *Dendronotus* sp. and the shallow-water species are similar.

Discussion

According to literature data, the nudibranchs of ~~the~~ genera *Aeolidia*, *Coryphella*, and *Dendronotus* prey on different Cnidaria ~~groups~~ (*Hall & Todd, 1986*). The nudibranch *A. papillosa* is known to prey on sea anemones and consume their nematocyst (stinging capsular organelles) to protect against other predators (*Hall & Todd, 1986*). The considerable levels of 22:5n-6 and C_{20-22} MUFAs are characteristic for the FA composition of shallow- and deep-water sea anemones (*Kiyashko et al., 2014; Revel et al., 2016*). Obviously, the noticeable amounts of 22:5n-6 and C_{20} MUFAs that we found in *A. papillosa* originate from sea anemone lipids consumed by this nudibranch species. Different feeding specializations on polyps of scyphoid jellyfish (*Hernroth & Grondahl, 1985; Ostman, 1997*), soft corals (*Sebens, 1983; Allmon & Sebens, 1988*), and hydroids of ~~the~~ orders Anthoathecata (the genera *Tubularia*, *Clava*, and *Hydractinia*) and Leptothecata (the genus *Obelia*) (*Kuzirian, 1979*) were reported for another shallow-water nudibranch, *C. verrucosa*. Very-long-chain C_{24} PUFAs are FATM of soft corals and jellyfishes (*Svetashev & Vysotskii, 1998; Imbs et al. 2010, 2016; Svetashev, 2019*). TPA are proposed as biomarkers for marine food web studies (*Drazen et al. 2008; Blanchet-Aurigny et al. 2015*). Trace amounts of C_{24} PUFAs in *C. verrucosa* indicate that this species from the Kuril Islands probably preys on anthoathecates hydroids, which may be a source of nematocysts (*Frick, 2003*) and an explanation for the increasing levels of 22:5n-6 and C_{20} MUFAs in ~~*C. verrucosa*~~.

The radula morphology in *Dendronotus* sp. is very similar to that of *Dendronotus lacteus* and *D. rufus* and has a large number of knife-like lateral teeth that nudibranch may use for biting off soft tissues of polyps (*Ekimova et al., 2019*). There is some evidence that *D. lacteus* and *D. rufus* feed on hydroids of ~~the~~ family Sertulariidae (order Leptothecata), scyphistomaes, and anemones (*Ekimova et al., 2019*). Considering the close similarity between the FATM profiles of *A. papillosa*, *C. verrucosa*, and *Dendronotus* sp., we assume that the increased 22:5n-6 and C_{20} MUFAs levels in the deep-sea nudibranch *Dendronotus* sp. from the Sea of Okhotsk indicate its preying on sea anemones and/or anthoathecates hydroids, similarly to ~~the~~ shallow-water species~~.~~ *A. papillosa* and *C. verrucose*.

Several species of the genus *Tritonia* are known to be obligate predators feeding on soft corals (*Allmon & Sebens, 1988; Goddard, 2006*). Recently, an analysis of the FA composition of the nudibranch *Tritonia tetraquetra* preying on soft corals (the Sea of Okhotsk) has shown an intensive transfer of a soft coral FATM (24:5n-6 and 24:6n-3) from prey to predator (*Imbs, 2016*). The ratio 24:6n-3/24:5n-6 was compared between *T. tetraquetra* (1.1 ± 0.2) and several soft coral species. As a result, the soft coral *Gersemia rubiformis* was suggested as most probable food source of *T. tetraquetra*~~.~~ (*Imbs, 2016*). The high percentage of TPA in the deep-sea nudibranch *Tritonia* sp. also ~~indicates~~ preying on soft corals. Based on the ratio 24:6n-

3/24:5n-6, we can assume that *Tritonia* sp. at a depth of 250 m mainly feed on the *Gersemia* soft corals similarly to *T. tetraquetra*. The significant increase in the ratio 24:6n-3/24:5n-6 in *Tritonia* sp. with increasing depth indicates a change in the taxonomic group of soft corals consumed. Among ~~of the~~ deep-sea soft corals ~~of~~ the Sea of Okhotsk, the high ratio 24:6n-3/24:5n-6 is characteristic ~~only~~ of soft corals ~~of~~ the family Primnoidae (*Imbs et al., 2016*) ~~that can be~~ the major food source of *Tritonia* sp. at a depth of 500 m.

To our knowledge, food sources of *C. pacifica* have not been identified to date. There is only a brief mention that species of ~~the~~ genus *Colga* can feed on members of ~~the~~ phylum Bryozoa (*Grischenko & Martynov, 1997; Behrens, 2004*). A noticeable level of Δ7,13-22:2 and 22:6n-3 has been detected in total FAs of the bryozoan *Dendrobeania flustroides* from the Sea of Okhotsk (*Demidkova, 2010*). The high content of these two FAs in *C. pacifica* confirms that this deep-sea species ~~can~~ feed on bryozoans. Other characteristic FAs of *C. pacifica* such as 22:5n-6, odd-chain and branched SFAs may originate from own biosynthesis or associated organisms.

The unexpectedly high content of 22:5n-6 found in *C. pacifica* may be a result of high activity of C_2 elongase and Δ4 desaturase that convert 20:4n-6 into 22:5n-6. Such activity has been ~~supposed~~ in the hydrocoral Millepora to explain the extremely high levels of 22:5n-6 and 22:6n-3 (*Imbs et al., 2019, 2021*). The relatively low level of 20:5n-3 in *C. pacifica* can be due to either conversion of 20:5n-3 to 22:6n-3 or a deficiency ~~of~~ dietary 20:5n-3 in deep waters (*Kiyashko et al., 2014*). Odd-chain and branched SFAs in marine invertebrates indicate the presence of associated bacteria (*Kharlamenko & Kiyashko 2018*). Various bacteria have been found in visceral organs of nudibranchs (*Zhukova & Eliseikina, 2012*). An abundant bacterial community may be a cause of the highest level of "bacterial" SFAs in *C. pacifica*.

Conclusions

FA profiles of five nudibranch mollusk species belonging to ~~the~~ families Polyceridae, Tritoniidae, Dendronotidae, Coryphellidae, and Aeolidiidae, collected near Simushir Island, Sea of Okhotsk, were determined. The feeding specializations of ~~the~~ deep- and shallow-water species were compared on the base of ~~their~~ FATM ~~compositions~~. ~~The~~ different species from different depths, but with similar food sources, showed similar FATM profiles. ~~The~~ species composition of soft corals consumed by Tritonia sp. change~~s~~ with increasing depth. Deep-sea nudibranchs of ~~the~~ genus *Colga* are most promising objects for future studies. ~~T~~The proportion between dietary and ~~biosynthetic origins in their~~ PUFAs should ~~to~~ be assessed.

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Author Contributions

Anatolii Komisarenko collected the samples, performed the lipid analyses, analysed the data, authored or reviewed drafts of the paper, approved the final draft.
Vladimir Mordukhovich conceived and designed the experiments, analysed the data, authored or reviewed drafts of the paper, approved the final draft.
Irina Ekimova collected the samples, determine the biological species, performed biological and morphological studies, approved the final draft.
Andrey Imbs conceived and designed the experiments, contributed reagents/materials/analysis tools, analysed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.

Data Availability

The following information was supplied regarding data availability: Raw data in the format of Word Table documents are provided as the DOCX file in the Supplemental Materials.

Supplemental Information

Supplemental information for this article can be found online.

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Figure titles and legends

Figure 1 Results of a cluster analysis of the FA composition data for the five nudibranch species. The numerals on the branches represent are bootstrap probability (BP) value of a cluster and approximately unbiased (AU) probability values. TS, *Tritonia* sp.; CP, *Colga pacifica*; CV, *Coryphella verrucosa*; DS, *Dendronotus* sp.; AP, *Aeolidia papillosa*.

Figure 2 Results of a principal component analysis (PCA) of the FA composition data for the five nudibranch species. (A) The plot of the first two principal components; variables were the major fatty acids (see Table 1). Ellipses were drawn manually to outline three groups according to results of the cluster analysis (see Figure 1). (B) The projectiosn of 12 variables is shown. TS, *Tritonia* sp.; CP, *Colga pacifica*; CV, *Coryphella verrucosa*; DS, *Dendronotus* sp.; AP, *Aeolidia papillosa*.

Table 1(on next page)

Fatty acid composition (% of total FAs) of nudibranch molluscs.

The species were collected at different depths near Simushir Island (Kuril Islands, Sea of Okhotsk). Values are means ± SD; asterisks indicate significant differences ($p < 0.05$) between the groups of shallow-water species (*C. verrucosa* and *A. papillosa*) and deep-sea species (*C. pacifica*, *Tritonia* sp., and *Dendronotus* sp.).

TABLE 1 Fatty acid composition (% of total FAs) of nudibranch molluscs. The species were collected at different depths near Simushir Island (Kuril Islands, Sea of Okhotsk). Values are means ± SD; asterisks indicate significant differences ($p < 0.05$) between the groups of shallow-water species (*C. verrucosa* and *A. papillosa*) and deep-sea species (*C. pacifica*, *Tritonia* sp., and *Dendronotus* sp.).

Fatty acids	Species names and sampling depths					Comparison of shallow- and deep-water groups by ANOVA	
	Shallow-water group		Deep-water group				
	Coryphella verrucosa, 0–20 m, $n = 3$	*Aeolidia papillosa*, 0–20 m, $n = 2$	*Colga pacifica*, 285–304 m, $n = 3$	*Tritonia* sp., 210–516 m, $n = 5$	*Dendronotus* sp., 210–516 m, $n = 3$	$F_{1,14}$	p
14:0*	4.2 ± 2.4	0.8 ± 0.0	1.3 ± 0.6	0.4 ± 0.2	0.9 ± 0.4	7.612	0.015
16:0	11.2 ± 2.9	7.7 ± 0.9	9.3 ± 1.3	14.7 ± 2.2	12.6 ± 1.7	2.456	0.139
16:1n-7*	2.8 ± 1.0	0.7 ± 0.1	1.4 ± 0.2	0.6 ± 0.1	0.8 ± 0.2	7.436	0.016
18:0	2.7 ± 1.8	5.1 ± 0.0	3.7 ± 0.4	5.5 ± 1.0	8.2 ± 1.0	3.798	0.072
18:1n-9	3.5 ± 1.7	1.5 ± 0.1	1.3 ± 0.1	2.8 ± 0.6	2.5 ± 0.6	0.495	0.493
18:3n-3	1.1 ± 0.2	3.5 ± 0.6	3.5 ± 1.1	0.5 ± 0.1	0.8 ± 0.2	0.679	0.424
20:1n-11*	2.8 ± 0.1	1.3 ± 0.0	1.1 ± 0.1	0.6 ± 0.1	1.1 ± 0.5	6.982	0.019
20:1n-9	9.4 ± 2.6	1.8 ± 0.1	1.9 ± 0.5	1.5 ± 0.1	4.0 ± 1.1	3.053	0.102
20:1n-7*	5.9 ± 2.3	4.3 ± 0.0	1.9 ± 0.1	2.6 ± 0.2	3.9 ± 1.3	5.417	0.035
Δ5,11-20:2	1.3 ± 0.9	2.5 ± 0.1	0.6 ± 0.2	1.6 ± 0.5	5.5 ± 0.3	0.389	0.543
20:4n-6*	3.2 ± 0.9	3.8 ± 0.2	4.3 ± 3.0	15.1 ± 4.1	8.3 ± 2.5	6.290	0.025
20:5n-3*	25.8 ± 12.4	15.5 ± 2.8	7.4 ± 3.1	13.7 ± 2.8	18.4 ± 0.5	4.647	0.049
Δ7,13-22:2*	0.6 ± 0.2	2.6 ± 0.4	10.8 ± 4.7	8.8 ± 2.1	3.2 ± 1.1	11.629	0.004
Δ7,15-22:2	0.3 ± 0.2	1.8 ± 0.4	0.7 ± 0.1	2.5 ± 0.4	1.1 ± 0.4	1.249	0.282
22:4n-6*	3.1 ± 1.8	4.1 ± 0.1	0.9 ± 0.5	0.6 ± 0.1	1.6 ± 1.5	7.726	0.015
22:5n-6	0.1 ± 0.1	3.5 ± 0.1	18.7 ± 5.9	0.6 ± 0.2	2.8 ± 1.0	0.504	0.489
22:5n-3*	2.5 ± 1.1	8.5 ± 1.9	1.4 ± 0.6	0.9 ± 0.2	2.0 ± 0.5	11.292	0.005
22:6n-3	8.5 ± 2.1	8.3 ± 0.0	12.0 ± 3.8	0.6 ± 0.1	11.0 ± 0.7	0.274	0.609
24:5n-6	0.0 ± 0.0	0.4 ± 0.1	0.5 ± 0.4	4.8 ± 2.8	0.0 ± 0.0	2.548	0.133

24:6n-3	0.9 ± 0.1	0.4 ± 0.0	0.7 ± 0.5	12.9 ± 2.6	0.3 ± 0.1	2.933	0.109

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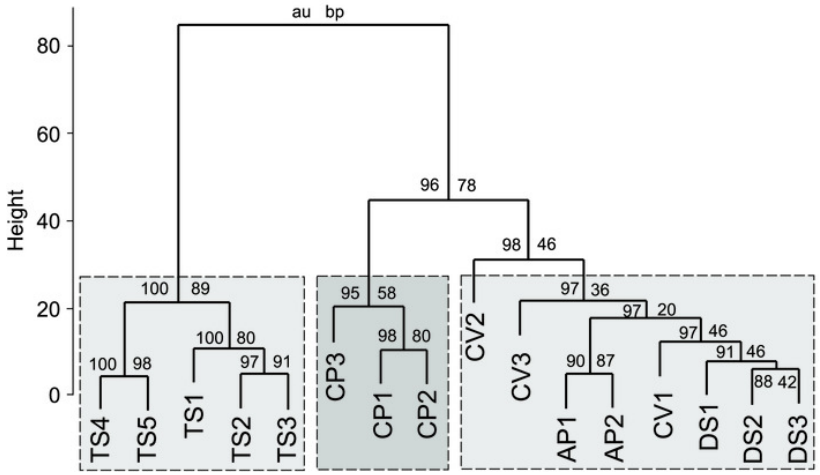
Figure 1

Results of a cluster analysis of the FA composition data for the five nudibranch species.

The numeral on the branches represent is bootstrap probability (BP) value of a cluster and approximately unbiased (AU) probability values. TS, *Tritonia* sp.; CP, *Colga pacifica*; CV, *Coryphella verrucosa*; DS, *Dendronotus* sp.; AP, *Aeolidia papillosa*.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88 42
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CV2
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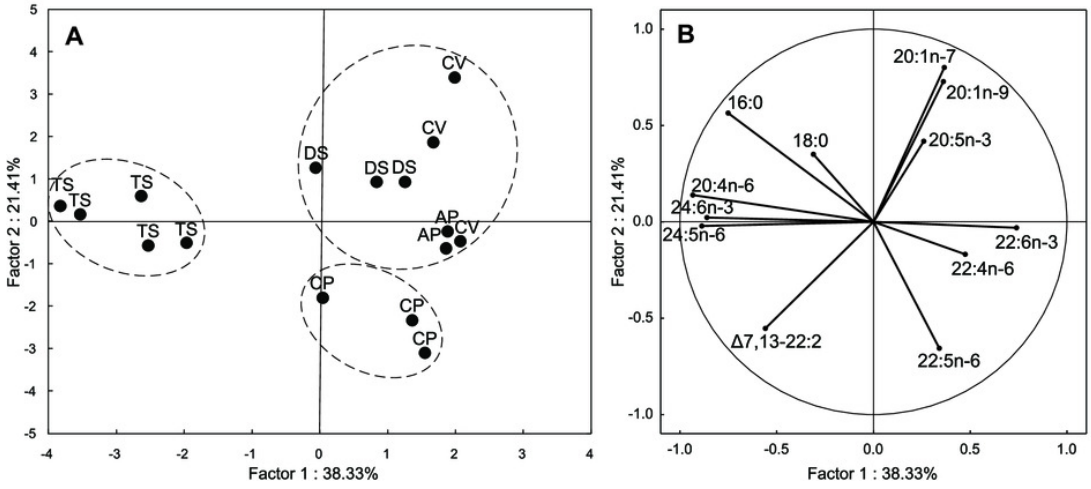
Figure 2

Results of a principal component analysis (PCA) of the FA composition data for the five nudibranch species.

(A) The plot of the first two principal components; variables were the major fatty acids (see Table 1). Ellipses were drawn manually to outline three groups according to results of the cluster analysis (see Figure 1). (B) The projectiosn of 12 variables is shown. TS, *Tritonia* sp.; CP, *Colga pacifica*; CV, *Coryphella verrucosa*; DS, *Dendronotus* sp.; AP, *Aeolidia papillosa*.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Factor 2 : 21.41%
Factor 1 : 38.33%
B
20:1n-7
20:1n-9
16:0
18:0
20:5n-3
20:4n-6
24:6n-3
24:5n-6
22:6n-3
22:4n-6
Δ7,13-22:2
22:5n-6
Factor 2 : 21.41%
Factor 1 : 38.33%