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ORIGINAL ARTICLE

Aquaculture

Effect of short-term oral supplementation with marine-derived n-3 polyunsaturated fatty acids on gene expression of synaptosome-associated protein 25 in the olfactory organ and brain of juvenile chum salmon *Oncorhynchus keta*

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Abstract

In Japan, the release of high-quality hatchery-reared juvenile fish is intended to mitigate against recent declines in adult returns of chum salmon *Oncorhynchus keta*. One approach to accomplish this aim is through oral supplementation with functional ingredients prior to release of the juveniles. This study aimed to clarify the effect of docosahexaenoic acid (DHA), a marine-derived n-3 polyunsaturated fatty acid (PUFA), on the neural function of juvenile salmon. We examined the messenger RNA (mRNA) expression of synaptosome-associated protein 25 (SNAP25), a molecule related to synaptic vesicle endocytosis, in the brain of hatchery-reared chum salmon juveniles whose feed was supplemented with a DHA-enriched oil for a short period of time. In addition, we tested critical swimming speed to clarify the effect of this supplementation on swimming ability. *snap25b* expression in the rostral and mid parts of brain was higher in the PUFA-supplemented fish than in the control groups. Both *snap25a* expression in the mid and caudal parts of brain, including the motor centers, and the results of the swimming ability test were higher in the n-3 PUFA-supplemented group. These findings suggest that short-term oral DHA supplementation in juvenile chum salmon via commercial salmon feed contributes to the enhancement of synaptic plasticity in the central nervous system.

Keywords DHA · Functional ingredients · Chum salmon · Brain · Swimming ability

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Introduction

Stocks of chum salmon *Oncorhynchus keta* in Japan have been increasing since the 1970s through stock enhancement programs (Mayama and Ishida 2003). However, returns of adult chum salmon have recently shown rapid declines (Miyakoshi et al. 2013; Kitada 2014; Kaeriyama and Sakaguchi 2023), even though the stock enhancement programs have been conducted consistently (e.g., Ban et al. 2022). The programs use artificial dry diets during the juvenile rearing period, namely from the time of emergence after the yolk sac is nearly absorbed to their release from the hatchery (Nogawa and Yagisawa 2011). The feeding method has the effect of reducing post-release mortality as it results in larger sized juveniles ready for release when the coastal environmental conditions are favorable (e.g., Miyakoshi et al. 2013). Although efficient fish feed formulations have been established, attempts have been made to enhance the quality of salmon feeds for hatchery-reared juveniles through formulations containing functional ingredients. For example, when cultured juvenile chum salmon were fed an iron citrate-supplemented feed for 2.5 months, factors indicating high-quality, healthy fry improved (e.g., adenosine triphosphate content, burst swimming velocity, tolerance to fasting; see Mizuno et al. 2008).

Docosahexaenoic acid (DHA), a functional ingredient derived from marine organisms of interest in the present study, is an n-3 polyunsaturated fatty acid (n-3 PUFA) that has a double bond at the third carbon from the omega terminal. Other n-3 PUFAs include α-linolenic acid, which is abundant in vegetable oil, and eicosapentaenoic acid (EPA), which is contained in fish oil along with DHA. Fish oils derived from marine fish generally contain 8.4–18.8% EPA and 8.7–12.4% DHA (Sprague et al. 2016). In vertebrates, DHA is the most abundant long-chain unsaturated fatty acid in the brain, and supplemented EPA is converted to DHA in the brain and other tissues (Ishiguro et al. 1988). Spatial cognitive and memory-learning tests of n-3 PUFA-deficient mice *Mus musculus* showed that administration of a DHA-containing diet enhanced learning and memory ability (Hiratsuka et al. 2009; Janssen et al. 2015). Ingestion of DHA has also been shown to reduce the incidence of memory impairment in humans *Homo sapiens* (Gómez-Pinilla 2008). DHA has a profound effect on cell membrane fluidity and cell membrane function (Krigman and Hogan 1976) and is important for brain function because it affects nerve growth and signal transduction (Calderon and Kim 2004).

Ueda (2015) showed upregulated gene expression of zeta-1, an essential subunit component of the NMDA receptor, also known as receptor subtype 1 (NR1), in the brain of chum salmon fry fed a diet containing DHA. When masu salmon *Oncorhynchus masou* smolts reared for a stock enhancement program were given a DHA-enriched feed for 1 week and then released to the river, their return to the natal river as adults occurred at a higher rate (Ueda et al., unpublished data). However, the authors were unclear based on their results regarding which physiological function was affected to improve the return rate as adult fish. Recently, it has been shown that the synaptosome-associated protein 25 (Snap25/SNAP25) gene (*snap25*), which regulates synaptic exocytosis and is involved in olfactory memory formation, plays a role in chum salmon natal river imprinting and neuroplasticity during memory retrieval (Abe et al. 2018). In salmonids, as in higher vertebrates, Snap25 has two isoforms, Snap25a and Snap25b, but the details of their functional differentiation have not been elucidated (Abe et al. 2018, 2020). Furthermore, the effect of DHA administration to salmonids on *snap25s* expression is largely unknown.

The impact of predation by fishes and seabirds on chum salmon populations has been evaluated in coastal waters of northern Japan (e.g., Nagasawa 1998). For example, the rhinoceros auklet *Cerorhinca monocerata*, a pelagic seabird that breeds on Daikoku Island, off the Pacific coast of eastern Hokkaido, consumes large numbers of chum salmon juveniles migrating along the coase of Okhotsk Sea, by bringing the fish to their chicks (Okado et al. 2020). Depredation of juvenile chum salmon by other fish and seabirds may be contributing to the recent decline in adult returns and, therefore, predator avoidance ability, including motor performance of the fish, is also expected to strongly influence survival. Salmonids in their natural marine environment obtain sufficient DHA and EPA through their diet, which primarily consists of zooplankton and other marine organisms. However, for improved survival of released juveniles, the enhancement process must be completed before release.

n-3 PUFAs such as DHA and EPA are essential for survival and development, even in marine fish, as they play important physiological roles (Izquierdo et al. 1989). Fish store long-chain fatty acids (those with ≥ 14 carbons) in tissues (Iverson et al. 2004), especially tissues important for physiological functions, such as the retina (Copeman et al. 2002) and brain (Masuda et al. 1999). DHA in the diet is required for the normal formation and development of organs such as the nervous system and sensory organs (Izquierdo 1996; Masuda et al. 1999). Bell et al. (1995) found that the eyesight of Atlantic herring *Clupea harengus* fry raised on a DHA-deficient diet deteriorated, suggesting that fatty acid nutritional composition might ultimately affect the predator avoidance behavior of fry. When late-stage larvae of the gilt-head bream *Sparus aurata* were given feeds containing either fish oil or soybean oil, an experiment on instantaneous avoidance conducted by sound stimulation

found that the instantaneous swimming speed was significantly faster in the group given the fish oil-supplemented feed (Benítez-Santana et al. 2012).

This study aimed to clarify the effect of oral DHA, a marine-derived n-3 PUFA, supplementation on neural function in juvenile salmon, with the ultimate goal to produce high-quality juvenile in chum salmon stock enhancement programs. To achieve this, we analyzed the gene expression of the synaptic endocytosis-related molecule *snap25a/b* in the central and olfactory nervous systems of chum salmon juveniles supplemented a DHA-enriched oil for a short period of time. In addition, a test of critical swimming speed (U_{crit}) was conducted to clarify the effect of this supplementation on swimming performance, based on DHA having been shown to be effective in other fish species.

Material and methods

Fish

Chum salmon juveniles (fork length [FL]: 49.4 ± 0.75 mm; body weight [BW]: 0.93 ± 0.04 g; condition factor [CF]: 7.64 ± 0.14) were produced through artificial fertilization of upriver adults collected from the Chitose River, Hokkaido, Japan, in autumn 2016. After emergence, the juveniles were reared in an indoor PVC-lined pond system (length × width × height: 3400 × 330 × 275 mm; water depth: 200 mm) supplied with a continuous spring-water supply (temperature: 9.3 °C; dissolved oxygen: 10.97 mg/L), at the Salmon and Freshwater Fisheries Research Institute, Eniwa, Hokkaido, Japan, until 12 April 2017. Fish were fed a commercial salmon crumble diet (Alpha Crumble EX-MASU #1 or #2; Nosan Corp., Yokohama, Japan) and kept under a natural photoperiod.

Oral supplementation of n-3 PUFAs

A total of 500 juveniles were randomly selected and then divided into four groups of 125 fish each. The four groups were introduced into separate indoor acrylic fish tanks (length × width × height: 600 × 300 × 360 mm) with spring water continuously supplied from above. After the acclimatization period, the four groups were each fed a different diet: (1) commercial salmon crumble diet (control); (2) supplementation of the commercial salmon crumble diet with olive oil (the crumble diet containing 10% olive oil [Wako Pure Chemical, Tokyo, Japan]) as an isocaloric control; (3) a low n-3 PUFA-enriched diet (the commercial salmon crumble diet containing 5% purified fish oil [DHA-27; Nippon Suisan Kaisha Ltd., Tokyo, Japan]); or (4) a high n-3 PUFA-enriched diet (the commercial salmon crumble diet containing 10% purified fish oil [DHA-27]). The fish were fed once a day to satiation, from 16 to 25 April 2017. All oils were added to the salmon crumble diet as an outer percentage of the feed just prior to feeding. The product DHA-27 contains 29.2% and 8.4% of fish-derived DHA and EPA, respectively, as triglycerides contained in n-3 PUFAs. The chemical composition of each diet was derived from the ingredient composition disclosed by the manufacturer and is shown in Table 1.

Table 1 Chemical composition of diets fed to juvenile chum salmon *Oncorhynchus keta* in this experiment

Chemical composition of diets	Amount (g/kg diet)[a]			
	Control	10% olive oil	Low n-3 PUFA 5% DHA-27[b]	High n-3 PUFA 10% DHA-27[b]
Protein	481.0	437.2	458.1	437.2
Carbohydrate	210.0	190.9	200.0	190.9
Ash	134.0	121.8	127.3	121.8
Moisture	98.0	89.1	93.3	89.1
Base lipids	77.0	70.0	73.3	70.0
DHA	9.2	8.3	8.7	8.3
EPA	8.6	7.8	8.2	7.8
Olive oil or DHA-27[b]	0.0	90.9	47.6	90.9
DHA added	0.0	0.0	13.9	26.5
EPA added	0.0	0.0	4.0	7.6
Total DHA contents	9.2	8.3	22.6	34.8
Total EPA contents	8.6	7.8	12.2	15.4
Energy (MJ)	14.5	16.6	15.6	16.6

DHA Docosahexaenoic acid, *EPA* eicosapentaenoic acid, *PUFA* polyunsaturated fatty acid

[a]Amounts are given in g/kg diet unless indicated otherwise

[b]Purified fish oil "DHA-27" (Nippon Suisan Co., Fine Chemical Business Group, Tokyo, Japan) contained 29.2% of fish-derived DHA, triglyceride type (TG type) PUFA

The calculated DHA and EPA contents in the control, olive oil, and low and high n-3 PUFA test diets, respectively, 9.2, 8.3, 22.6, and 34.8 g DHA/kg diet, and 8.6, 7.8, 12.2, and 15.4 g EPA/kg diet. Each group was examined on 25 April. After 10 days of feeding, all fish were immobilized with carbonated water and then measured individually for FL (mm) and BW (g). Their CF was calculated as: $CF = (10^6 \times BW)/FL^3$ (Bagenal and Tesch 1978). The lipid compositions of the commercial salmon crumble diet and of juvenile chum salmon from the same population under identical rearing conditions have been previously reported in another study (Torao 2022).

Total RNA extraction and complementary DNA preparation

For the molecular biological analyses, four or five individual fish were sampled from each group. The olfactory organ and the rostral, mid, and caudal parts of the brain (Fig. 1) were dissected from each fish immobilized in carbonated water. Tissues were immediately submerged in RNA*later* solution (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) and stored at − 30 °C until total RNA extraction. The above tissues collected from each group of juveniles were homogenized in ISOGEN reagent (Nippon Gene, Tokyo, Japan) using a disposable homogenizer (TaKaRa BioMasher Standard; Takara, Tokyo, Japan). Then, each total RNA extract was prepared using an acid guanidium thiocyanate–phenol–chloroform extraction method according to the manufacturer's instructions (ISOGEN). RNA purification was conducted using the NucleoSpin RNA Clean-up kit (Macherey–Nagel, Düren, Germany). Total RNA concentration and purity were measured using the NanoDrop 1000 Spectrometer (Thermo Fisher Scientific).

The oligonucleotides used as PCR primers are listed in Table 2. These primer sets were used for quantitative real-time PCR (qPCR) and are described in detail in our previous study (Abe et al. 2018) that characterized *snap25* gene expression in chum salmon. Briefly, poly(A)$^+$ RNA was reverse transcribed using a random primer (Random pd(N)6; Takara) and PrimeScript reverse transcriptase (Takara) with RNase inhibitor (Recombinant RNase Inhibitor; Takara) in accordance with the manufacturer's instructions.

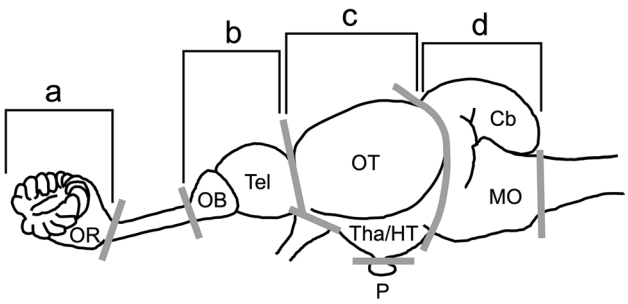

Fig. 1 Schematic illustration of division of the brain region for analyzing gene expressions of the synaptosome-associated protein 25 gene (*snap25s*) in juvenile chum salmon *Oncorhynchus keta*. **a** Olfactory rosette (*OR*), **b** rostral part of brain (mixture of olfactory bulb [*OB*] and telencephalon [*Tel*]), **c** mid part of brain (mixture of optic tectum [*OT*], thalamus [*Tha*], and hypothalamus [*HT*], excluding the pituitary [*P*]), **d** caudal part of brain (mixture of cerebellum [*Cb*] and medulla oblongata [*MO*])

qPCR of *snap25a/b*

The expression levels of the *snap25* genes were quantified using the LightCycler Nano qPCR System (Roche, Basel, Switzerland) with intercalating dye (FastStart Essential DNA Green Master; Roche). For absolute quantification, *snap25a* and *snap25b* qPCRII-TOP10 plasmid solutions (Abe et al. 2018) were each prepared as standards using the following dilution series: 10^{-2} to 10^{-6}. The qPCR reaction was performed at: 95 °C for 10 min; 45 cycles of a 3-step amplification (annealing at 65 °C for *snap25a* and *snap25b*); with a final melting step (60–97 °C, at 0.1 °C per second) to discriminate specific amplifications. Primer specificities were checked by melting analyses and 1.5% agarose gel electrophoresis of qPCR amplicons. Every sample was duplicated to exclude outliers. Melting analyses showed specific single melting peaks with each primer set: *snap25a* was approximately 80 °C, whereas *snap25b* was approximately 87 °C, which confirmed the isoform specificity and distinguishable amplification by qPCR. The quantification cycle (C_q) of all amplicons was within 15–25 cycles and ranged within the plasmid standard C_q values, indicating they were

Table 2 Primer sets used in real time PCR of chum salmon *Oncorhynchus keta* synaptosome associated protein 25 isoform a and isoform b

Primer name[a, b]	Sequences	Source
sSNAP-25a-F	5′-ACTGGATCGTGTTGAAGATGGC-3′	Modified from Abe et al. (2018)
sSNAP-25b-F	5′-CTGTGGTCTTTGCTCCTGTCCT-3′	Abe et al. (2018)
sSNAP-25ab-R	5′-TCCTCCACTGATGGCCATCTGT-3′	Abe et al. (2018)

[a]Small letter "s" means "chum

[b]snap25a, Synaptosome associated protein 25 isoform a (GenBank accession no. LC315096.1); snap25b, synaptosome associated protein 25 isoform b (GenBank accession no. LC315097.1)

sufficient for quantification. The experimental information from the present study was documented in accordance with The Minimum Information for Publication of Quantitative Real-time PCR Experiments (MIQE) guidelines (Bustin et al. 2009).

Swimming performance

The U_{crit} was measured as one evaluation of swimming performance, modified from the method of Makiguchi and Ueda (2009). Relative U_{crit} (BL s^{-1}) was calculated using the formula described by Brett (1964): $U_{crit} = U_p + (T_p\ T_i^{-1})\ U_i$, where U_p is the velocity at which the fish last swam for the full period, U_i is the velocity increment (0.5 BL s^{-1}), T_p is the time in minutes that the fish was able to swim against the water velocity which produced fatigue, and T_i is the time between velocity increments (5 min). Mean FL of each experimental group was used for the BL value. The U_{crit} trials were conducted at the Salmon and Freshwater Fisheries Research Institute, Eniwa, Hokkaido, Japan. The U_{crit} of each group was measured using eight or ten juveniles, in a swim chamber (volume: approx. 6.8 L water) containing a swimming section of φ120 × 600 mm (swim tunnel at the West Japan Fluid Engineering Laboratory, Nagasaki, Japan). The swim chamber was filled with spring water (9.3 °C) before each trial; the water flow in the swimming section was generated by a voltage-controlled motor and propeller, with the voltage calibrated against water velocity.

Statistical analysis

Statistical calculations were performed using GraphPad Prism version 8.4.1 (GraphPad Software Inc., San Diego, CA, USA). Gene expression levels were calculated as copies/total RNA (ng) for normalization among samples with different masses. All values are reported as the mean ± standard error (SE). Tukey's multiple comparisons test was used to compare body size among the groups. Dunn's multiple comparisons test was used to compare *snap25a/b* expression levels and U_{crit} among groups. Differences were considered significant at $p < 0.05$.

Results

The three body size parameters (FL, BW, and CF) of each group showed extremely similar values at the end of the supplementary experiment, after 10 days of feeding (Fig. 2).

The level of *snap25a* expression in the mid part of the brain was significantly higher in the high n-3 PUFA-supplemented group than in the control group ($p < 0.0166$; Fig. 3c). In the caudal part of the brain, *snap25a* expression level was significantly higher in the low n-3 PUFA-supplemented group compared with the control and the olive oil-supplemented groups ($p < 0.0067$ and $p < 0.0327$, respectively; Fig. 3d). The differences in the expression of *snap25a* in the olfactory rosette and rostral part of the brain were not significant among the groups fed different diets (Fig. 3a, b).

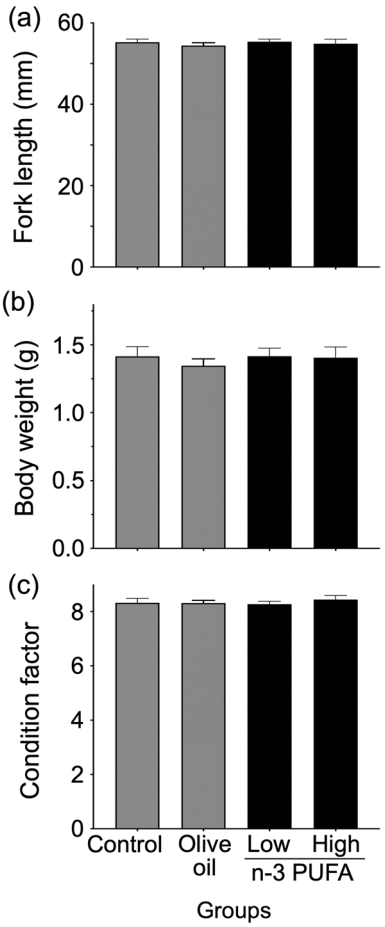

Fig. 2 Effects of oral supplementation with marine-derived n-3 polyunsaturated fatty acids (*n-3 PUFAs*) on fork length (**a**), body weight (**b**), and condition factor (**c**) of juvenile chum salmon *O. keta*. Shaded and solid columns show control groups (Control: commercial salmon crumble diet; Olive oil: commercial salmon crumble diet containing 10% olive oil with outer percentage) and experimental groups (Low n-3 PUFA: commercial salmon crumble diet containing 5% purified fish oil [DHA-27; Nippon Suisan Co.]; High n-3 PUFA: commercial salmon crumble diet containing 10% DHA-27 with outer percentage), respectively. Values represent mean ± standard error ($n = 11$–15). *DHA* Docosahexaenoic acid

The level of *snap25b* expression in the rostral part of the brain was significantly higher in the high n-3 PUFA-supplemented group than in the olive oil-supplemented group ($p < 0.0166$; Fig. 4b). In the mid part of the brain, *snap2ab* expression level was significantly higher in the high n-3 PUFA-supplemented group compared with the control group ($p < 0.0116$; Fig. 4c). The differences in the expression of *snap25b* in the olfactory rosette and caudal part of

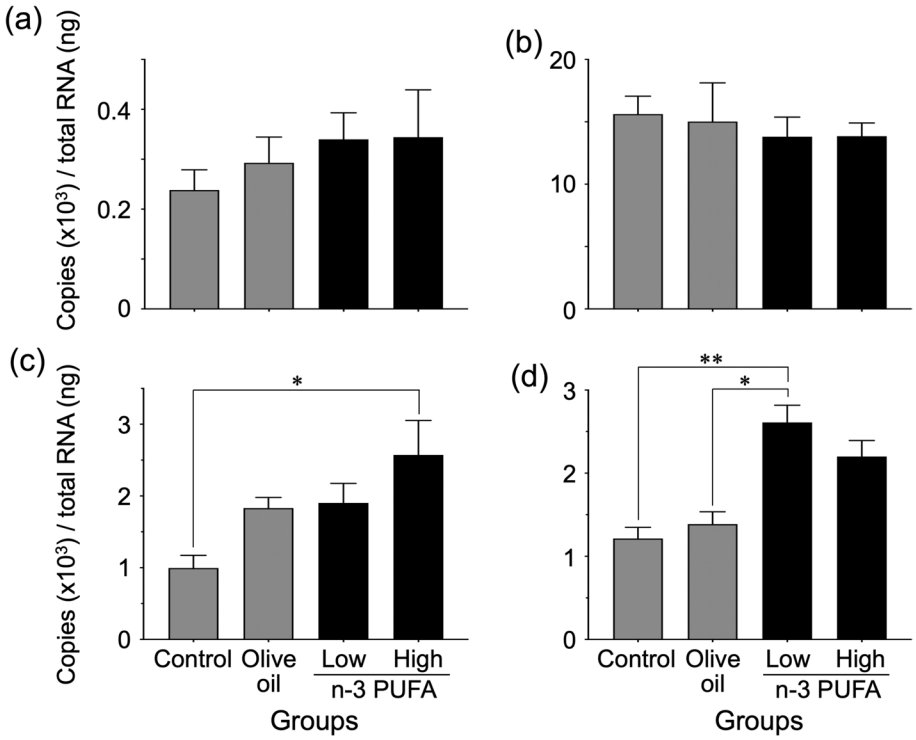

Fig. 3 Effects of oral supplementation with marine-derived n-3 PUFAs on the expression of synaptosome-associated protein 25 isoform-a (*snap25a*) in the olfactory rosette (**a**), rostral part of brain (**b**; mixture of olfactory bulb and telencephalon), mid part of brain (**c**; mixture of optic tectum, thalamus, and hypothalamus), and caudal part of brain (**d**; mixture of cerebellum and medulla oblongata) of juvenile chum salmon *O. keta* using quantitative real-time PCR. Shaded and solid columns show control groups (Control: commercial salmon crumble diet; Olive oil: commercial salmon crumble diet containing 10% olive oil with outer percentage) and experimental groups (Low n-3 PUFA: commercial salmon crumble diet containing 5% purified fish oil [DHA-27; Nippon Suisan Co.]; High n-3 PUFA: commercial salmon crumble diet containing 10% DHA-27 with outer percentage), respectively. Values represent mean ± standard error ($n = 4$–5) of copies per sample RNA (ng). Asterisks indicate significant differences between groups at $^{*}p < 0.05$ and $^{**}p < 0.01$ by Dunn's multiple comparisons test

the brain were not significant among the groups fed different diets (Fig. 4a, d).

The value of U_{crit} was significantly higher in the low n-3 PUFA-supplemented group than in the control group ($p < 0.0041$; Fig. 5).

Discussion

The level of *snap25b* expression in the rostral part of the brain was higher in the high n-3 PUFA-supplemented group than in the olive oil-supplemented group functioning as an isocaloric control, suggesting that DHA, not the amount of heat (joules), upregulates *snap25b* expression in this part of the brain. The rostral part of the brain, where enhanced *snap25b* expression was confirmed, consists of the olfactory bulb and telencephalon. In salmonids, axons of the olfactory neuron with odorant receptors are primarily projected to the glomerular layer of the olfactory bulb (Satou 1990; Kudo et al. 1996; Ochs et al. 2017). Blood oxygen-dependent functional magnetic resonance imaging (BOLD fMRI) has shown that the telencephalon region of mature lacustrine sockeye salmon (*Oncorhynchus nerka*) responds specifically to the natal river water stimulus (Bandoh et al. 2011). Furthermore, the fish telencephalon is analogous to the cerebrum of higher vertebrates and has several functions other than olfaction (Yamamoto 2009; Ebbesson and Braithwaite 2012). The gene expression of *snap25b* in the telencephalon of the chum salmon appears to correlate more closely with the expression of other synaptic exocytosis-associated proteins, such as syntaxin 1 (STX1) and vesicle-associated membrane protein 2 (VAMP2; Abe and Kudo 2019). This physiology may be strongly related to the characteristics of *snap25b* expression found in the present study.

Both the level of *snap25a* expression in the caudal part of the brain, including the motor center (i.e., cerebellum and medulla oblongata: Roberts et al. 1992; Pushchina et al. 2022), and U_{crit} in the swimming test were higher in the low n-3 PUFA-supplemented group than in the control group, suggesting that these factors may be involved in improving motor performance at the central nervous system level. Larvae of gilt-head bream given a DHA-supplemented diet

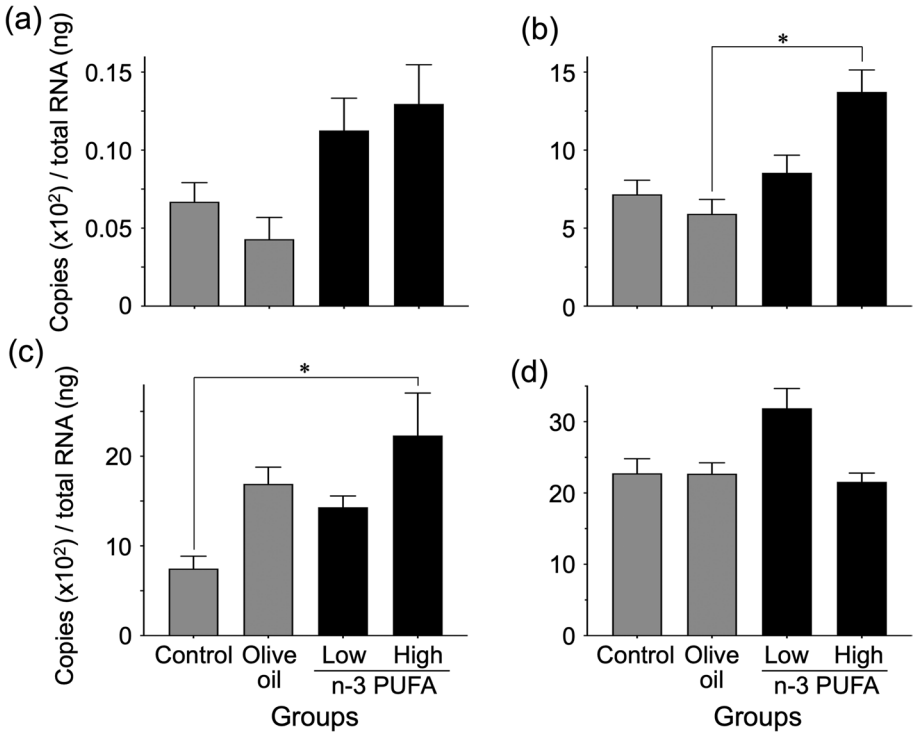

Fig. 4 Effects of oral supplementation with marine-derived n-3 PUFAs on the expression of synaptosome-associated protein 25 isoform-b (*snap25b*) in olfactory rosette (**a**), rostral part of brain (**b**; mixture of olfactory bulb and telencephalon), mid part of brain (**c**; mixture of optic tectum, thalamus, and hypothalamus), and caudal part of brain (**d**; mixture of cerebellum and medulla oblongata) of juvenile chum salmon *O. keta* using quantitative real-time PCR. Shaded and solid columns show control groups (Control: commercial salmon crumble diet; Olive oil: commercial salmon crumble diet containing 10% olive oil with outer percentage) and experimental groups (Low n-3 PUFA: commercial salmon crumble diet containing 5% purified fish oil [DHA-27, Nippon Suisan Co.]; High n-3 PUFA: commercial salmon crumble diet containing 10% DHA-27 with outer percentage), respectively. Values represent mean ± standard error ($n = 5$) of copies per sample RNA (ng). Asterisk indicates a significant difference between groups at $*p < 0.05$ by Dunn's multiple comparisons test

showed increased swimming speed and activation of the Mauthner cells in the medulla oblongata (Benítez-Santana et al. 2012). However, in the present study there was no significant difference between the low n-3 PUFA-supplemented group and the olive oil group and no effect was observed in the low n-3 PUFA-supplemented group, which may be related to the appropriate amount of total energy and/or n-3 PUFA in the swimming test. Our results suggest that the appropriate amount of DHA may likewise act on the neural mechanisms involved in the motor performance, which would affect the survival of the chum salmon early in their ocean phase. However, because various factors are involved in predator avoidance in juvenile salmon, the results of this study could not clarify how the enhancement of synaptic plasticity in the motor center is involved.

In the mid part of the brain, *snap25a* and *snap25b* expressions were higher in the high n-3 PUFA-supplemented group than in the control group. Differences in heat as well as DHA content may explain the significant effect of DHA on *snap25s* expression in this region (the amount of heat in 1 kg of diet was 14.5 MJ in the normal diet, and 16.6 g in the high n-3 PUFA diet; Table 1). The nucleus of the medial longitudinal fasciculus (nflm), as the main higher locomotor center, is where neurons responsible for initiating the signal to begin swimming are located in teleosts (Oka et al. 1986; Uematsu and Todo 1997). This indicates that increasing synaptic plasticity in this region may be involved in improving motor ability. On the other hand, this region also includes the hypothalamus, which controls appetite, stress response, and various other actions through the neuroendocrine system (e.g., Rønnestad et al. 2017; Wendelaar Bonga 1997). Therefore, identifying the functions affected by DHA in this particular region is challenging based on the findings of this study.

Exocytosis of pre-synaptic neurotransmitter vesicles is regulated by the soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex (Lin and Scheller 2000). SNAP25 is a component of the SNARE complex which regulates synaptic transmission and neuronal plasticity, and is associated with learning or memorization in rats *Rattus norvegicus* and mice (e.g., Osen-Sand et al. 1993; Johansson et al. 2008). Repeated formaldehyde inhalation

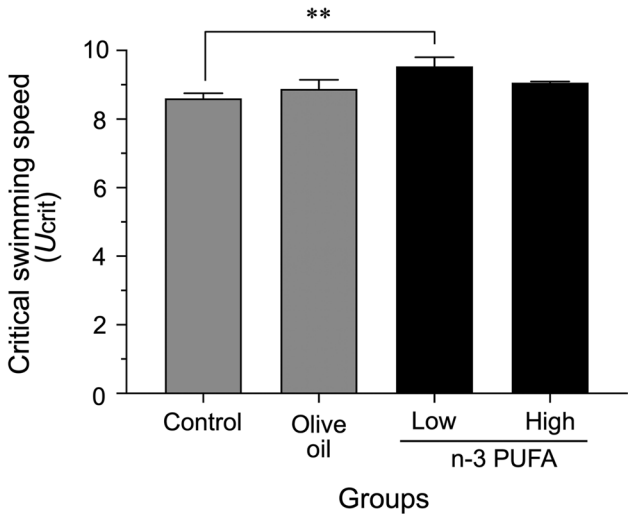

Fig. 5 Effects of oral supplementation with marine-derived n-3 PUFAs on critical swimming speed (U_{crit}, in body length s^{-1}) of juvenile chum salmon *O. keta* using a swim chamber. Body length was mean fork length of each experimental group. Shaded and solid columns show control groups (Control: commercial salmon crumble diet; Olive oil: commercial salmon crumble diet containing 10% olive oil with outer percentage) and experimental groups (Low n-3 PUFA: commercial salmon crumble diet containing 5% purified fish oil [DHA-27, Nippon Suisan Co.]; High n-3 PUFA: commercial salmon crumble diet containing 10% DHA-27 with outer percentage), respectively. Values represent mean ± standard error ($n = 8$–10). Asterisks indicate a significant difference between groups at $^{**}p < 0.01$ by Dunn's multiple comparisons test

downregulated SNAP25a/b protein expression levels in the mouse olfactory bulb and impaired learning abilities to detect buried food (Zhang et al. 2014). In the present study, only limited significant differences among groups were observed in the expression of *snap25s* mRNA. Although protein expression levels were not analyzed in the present study, it is assumed that even if no increase in *snap25s* mRNA expression is observed, the required amount is maintained by membrane recycling involved in the endocytosis. In other words, increases of *snap25s* mRNA expression in the brain suggests that the brain is in a state of experiencing significantly high synaptic plasticity, such as neurotransmitter release and neurite outgrowth.

Vertebrate SNAP25/Snap25 has two isoforms (SNAP25a/Snap25a and SNAP25b/Snap25b) due to selective splicing of exon 5 (e.g., Bark 1993; Bark and Wilson 1994; Risinger et al. 1998). In higher vertebrate brains, the expression of SNAP25a protein and mRNA has been found to be distributed in the olfactory bulb, telencephalon, and thalamus. Conversely, SNAP25b expression has been found to be high in the optic tectum, cerebellum, and medulla oblongata (Boschert et al. 1996; Prescott and Chamberlain 2011; Yamamori et al. 2011). It is important to note the distribution patterns of these proteins and mRNA in different brain regions. In fact, the primary isoform of *snap25s* that displays high mRNA expression in the olfactory bulb of normal juvenile chum salmon is *snap25a* according to Abe et al. (2018). Functional differentiation between the two isoforms is suggested by their tissue distribution and timing of expression (Delgado-Martínez et al. 2007). Nevertheless, the functional distinction between the two isoforms, which stems from the variance in molecular structure, remains unknown in higher vertebrates. In the present study, partial increases in the expression of *snap25a* and *snap25b* were observed mainly in the caudal (including the cerebellum and medulla oblongata) and rostral (including the olfactory bulb and telencephalon) parts of the brain, respectively, after n-3 PUFA supplementation. It is likely that this disparity in isoform-specific expression patterns is related to differences between normal physiological status and n-3 PUFAs-induced neuroplasticity activation status.

In conclusion, short-term oral supplementation of marine-derived n-3 PUFAs to chum salmon juveniles resulted in an upregulation of *snap25a* and *snap25b* expression, mainly in the caudal and rostral parts of brain, respectively. This finding suggests the presence of functional disparities between Snap isoforms in the salmon brain. Additionally, our findings suggest that this dietary supplementation can partially affect the neurological function and swimming ability of these juvenile salmonids. In stock enhancement programs for chum salmon, an efficious timing for supplementation of feed with a DHA-enriched oil may be when juveniles have finished using components derived from the female parent fish via the yolk sac, even if such supplementation is for a short period of time. However, results from ecological field research studies to determine the duration of this effect and the impact of DHA supplementation on salmon juveniles after release into rivers will be crucial to incorporating this fish oral supplement into Japan's salmon stock enhancement programs.

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Data availability All data that supports the findings of this study is available within the article and/or supplementary materials.

Declarations

Conflict of interest The authors have no conflicts of competing interest to declare that are relevant to the content of this article.

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