

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

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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* ~~*tetraquetra*~~ 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<sub>20</sub> monounsaturated FAs indicated that *Dendronotus* sp. preys on sea anemones and/or anthothecates hydroids similarly to ~~that of the~~ shallow-water ~~species~~ *A. papillosa* and *C. verrucosa*. The high percentage of tetracosapolyenoic acids and the ratio 24:6n-3/24:5n-6 indicated that ~~*Tritonia. tetraquetra*~~ sp. preys on soft corals such as *Gersemia rubiformis* and/or *Acanella* at a depth of 250 m, but soft corals of the family Primnoidae may be the main item in the diet of ~~*T. tetraquetra Tritonia*~~ sp. at a depth of 500 m. The high content of Δ<sup>7,13</sup>-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 ~~associated~~ bacteria-associated.


**Subjects** Biochemistry, Marine Biology, Aquatic and Marine Chemistry

**Keywords** Nudibranchia, Cold-water species, Dietary fatty acids, Lipids~~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; Bertsch, 2020). Identification of food sources of nudibranchs is important for understanding their ecology and description of trophic interactions in marine benthic ecosystems (Ekimova et al., 2019b). Nudibranchs are mostly carnivorous, but detritus and microalgae may comprise some part of their diet (Ekimova, ~~Deart & Schepetov et al.~~, 2019a; Ekimova et al., 2019b). 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., 2019b; Imbs & Grigorchuk, 2019; Mikhlin et al., 2019<sup>8</sup>, Mikhlin, Ekimova & Vortsepneva, 2020). In contrast to shallow-water species, data on feeding regimes of deep-sea nudibranch species still remain limited (Chimienti et al., 2020).

Fatty acids (FAs) have been used as biochemical markers to trace predator–prey relationships in marine ecosystems for more than 40 years (Budge, Iverson & Koopman, 2006; Kelly & Scheibling, 2012; Braeckman et al., 2015; Calado & Leal, 2015; Budge et al., 2006). The method of FA trophic markers (FATM) was already 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* (Pallas, 1788), *Dendronotus* sp., and *D. robustus* A.E. Verril, 1870 collected in the Kurile Islands region (Imbs, 2016; Imbs & Chernyshev, 2019; Imbs & Grigorchuk, 2019). FATM showed 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). The difference in food sources between these two species belonging to the same genus (*Dendronotus*) and inhabiting the same waters was detected by using FATM. The detection of large amounts of dietary FAs in *T. tetraquetra* (Imbs, 2016; Imbs & Chernyshev, 2019) showed that the FATM method could be successfully apply for the study of trophic ecology of cold-water nudibranchs.

Waters around the Kuril Islands, with their significant depth differences, are one of the world's most productive marine ecosystem (Shuntov, Ivanov & Dulepova, 2019). Nudibranchs are a common animal group of this area and, therefore, play an important role on trophic dynamics in the ecosystem studied. To expand our knowledge on trophic ecology of deep-sea mollusks, FA comptions of total lipids of three nudibranch species (*Colga pacifica* (Bergh, 1894), *Tritonia tetraquetra* (Pallas, 1788), and *Dendronotus* sp.) collected from deep waters (up to 500 m) were analyzed and compared with those of two nudibranch species (*Aeolidia papillosa* (Linnaeus, 1761) and *Coryphella verrucosa* (M. Sars, 1829))

from the offshore zone (about 20 m) of the Kurile Islands. Dietary preferences of these five species were studied using the method of FATM. A possible influence of depth on nudibranch feeding specialization was discussed. 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). Nudibranchs are a common animal group of this area and, therefore, are important for trophic relationships in the ecosystem studied. 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 collection 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. In total, 18 individuals of mollusks were taken. For the nudibranchs *C. verrucosa* and *A. papillosa*, three individuals of each species were collected at a depth of 20 m by SCUBA and referred as a shallow-water group. Three specimens of *C. pacifica*, 3 specimens of *Dendronotus* sp., and 6 specimens of *T. tetraquetra* were collected at the depth of 250–500 m by dredging and referred as a deep-sea group. The nudibranch *Dendronotus* sp. was different from the one already reported (Imbs & Grigorchuk, 2019). Each specimen was photographed (Fig. 1); one piece of foot tissue was fixed in 96% EtOH for molecular analysis to confirm the identification of nudibranchs according to Ekimova et al. (2019b); the other piece of foot tissue was frozen at –80 °C for lipid analysis. Unfortunately, two frozen samples (one for *A. papillosa* and one for *T. tetraquetra*) were lost in transit.

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).

### Morphological analysis

To study modular morphology of each species, the buccal mass was extracted and soaked in proteinase K solution for 2 hours at 60 °C. When connective and muscle tissues were dissolved, the samples were rinsed in distilled water, air-dried, mounted on an aluminium stub, and sputter-coated with gold for visualization under a JEOL JSM 6380 scanning electron microscope. The modular morphology are shown in Figure 2.

### **Lipid preparation and Fatty acid analysis**

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)*. A gas chromatography analysis of FAME was conducted with a GC-2010 chromatograph (Shimadzu, Kyoto, Japan) with a flame ionization detector. An *Equity-5Supelcowax 10* (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<sup>-1</sup> ramp to 240 °C that was held for 5 min. A sample volume of 1 µL (about 1 mg · mL<sup>-1</sup>) was injected. 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<sup>-1</sup>. The FAME peaks were analyzed by comparing their retention time with those of the standards (a mixture of PUFA methyl esters No. 3 from menhaden oil, Sigma-Aldrich Co., USA). The concentrations of individual FAs were calculated from the integrated area (% of total FAs). 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<sup>-1</sup>. The GC–MS analysis of FAME was performed at 160 °C with a 2 °C · min<sup>-1</sup> 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<sup>-1</sup> 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**

The differences in the FA composition (only for species with 3 replicates) were investigated using PERMANOVA (*Anderson, Gorley & ClarkeAnderson et al., 2008; Clarke & Gorley, 2015*). The PERMANOVA analysis was based on Bray-Curtis similarity matrices, using 9999 random permutations of raw data. After the PERMANOVA routines, pairwise Monte Carlo tests were performed between all pairs of species. PERMDISP routines was performed to test homogeneity of multivariate dispersions. A nMDS ordination plot was used to visualize the similarity relationship among individuals and groups of individuals. The FAs that characterized and discriminated these groups were identified by SIMPER. The tests mentioned above were carried out using Primer 7+ software (PRIMER-e, New Zealand). 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). ~~These~~ 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  $\pm$  standard deviation.~~ Cluster analysis was performed using Ward's method (Minimum variance method) and the pvcust( ) function in the pvcust 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). ~~A statistical probability of  $p < 0.05$  was considered significant. Values are represented as mean  $\pm$  standard deviation.~~

## Results

The full FA composition of total lipids in the five nudibranch species from different depths is summarized in [Supplement 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 *T. tetraquetra*~~*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 *T. tetraquetra*~~*Tritonia* sp.~~. The ratio 24:6n-3/24:5n-6 in the *Tritonia* specimens from a depth of 450–516 m ( $7.0 \pm 2.2$ ) was higher than that in the *Tritonia* specimens from a depth of 210–247 m ( $1.3 \pm 0.1$ ). 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). *Individuals of A. papillosa* contained the highest level of 22:5n-3 (up to 9.9% of total FAs). 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  $\Delta 5,11$ -20:2 in the ~~*Tritonia*-*T. tetraquetra* sp.~~ specimens distinguished them from other nudibranchs. All species (except for *C. verrucosa*) contained noticeable amounts of  $\Delta 7,13$ -22:2.

The PERMANOVA results (Table 2) were corroborated by the nMDS plot (Supplement Fig. S1) and revealed significant differences ( $p < 0.001$ ) between species. The pairwise comparison has shown significant ( $p < 0.05$ ) differences for all pairs of species (Table 2), with the exception of the pair *Dendronotus* sp. and *C. verrucosa*. To detail the impact of each FA in similarity and dissimilarity among all nudibranch studied, the FA composition data were analyzed by SIMPER. Supplement Table S2 shows the first five FAs that contribute more than 7% in the similarity or dissimilarity. The high level of arachidonic acid (20:4n-6) in the *T. tetraquetra* specimens distinguished them from other nudibranchs studied. The level of 20:4n-6 in the *Tritonia* specimens from a depth of 450–516 m ( $10.4$ –



13.6%) was lower than that in the *Tritonia* specimens from a depth of 210–247 m (18.5–20.0%) (Supplement Table S1).

Analyses of the FA composition data by ANOVA identified certain FAs that were mainly responsible for the difference between species from deep and shallow waters (Table 1). Compared to the group of the shallow-water species, the deep-sea ones contained significantly higher ( $p < 0.05$ ) levels of 20:4n-6 and  $\Delta 7,15$ -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 3. All the studied specimens were subdivided into three groups: the first and the second groups consisted of deep-sea specimens of *T. tetraquetra* 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*.

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 *T. tetraquetra* 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 *T. tetraquetra* and *C. pacifica*. The PCA results (Fig. 4) agree with the results of cluster analysis (Fig. 3) and show a significant difference in FA profiles between the deep-sea *T. tetraquetra*, *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 representatives of nudibranchs of the genera *Aeolidia*, *Coryphella*, and *Dendronotus* prey on different various groups of Cnidaria (Hall & Todd, 1986). The nudibranch *A. papillosa* is known to prey on sea anemones, grabbing their soft tissues by highly denticulated uniserial radula (Fig. 2A), and consume their nematocyst (stinging capsular organelles) to protect against other predators (Hall & Todd, 1986; Vorobyeva, Malakhov & Ekimova, 2021). The considerable levels of 22:5n-6 and C<sub>20-22</sub> 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<sub>20</sub> MUFAs that we found in *A. papillosa* most likely originate from sea anemone lipids consumed by this nudibranch species.

The nudibranchs *A. papillosa* and *C. verrucosa* occur in the same shallow-water community, but *C. verrucosa* demonstrates non-specified feeding mode (Mikhlin, Vortsepneva & Tzetlin, 2015), and its radula (Fig. 2C,D) does not take part in biting the prey.

This species is known as a non-specialized cnidarian feeder preying on ~~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). C<sub>24</sub> PUFAs are proposed as biomarkers for marine food web studies (Drazen et al. 2008; Blanchet-Aurigny et al. 2015). ~~were reported for another shallow-water nudibranch, *C. verrucosa*.~~ Very-long-chain C<sub>24</sub> PUFAs are FATM of jellyfish and 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<sub>24</sub> 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<sub>20</sub> MUFAs in this nudibranch species *C. verrucosa*. *C. verrucosa* is characterized by the least intraspecific similarity in the composition of fatty acids. It may also indicate a wide range of food supplies for this species.

The radula morphology in *Dendronotus* sp. (Fig. 2B) is very similar to that of *Dendronotus lacteus* and *D. rufus* and has a large number of knife-like lateral teeth that nudibranchs may use for biting off soft tissues of polyps (Ekimova et al., 2019b). There is some evidence that *D. lacteus* and *D. rufus* feed on hydroids of ~~the~~ family Sertulariidae (order Leptothecata), scyphistomae, and anemones (Ekimova et al., 2019b). 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<sub>20</sub> MUFAs levels recorded in the deep-sea nudibranch *Dendronotus* sp. from the Sea of Okhotsk likely indicate its preying on sea anemones and/or anthoathecates hydroids, similarly to ~~the~~ shallow-water species, *A. papillosa* and *C. verrucosa*. A dietary resemblance and smoothing of lipid profiles by dietary FAs may be a possible reason of the resemblance in FATM between evolutionary distant species.

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 *T. ~~ritonia~~ 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 corals *Gersemia rubiformis* and *Acanella* sp. were suggested as the most probable food sources of *T. tetraquetra*. (Imbs, 2016; Imbs & Chernyshev, 2019). No significant differences in the ratio 24:6n-3/24:5n-6 were earlier found between *T. tetraquetra* specimens collected at different depths.

In the present study, the high percentage levels of 24:6n-3 and 24:5n-6, which are observed TPA in *T. tetraquetra* from Simushir Island, the deep-sea nudibranch *Tritonia* sp. also indicates confirm preying on soft corals. Based on the ratio 24:6n-3/24:5n-6, we can assume that *T. tetraquetra* ~~*Tritonia* sp.~~ at a depth of 250 m mainly feed on the *Gersemia* and/or *Acanella* soft corals ~~similarly to *T. tetraquetra*.~~ The ~~significant~~ increase in the ratio 24:6n-3/24:5n-6 accompanying by the decrease in the 20:4n-6 level the in *T. tetraquetra* ~~*Tritonia* sp.~~ with increasing depth indicates a change in the taxonomic group of

soft corals consumed. Among ~~of the~~ deep-sea soft corals ~~of that occur in~~ the Sea of Okhotsk, the very high ratio  $24:6n-3/24:5n-6 = 95 \div 310$  and the lowest level of  $20:4n-6$  ( $1.7 \pm 0.3\%$ ) is characteristic ~~only~~ of soft corals ~~of within~~ the family Primnoidae (Imbs *et al.*, 2016), which most likely make a considerable contribution in that can be the major food source diet of *T. tetraquetra* *Tritonia* sp. at a depth of 500 m. Our field observations show that *T. tetraquetra* is often found in communities of various groups of soft corals (Octocorallia) (Supplement Fig. S3), which apparently dominate food sources of this nudibranch.

~~To our knowledge, food sources of *C. pacifica* have not been identified to date. There is only a brief mention~~ Species of ~~the~~ genus *Colga* can feed on members of ~~the~~ phylum Bryozoa (Grischenko & Martynov, 1997; Behrens, 2004). At least 18 species of bryozoans were earlier found in a digestive tract of *C. pacifica* (Martynov & Baranets, 2002). A noticeable level of  $\Delta 7,13-22:2$  and  $22:6n-3$  has been detected in total FAs of the bryozoan *Dendrobeatia flustroides* from the Sea of Okhotsk (Demidkova, 2010). The high content of these two FAs in *C. pacifica* confirms that this deep-sea species likely can feed on bryozoans. The low intraspecific similarity in the FA composition revealed for *C. pacifica* may indicate the lack of food specialization in any particular species of bryozoans.

Other characteristic FAs of *C. pacifica* such as  $22:5n-6$ , and odd-chain and branched SFAs may originate from own biosynthesis or and associated microorganisms, respectively.

The unexpectedly high content of  $22:5n-6$  found in *C. pacifica* may be a result of high activity of  $C_2$  elongase and  $\Delta 4$  desaturase that convert  $20:4n-6$  into  $22:5n-6$ . Such activity has been supposed hypothesized in the hydrocoral *Millepora* to explain the extremely high levels of  $22:5n-6$  and  $22:6n-3$  (Imbs, Dang & Nguyen, 2019; 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 ~~in~~ 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-sea and shallow-water species were compared on the base of ~~their~~ FATM ~~compositions present in their body tissues. The d~~ Different species originating from different depths, but with similar food sources, showed similar FATM profiles. ~~The s~~ Species composition of soft corals consumed by *T. tetraquetra* *Tritonia* sp. appear to changes with increasing depth. Deep-sea nudibranchs of ~~the~~ genus *Colga* are most promising objects for future studies, as. ~~The~~ the proportion between dietary and self-synthesize biosynthetic origins in their PUFAs that they feature should ~~to~~ be assessed. The use of molecular barcodes of gut content or a stable isotope ratio in future studies can specify our assumptions on the feeding regimes of the deep-sea species.



because FATM provides indirect evidence of trophic interactions and the discrimination of prey at genus or species level is not able.

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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~~Excel Table documents are provided as the ~~DOC~~XLSX file in the Supplemental Materials.

### Supplemental Information

Supplemental information for this article can be found online.

## References

Allmon RA, Sebens KP. 1988. Feeding biology and ecological impact of an introduced nudibranch, *Tritonia plebeia*, New England, USA. *Marine Biology* **99**(3):375–385 DOI: 10.1007/BF02112130.

Anderson MJ, Gorley RN, Clarke KR. 2008. *PERMANOVA + for PRIMER: Guide to Software and Statistical Methods*. UK: PRIMER-E Ltd.

Barnes DKA, Bullough LW. 1996. Some observations on the diet and distribution of nudibranchs at Signy Island, Antarctica. *Journal of Molluscan Studies* **62**:281–287 DOI: 10.1093/mollus/62.3.281.

Behrens DW. 2004. Pacific Coast Nudibranchs, Supplement II. New Species to the Pacific Coast and New Information on the Oldies. *California Academy of Sciences* **55**:11–54.

Bertsch H. 2020. A history of eastern Pacific marine heterobranch research. *The nautilus* **134**(2):71–88.

Blanchet-Aurigny A, Dubois SF, Quere C, Guillou M, Pernet, F. 2015. Trophic niche of two co-occurring ophiuroid species in impacted coastal systems, derived from fatty acid

- and stable isotope analyses. *Marine Ecology Progress Series* **525**:127–141 DOI: 10.3354/meps11169.
- Bligh EG, Dyer WJA. 1959.** A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* **37**:911–917 DOI: 10.1139/y59-099.
- Braeckman U, Provoost P, Sabbe K, Soetaert K, Middelburg JJ, Vincx M, Vanaverbeke J. 2015.** Temporal dynamics in a shallow coastal benthic food web: Insights from fatty acid biomarkers and their stable isotopes. *Marine Environmental Research* **108**:55–68 DOI: 10.1016/j.marenvres.2015.04.010.
- Budge SM, Iverson SJ, Koopman HN. 2006.** Studying trophic ecology in marine ecosystems using fatty acids: A primer on analysis and interpretation. *Marine Mammal Science* **22**(4):759–801 DOI: 10.1111/j.1748-7692.2006.00079.x.
- Calado R, Leal MC. 2015.** Trophic ecology of benthic marine invertebrates with bi-phasic life cycles: What Are We Still Missing? *Advances in Marine Biology* **71**:1–70 DOI: 10.1016/bs.amb.2015.07.001.
- Carreau JP, Dubacq JP. 1978.** Adaptation of macro-scale method to the micro-scale for FA methyl transesterification of biological lipid extracts. *Journal of Chromatography A* **151**:384–390 DOI: 10.1016/S0021-9673(00)88356-9.
- Chimienti G, Angeletti L, Furfaro G, Canese S, Taviani M. 2020.** Habitat, morphology and trophism of *Tritonia callogorgiae* sp. nov., a large nudibranch inhabiting *Callogorgia verticillata* forests in the Mediterranean Sea. *Deep Sea Research Part I: Oceanographic Research Papers* **165**:103364 DOI: [10.1016/j.dsr.2020.103364](https://doi.org/10.1016/j.dsr.2020.103364).
- Christie WW. 2020.** Methyl esters of FA. Archive of mass spectra. The Lipid Web. Available at <https://www.lipidhome.co.uk/ms/methesters/me-arch/index.htm>. (accessed 22 June 2021).
- Clarke KR, Gorley RN. 2015.** *PRIMER v7: User Manual/Tutorial*. UK: PRIMER-E Ltd.
- Demidkova DA. 2010.** The composition of fatty acids and aldehydes of the marine bryozoans *Berenicea meandrina* and *Dendrobeania flustroides* (Bryozoa: Gymnolaemata). *Russian Journal of Marine Biology* **36**:300–304.
- Drazen JC, Popp BN, Choy CA, Clemente T, De Forest L, Smith, KL. 2008.** Bypassing the abyssal benthic food web: Macrourid diet in the eastern North Pacific inferred from stomach content and stable isotopes analyses. *Limnology and Oceanography* **53**(6):2644–2654 DOI: 10.4319/lo.2008.53.6.2644.
- Ekimova I, Deart Y, Schepetov D. 2019a.** Living with a giant parchment tube worm: a description of a new nudibranch species (Gastropoda: Heterobranchia) associated with the annelid *Chaetopterus*. *Marine Biodiversity* **49**(1):289–300 DOI: <https://doi.org/10.1007/s12526-017-0795-z>.
- Ekimova I, Korshunova T, Schepetov D, Neretina T, Sanamyan N, Martynov A. 2015.** Integrative systematics of northern and Arctic nudibranchs of the genus *Dendronotus* (Mollusca, Gastropoda), with descriptions of three new species. *Zoological Journal of the Linnean Society* **173**(4):841–886 DOI: 10.1111/zoj.12214.
- Ekimova I, Valdes A, Chichvarkhin A, Antokhina T, Lindsay T, Schepetov D. 2019b.** Diet-driven ecological radiation and allopatric speciation result in high species diversity in a temperate-cold water marine genus *Dendronotus* (Gastropoda: Nudibranchia). *Molecular Phylogenetics and Evolution* **141**:106609 DOI: 10.1016/j.ympev.2019.106609.

- Goddard JHR. 2006.** Stealthy slugs and communicating corals: polyp withdrawal by an aggregating soft coral in response to injured neighbors. *Canadian Journal of Zoology* **84**:66–71 DOI: 10.1139/Z05-178.
- Grischenko AV, Martynov AV. 1997.** Bryozoa as food items for the genera *Colga* and *Triopha* (Nudibranchia, Doridacea). [Abstract available from: <http://www.bryozoa.net/russabst.html> ] Russian and International Conference on Bryozoa.
- Hall SJ, Todd CD. 1986.** Growth and reproduction in the aeolid nudibranch *Aeolidia papillosa* (L). *Journal of Molluscan Studies* **52**:193–205 DOI: 10.1093/mollus/52.3.193.
- Hernroth L, Grondahl F. 1985.** On the biology of *Aurelia aurita* (L) 2. Major factors regulating the occurrence of ephyrae and young medusae in the gullmar fjord, Western Sweden. *Bulletin of Marine Science* **37**(2):567–576.
- Hoover RA, Armour R, Dow I, Purcell JE. 2012.** Nudibranch predation and dietary preference for the polyps of *Aurelia labiata* (Cnidaria: Scyphozoa). *Hydrobiologia* **690**:199–213 DOI: 10.1007/s10750-012-1044-x.
- Imbs AB. 2016.** High level of tetracosapolyenoic fatty acids in the cold-water mollusk *Tochuina tetraquetra* is a result of the nudibranch feeding on soft corals. *Polar Biology* **39**(8):1511–1514 DOI: 10.1007/s00300-015-1865-y.
- Imbs AB, Chernyshev AV. 2019.** Tracing of lipid markers of soft corals in a polar lipidome of the nudibranch mollusk *Tritonia tetraquetra* from the Sea of Okhotsk. *Polar Biology* **42**(2):245–256 DOI: 10.1007/s00300-018-2418-y.
- Imbs AB, Dang LPT, Nguyen KB. 2019.** Comparative lipidomic analysis of phospholipids of hydrocorals and corals from tropical and cold-water regions. *Plos One* **14**(4):e0215759 DOI: 10.1371/journal.pone.0215759.
- Imbs AB, Ermolenko EV, Grigorchuk VP, Dang LPT. 2021.** Seasonal variation in the lipidome of two species of *Millepora hydrocorals* from Vietnam coastal waters (the South China Sea). *Coral Reefs* **40**:719–734 DOI: 10.1007/s00338-021-02073-2.
- Imbs AB, Grigorchuk VP. 2019.** Lipidomic study of the influence of dietary fatty acids on structural lipids of cold-water nudibranch mollusks. *Scientific Reports* **9**:20013 DOI: 10.1038/s41598-019-56746-8.
- Imbs AB, Yakovleva IM, Latyshev NA, Pham LQ. 2010.** Biosynthesis of polyunsaturated fatty acids in zooxanthellae and polyps of corals. *Russian Journal of Marine Biology* **36**(6):452–457 DOI: 10.1134/S1063074010060076.
- Kelly JR, Scheibling RE. 2012.** Fatty acids as dietary tracers in benthic food webs. *Marine Ecology Progress Series* **446**:1–22 DOI: 10.3354/meps09559.
- Kharlamenko VI, Kiyashko SI. 2018.** Fatty acid and stable isotope compositions in shallow-water bivalve mollusks and their food. *Russian Journal of Marine Biology* **44**(2):100–111 DOI: 10.1134/S1063074018020050.
- Kiyashko SI, Kharlamenko VI, Sanamyan K, Alalykina IL, Wurzburg L. 2014.** Trophic structure of the abyssal benthic community in the Sea of Japan inferred from stable isotope and fatty acid analyses. *Marine Ecology Progress Series* **500**:121–137 DOI: 10.3354/meps10663.
- Kuzirian AM. 1979.** Taxonomy and biology of 4 New England coryphellid nudibranchs (gastropoda-opisthobranchia). *Journal of Molluscan Studies* **45**:239–261.

- Martynov AV, Baranets ON. 2002.** A revision of the genus *Colga* Bergh (Opisthobranchia, Polyceridae), with description of a new species from the North Pacific. *Ruthenica* **12**(1):23–43.
- McDonald G, Nybakken J. 1997.** A worldwide review of the food of nudibranch mollusks 1. Introduction and the suborder Arminacea. *Veliger* **40**(2):157–159.
- McDonald G, Nybakken J. 1999.** A worldwide review of the food of nudibranch mollusks. Part II. The suborder Dendronotacea. *Veliger* **42**:62–66.
- Mikhlina A, Ekimova I, Vortsepneva E. 2020.** Functional morphology and post-larval development of the buccal complex in *Eubranchius rupium* (Nudibranchia: Aeolidida: Fionidae). *Zoology* **143**:125850 DOI: [10.1016/j.zool.2020.125850](https://doi.org/10.1016/j.zool.2020.125850).
- Mikhlina AL, Tzetlin AB, Ekimova IA, Vortsepneva EV. 2019.** Drilling in the dorid species *Vayssierea* cf. *elegans* (Gastropoda: Nudibranchia): Functional and comparative morphological aspects. *Journal of Morphology* **280**(1):119–132 DOI: [10.1002/jmor.20922](https://doi.org/10.1002/jmor.20922).
- Mikhlina AL, Vortsepneva EV, Tzetlin AB. 2015.** Functional morphology of the buccal complex of *Flabellina verrucosa* (Gastropoda: Opisthobranchia). *Invertebrate Zoology* **12**, 175–196 DOI: [10.15298/invertzool.12.2.04](https://doi.org/10.15298/invertzool.12.2.04).
- Ostman C. 1997.** Abundance, feeding behaviour and nematocysts of scyphopolyps (Cnidaria) and nematocysts in their predator, the nudibranch *Coryphella verrucosa* (Mollusca). *Hydrobiologia* **355**:21–28 DOI: [10.1023/A:1003065726381](https://doi.org/10.1023/A:1003065726381).
- Revel J, Massi L, Mehiri M, Boutoute M, Mayzaud P, Capron L, Sabourault C. 2016.** Differential distribution of lipids in epidermis, gastrodermis and hosted *Symbiodinium* in the sea anemone *Anemonia viridis*. *Comparative Biochemistry and Physiology A* **191**:140–151 DOI: [10.1016/j.cbpa.2015.10.017](https://doi.org/10.1016/j.cbpa.2015.10.017).
- Sebens KP. 1983.** The larval and juvenile ecology of the temperate octocoral *Alcyonium siderium* Verrill 2. Fecundity, survival, and juvenile growth. *Journal of Experimental Marine Biology and Ecology* **72**(3):263–285 DOI: [10.1016/0022-0981\(83\)90111-9](https://doi.org/10.1016/0022-0981(83)90111-9).
- Shuntov VP, Ivanov OA, Dulepova EP. 2019.** Biological resources in the Sea of Okhotsk large marine ecosystem: their status and commercial use. *Deep Sea Research* **163**:33–45 DOI: [10.1016/j.dsr2.2019.01.006](https://doi.org/10.1016/j.dsr2.2019.01.006)
- Suzuki R, Shimodaira H. 2006.** Pvcust: an R package for assessing the uncertainty in hierarchical clustering. *Bioinformatics* **22**(12): 1540–1542 DOI: [10.1093/bioinformatics/btl117](https://doi.org/10.1093/bioinformatics/btl117)
- Svetashev VI. 2011.** Mild method for preparation of 4,4-dimethyloxazoline derivatives of polyunsaturated fatty acids for GC–MS. *Lipids* **46**(5):463–467 DOI: [10.1007/s11745-011-3550-4](https://doi.org/10.1007/s11745-011-3550-4).
- Svetashev VI. 2019.** Fatty acids of the medusae *Aurelia aurita* (Linnaeus, 1758) and *Rhopilema esculentum* (Kishinouye, 1891): the presence of families of polyenoic acids with 24 and 26 carbon atoms. *Russian Journal of Marine Biology* **45**(2):113–117 DOI: [10.1134/S1063074019020123](https://doi.org/10.1134/S1063074019020123).
- Svetashev VI, Vysotskii MV. 1998.** Fatty acids of *Heliopora coerulea* and chemotaxonomic significance of tetracosapolyenoic acids in coelenterates. *Comparative biochemistry and Physiology B* **119**:73–75 DOI: [10.1016/S0305-0491\(97\)00231-9](https://doi.org/10.1016/S0305-0491(97)00231-9).



**Vorobyeva OA, Malakhov VV, Ekimova IA. 2021.** General and fine structure of *Aeolidia papillosa* cnidosacs (Gastropoda: Nudibranchia). *Journal of Morphology* **282**: 754–768 DOI: 10.1002/jmor.21346.

**Zhukova NV. 2014.** Lipids and fatty acids of nudibranch mollusks: potential sources of bioactive compounds. *Marine Drugs* **12**(8):4578–4592 DOI: 10.3390/md12084578.

**Zhukova NV, Eliseikina MG. 2012.** Symbiotic bacteria in the nudibranch mollusk *Dendrodoris nigra*: FA composition and ultrastructure analysis. *Marine Biology* **159**:1783–1794 DOI: 10.1007/S00227-012-1969-7.

## Figure titles and legends

**Figure 1 External views of the studied nudibranchs.** A and B, *Aeolidia papillosa* (dorsal and ventral view, respectively); C and D, *Colga pacifica* (dorsal and lateral view, respectively); E, *Coryphella verrucosa*; F, *Dendronotus* sp.; G and H, *Tritonia tetraquetra* (dorsal and ventral view, respectively). Scale bar 1 mm. Photos by A. Maiorova

**Figure 2 Radular morphology in studied nudibranch species.** A – *Aeolidia papillosa*, posterior portion of uniserial radula. B – *Dendronotus* sp., middle portion of polycerial radula. C – *Coryphella verrucosa*, triserial radula. D – *Coryphella verrucosa*, rachidian and lateral teeth. E – *Colga pacifica*. F – *Tritonia tetraquetra*, posterior portion of polyserial radula. G – *Tritonia tetraquetra*, rachidian teeth and innermost lateral teeth. H – *Tritonia tetraquetra*, middle lateral teeth. Abbreviations: rh, rachidian tooth; lt, lateral tooth; mt, marginal teeth. Scale bars: A–C, G – 100 µm; D – 30 µm; E, F – 500 µm; H – 200 µm.

**Figure 13 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. ~~TST~~, *Tritonia tetraquetra*~~Tritonia~~ sp.; CP, *Colga pacifica*; CV, *Coryphella verrucosa*; DS, *Dendronotus* sp.; AP, *Aeolidia papillosa*.

**Figure 24 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 13). (B) The projection of 12 variables is shown. ~~TTS~~, *Tritonia tetraquetra*~~Tritonia~~ sp.; CP, *Colga pacifica*; CV, *Coryphella verrucosa*; DS, *Dendronotus* sp.; AP, *Aeolidia papillosa*.