Membrane Lipids and Maximum Lifespan in Clownfish

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Abstract

The longevity-homeoviscous adaptation (LHA) theory of aging states that lipid composition of cell membranes is linked to metabolic rate and lifespan, which has been widely shown in mammals and birds but not sufficiently in fish. In this study, two species of the genus *Amphiprion* (*A. percula* and *A. clarkii*, with estimated maximum lifespan potentials [MLSP] of 30 and 9-16 years, respectively) and the damselfish *Chromis viridis* (estimated MLSP of 1-2 years) were chosen to test the LHA theory of aging in a potential model of exceptional longevity. Brain, livers and samples of skeletal muscle were collected for lipid analyses and integral part in the computation of membrane peroxidation indexes (PIn) from phospholipid (PL) fractions and PL fatty acid composition. When only the two anemonefish were compared, results pointed to the existence of a negative correlation between membrane PIn value and maximum life expectancy, well in line with the predictions from the LHA theory of aging. Nevertheless, contradictory data were obtained when the two clownfish were compared to the shorter-lived *C. viridis*. This results along with those obtained in previous studies on fish denote that the magnitude (and sometimes the direction) of the differences observed in membrane lipid composition and peroxidation index with MLSP cannot explain alone the diversity in longevity found among fishes.

1. Introduction

Reactive oxygen species (ROS) constitute the only known molecules endogenously and continuously produced by cells that have the capacity to break covalent bonds, causing damage to tissue macromolecules in biological systems (Barja 2019). Scientific evidence continues to support the mitochondrial oxygen free radical theory of aging (Barja 2013, 2019; Miwa et al. 2014; Shen et al. 2014; López-Lluch et al. 2015; Zsurka et al. 2018) both between and within animal species. Short-lived mammals and birds have species-specific high mitochondrial ROS production (mitROSp) rates at complex I of the electron transport chain (Ku et al. 1993; Barja and Herrero 1998; Herrero and Barja 1998; Barja 2004; Lambert et al. 2007; Csiszar et al. 2012). Although ROS damage affects all cell macromolecules, lipid peroxidation is quantitatively the main oxidative process in tissues due to the high sensitivity to oxidation of polyunsaturated fatty acids (PUFA), which are essential constituents of cell membrane phospholipids (PL) (Bielski et al. 1983). Besides, lipid peroxidation is an exponential reaction chain process that generates many toxic and mutagenic by-products like the aldehydes hydroxynonenal or malondialdehyde, which can diffuse throughout the cell including the nucleus, which is poor in lipids (Chaudhary et al. 1994). Reaching the nucleus by diffusion, those aldehydes chemically react with free amino groups in DNA and could contribute to DNA damage, both in the nucleus and mitochondria.

The longevity-homeovicous adaptation (LHA) theory of aging states that lipid composition of cell membranes (particularly that of mitochondria) is linked to metabolic rate and lifespan, which has been shown in a wide number of animal species (Pamplona et al. 1998; 2000). In comparative studies, performed on various species of mammals and birds, it has been found that species with a shorter lifespan have more unsaturated membranes than species with a longer life expectancy (Pamplona et al. 2002). Membranes with high levels of PUFA are more fluid and this can enable or promote higher

molecular activity of membrane proteins and, in turn, increase the metabolic activity of cells, tissues and, consequently, whole animals. At the same time, susceptibility to oxidative damage increases with the proportion of PUFA in membranes (Pamplona et al. 1998).

In order to test the LHA theory of aging in fish, where very little information is available, we recently published a study on fishes of genus *Nothobranchius* (de Costa et al. 2020), which includes some of the shortest-lived vertebrates in nature (3–18 months, depending on the species) and has proved to be a remarkable system for gerontological research (Lucas-Sanchez et al. 2014; Tozzini et al. 2013). In these fishes, the longer-lived fish species have more saturated membranes and therefore, a lower susceptibility to oxidative damage, as the LHA theory posits (de Costa et al. 2020). However, the magnitude of the observed differences among *Nothobranchius* species was much smaller than that of the inter-species differences in longevity this suggesting that the LHA theory of aging alone is not sufficient to explain those differences.

On the other hand, clownfish of genus *Amphiprion* have been proposed as the first experimental models for long-lived vertebrates as some of its species have been reported by hobbyists (in captivity) and by researchers (in the wild) to live for more than two decades (Sahm et al. 2019). These fishes evolved a specific adaptation that allows them to live in symbiosis with sea anemones (Buston and García 2007). Under the anemone´s protection, the overall mortality rate of these fish is low, which is related to longer life expectancies (Mariscal 1970; Aldenhoven 1986; Eckert 1987; Elliot et al. 1995; Buston 2003; Blanco and Sherman 2005).

In this study, two species of the genus *Amphiprion* (*A. percula* and *A. clarkii*, with estimated maximum lifespan potentials [MLSP] in the wild of 30 and 9–16 years, respectively) (Moyer 1986; Buston and García 2007; Sahm et al. 2019) were studied along with the damselfish *Chromis viridis*, to test how the LHA theory of aging applies to this fish group. *C. viridis* belong to the non-symbiotic sister-taxon to the *Amphiprion* genus, share with them general traits linked to their life in nature and show an interesting relationship with branching corals (Garcia-Herrera et al. 2017). However, despite the presence of a favourable microhabitat, *C. viridis* are predated by a wide range of generalist predator species, which has been suggested to be linked to a higher mortality rate (Hixon and Carr 1997; Sahm et al. 2019) and low life expectancy (estimated MLSP of 1–2 years) (Wantiez and Thollot 2001; Sahm et al. 2019).

2. Methods

2.1. Animal housing and sampling

Young adults (taken just after attaining adult size and sexual maturation, which is 2–3 years approx., for the clownfish and 1 year for the damselfish) of *Amphiprion percula* (total length, 45.2 ± 1.2 mm; total weight, 1.6 ± 0.4 g; n = 12), *Amphiprion clarkii* (L_T, 46.4 ± 5.1 mm; W_T, 2.3 ± 1.19 g; n = 12) and *Chromis viridis* (L_T, 43.0 ± 1.6 mm; W_T, 1.3 ± 0.4 g; n = 12) (*Perciformes*, *Pomacentridae*) were used for the present study (Fig. 1). Fishes were acquired from local dealers and subjected to acclimation during 1 month in

the facilities of the Marine Aquarium of the University of Murcia. Fish were kept in groups under exactly the same conditions (temperature, 27 ± 1ºC; salinity, 24 ± 1; pH = 8 ± 0.2; dissolved oxygen, 6.5 ± 0.2 mg/L) and fed ad libitum twice a day a standard diet to match their requirements (Mysis shrimp, enriched Artemia nauplii and red plankton).

Fishes were euthanized by exposure to the anesthetic MS222 (200 mg/L) for 10 min following the cessation of gill movement. Brain, livers and samples of skeletal muscle were collected from fishes and processed for lipid analyses.

2.2. Lipid extraction and phospholipid class composition

Total lipid content from fish tissues was obtained by extraction with chloroform/methanol (2:1, v/v) containing 0.01% (w/v) butylated hydroxytoluene as antioxidant, basically according to Folch et al. (1957). Briefly, fish samples were homogenized in 20 mL of ice-cold chloroform/methanol followed by the addition of 5 mL of 0.88% (w/v) KCl, mixing, and layers allowed to separate on ice for 1 h. The upper aqueous layer was aspirated and the lower organic layer was evaporated under a stream of oxygen-free nitrogen. All lipids extracts were stored at − 20°C under a N_2 atmosphere prior to analysis. PL classes were separated by high-performance thin-layer chromatography using 10- x 10-cm silica gel plates (VWR, Lutterworth, England) and methyl acetate/ isopropanol/ chloroform/ methanol/ 0.25% (w/v) KCl (25:25:25:10:9, by volume) as solvent system (Olsen and Henderson 1989). The lipid classes were visualized by charring at 160°C for 15 min after spraying with 3% (w/v) aqueous cupric acetate containing 8% (v/v) phosphoric acid and quantified by visible densitometry using Image Scanner II (Amersham Biosciences, UK). Scanned images were recorded automatically and analysed by computer using IQ-Image Quant TL 8.1 software (GE Healthcare Bio-Sciences AB, Sweden).

2.3. Phospholipid fatty acid composition

Individual phospholipid (PL) classes from tissue´s total lipid extract were separated by preparative-TLC, using silica gel plates (20 × 20 cm) (VWR) and the solvent system as above. Individual PL classes were identified by comparison with known standards after spraying with 1% (w/v) 2',7'-dichlorofluorescein in 97% (v/v) methanol containing 0.05% (w/v) BHT, and visualization under UV light. Each PL class was scraped from the plate into a test tube and subjected directly (on silica) to acid-catalyzed transmethylation at 50°C overnight following addition of 2 ml of 1% (v/v) sulphuric acid in methanol in order to prepare fatty acid methyl esters (FAME) (Christie 2003). FAME were separated and quantified by gas–liquid chromatography. For this, a Hewlett-Packard 5890 gas chromatograph with a capillary column (SPTH-2560, SUPELCO, 100 m×0.25 mm I.D., 0.20 µm film thickness) was used. The oven temperature, held at an initial value of 140°C for 5 min, was increased at a rate of 4°C per min to 230°C, then further increased at a rate of 1°C per min to 240°C, and finally held at that temperature for 6 min. The injector and flame ionization detector were set at 250°C. Helium at a pressure of 290 kPa was used as carrier gas. Peaks were identified by comparing their retention times with appropriate FAME standards purchased from Sigma Chemical Company (St. Louis, MO, USA). Individual fatty acid concentrations were expressed as percentages of the total content.

2.4. Fish life expectancy and its correlation with membrane peroxidation index.

Data on longevity in wild Clark's anemonefish (*Amphiprion clarkii*) are limited to a single study, which estimated that a female under periodic observation over a period of 11 years was at least 13 years old at the time she disappeared from her host anemone, with other anemones hosting females with estimated ages of 10 and 12 years (Moyer 1986; Fautin and Allen 1997). More recently, Buston and Garcia (2007) studied a wild population of *Amphiprion percula* in Papua New Guinea and their results suggested that females can live up to 30 years in the wild (lower 95% CI = 22.0 years, upper 95% CI = 89.9 years), although this has not been empirically proven through otolith examination.

In a previous study (Sahm et al. 2019), we corroborated the evidence for exceptional longevity in captivity of the clownfish. For six different species of genus *Amphiprion*, at least one individual was reported to have lived more than 10 years and for two different species, *A. ocellaris* and *A. melanopsus*, we obtained records of animals alive and spawning at an age of over 20 years.

Regarding *Chromis* fishes, they are considered a priori a model for short-lived reef inhabitants (Wantiez and Thollot 2001). These fishes undergo severe predation in the post-settlement phase (Hixon and Carr 1997) and have high juvenile and adult mortality. This, combined with a very rapid growth (80% of maximum size reached within the first year) clearly indicates that these animals are short-lived in the wild. Nevertheless, there are no reliable estimates of *C. viridis* lifespan in captivity, mainly due to the fact that these animals build large schools and it is not possible to identify them individually (Sahm et al. 2019).

Therefore, due to the little information available on *Amphiprion* species and *Chromis viridis*, no correlation analyses between fish maximum lifespan and membrane peroxidation indexes were performed in this study.

2.5. Indexes and statistical analysis

The peroxidation index (PIn) was used as an estimate of PL susceptibility to oxidation and was calculated using the formula: PIn = 0.025 × (percentage of monoenoics) + 1 × (percentage of dienoics) + 2 × (percentage of trienoics) + 4 × (percentage of tetraenoics) + 6 × (percentage of pentaenoics) + 8 × (percentage of hexaenoics) (Witting and Horwitt 1964). Results are presented as mean ± SD (n = 4). Data were checked for homogeneity of variances by the Levene's test and, where necessary, arc-sin transformed before further statistical analysis. One-way analysis of variance (ANOVA) was performed to determine statistical significance of differences between fish species and tissues for individual PL class, single fatty acids, group of fatty acids and index and Tukey's post-hoc test was used for multiple comparisons when pertinent. $p < 0.05$ was considered to be statistically different. Statistical analyses were performed using SPSS, version 22.0 (SPSS Inc., Chicago, IL).

3. Results

3.1. Phospholipid class composition

Percentages of the main phospholipid (PL) classes that integrate tissue membranes from *Amphiprion percula*, *A. clarkii* and *Chromis viridis* are represented in Figs. 2–4. Fish liver, skeletal muscle and brain showed different membrane PL compositions. Liver membranes had high sphingomyelin (SM) and cardiolipin (CL) and low phoshatidylserine (PS) contents compared to the other two tissues (Fig. 2), skeletal muscle membranes showed intermediate contents for the different PL classes (Fig. 3) and brain membranes showed higher PS and phosphatidylethanolamine (PE) and lower phosphatidylinositol (PI) and CL compared to liver and skeletal muscle (Fig. 4). Regarding inter-species comparisons, no significant differences in PL class percentages were found in liver membranes among *Amphiprion percula*, *A.clarkii* and *Chromis clarkii* (Fig. 2). Skeletal muscle from the coral reef damselfish *C. viridis* had higher contents of PS and lower of PC than the two anemonefish (Fig. 3) and brain membranes from *C. viridis* showed a higher content in PS and lower of PI and CL (Fig. 4).

3.2. Fatty acid compositions and peroxidation index values of individual PLs

Figures 5–7 show the fatty acid composition and peroxidation index (PIn) values of tissue membranes (liver, skeletal muscle and brain, respectively) from young adult *Amphiprion percula, Amphiprion clarkii* and *Chromis viridis* kept under equal temperature and rearing conditions and fed the same diet. For clarity reasons, pie charts in every figure represent only the main fatty acids and groups of fatty acids for each of the four most significant PLs (the three PL representing the highest percentages of the total plus CL). The complete fatty acid composition and indexes for every PL class and tissue for the three experimental fish species are included as Supplementary material (Supp. Tables 1–18).

Regarding fish liver, the peroxidation index (PIn) was significantly lower in *C. viridis* than in the two anemonefish for all PL classes, except for cardiolipin (just a trend was found) and sphingomyelin (SM), in which PIn was higher in *C. viridis* than in the other two fish species (Fig. 5, Supp. Tables 1–6). PIn values for *A. clarkii* were higher than those for *A. percula* for PC, PE and SM. PIn value for total PL was significantly lower in *C. viridis*. Regarding the two anemonefish, PIn of total PL was higher in *A. clarkii* than in *A. percula*. Liver membranes from *C. viridis* showed a lower content in n-3 polyunsaturated fatty acids (PUFA) in PC, PE and PS and higher in SM when compared with the two *Amphiprion* species.

There were significant differences in PIn values for skeletal muscle membranes among the experimental fish species for PC, PS and PI (Fig. 6, Supp. Tables 7–12). PIn values for PS and PI were lower in *A. percula* than in *A. clarkii* and *C. viridis*, while PC PIn was higher in *A. clarkii* compared to *A. percula* and *C. viridis*. Regarding the two anemonefish, PIn values for PC, PS and PI were lower in *A. percula* compared to *A. clarkii*. Total PL PIn value was significantly higher in *A. clarkii* than in *C. viridis* and *A. percula*. Skeletal muscle membranes from *A. percula* showed a lower n-3 PUFA content than the other two species.

Regarding fish brain, PIn values showed no significant differences among fish species for any of the four most significant PL classes (Fig. 7, Supp. Tables 13–18). Although not significant, a similar trend to that

found in liver was also observed in fish brain. PIn value for total PLs from *A. clarkii*˝s brain was higher than those from *A. percula* and *C. viridis*.

4. Discussion

Membrane lipid composition significantly differed among *Amphiprion percula, A. clarkii* and *Chromis viridis* in the three analysed tissues but the direction and magnitude of the observed differences did not explain the existing divergence in the estimated maximum lifespan potential (MLSP) among the species. Membranes from each tissue had distinctive phospholipid (PL) proportions and PL fatty acid compositions, which is in alignment with previous data in fish (Almaida-Pagán et al. 2012a, b) and rats (Paradies et al. 1992; Modi et al. 2008) and is very likely related to differential properties of membranes in each tissue, so as to different susceptibilities to lipid peroxidation. While liver membranes were richer in sphingomyelin (SM) and cardiolipin (CL), those from brain had a higher content in phophatidylserine (PS) than the other tissues. Regarding membrane PL fatty acid composition, clownfish brain had the most unsaturated membranes compared to liver and skeletal muscle within each species, which is correlated with faster turnover rates of individual membrane proteins and higher cellular metabolism (Hulbert et al. 2006).

Tissue membranes showed significant differences in PL proportions among the three species. PS content was statistically higher in skeletal muscle and brain from the shorter-lived *C. viridis* (estimated MLSP = 1–2 years) compared to the two anemonefish (estimated MLSP of 30 and 9–16 for *A. percula* and *A. clarkii*, respectively). This is in accordance with that observed in a previous study where three fish species of the short-lived annual genus *Nothobranchius* with different MLSP and the longer-lived outgroup species *Aphyosemion australe* were studied to test whether they conform to the predictions of the LHA theory of ageing (de Costa et al. 2020). A negative correlation between fish MLSP and PS content from cell membranes was also found in the *Nothobranchius* study and suggested to be linked to PS decarboxylation. PS descarboxylation leads to an increase in phosphatidylethanolamine (PE) intracellular levels, which was also found in the longer-lived *Nothobranchius* species and in *A. australe*. Since the abundance of PE positively regulates autophagy, regarded as one of the major cytoprotective mechanisms during aging (Feng et al. 2014), both a lower content of PS and higher of PE in cell membranes could be indicating that this mechanism is operating to protect cells and tissues from the aging-associated damage caused by ROS (de Costa et al. 2020). Nevertheless, no statistical differences among *Amphiprion* species and *C. viridis* in PE levels were found in any tissue´s membranes.

Regarding membrane PL fatty acid composition of fish tissues from the two anemonefish, *A. percula* had less unsaturated membranes and thus, lower peroxidation levels in liver and skeletal muscle (not statistical differences were found for brain) than *A. clarkii*, which has an estimated MLSP of half that of *A. percula* (9–16 vs. 30 years). This occurred at the level of the main PL classes from the two tissues, as it was shown in whole body of the *Nothobranchius* species previously studied (de Costa et al. 2020) and supports the longevity-homeovicous adaptation (LHA) theory of aging (Pamplona et al. 1998, 2000, 2002; Naudí et al. 2013). Besides, similar to what has been found in the *Nothobranchius* study, the magnitude

of the observed differences in these fishes was much smaller than that of the inter-species differences in longevity. This suggests that the LHA theory of aging alone is not sufficient to explain those differences and other aging effectors, such as increased mitochondrial ROS production, increased mtDNA fragments insertion inside nuclear DNA induced by mtROSp or decreased autophagy, among others, may be operating in an integrated way inside cells to determine longevity, as it has recently been proposed by Barja (2019).

When membrane PL fatty acid composition from tissues of the two *Amphiprion* species was compared with that of *C. viridis*, we found that the damselfish membranes had generally a lower PIn value than that from one (in skeletal muscle) or the two clownfish species (in liver), this being in contradiction with the LHA theory of aging. This is not the first time that we obtain data that apparently contradict the theory. Previous to the *Nothobranchius* study (de Costa et al. 2020), we compared data belonging to mitochondrial membrane lipids from whole *Nothobranchius rachovii* (MLSP = 14 months) and *N. furzeri* (MLSP = 7 months), which resulted from two separate experiments (Lucas-Sánchez et al. 2014b; Almaida-Pagán et al. 2019), and found that the shorter-lived species had the lowest PIn values. The *Nothobranchius* study, performed in fish raised in exactly the same conditions, resulted in a negative correlation between membrane total PIn and fish MLSP, meaning that the longer-lived *Nothobranchius* species have more saturated membranes and, therefore, a lower susceptibility to oxidative damage, as the LHA theory posits.

In conclusion, the present study showed differences in membrane composition (phospholipid class and fatty acid compositions) among fish tissues that point to the importance of particular PLs for tissue-specific functions. Significant changes in liver, skeletal muscle and brain membranes among *A. percula*, *A. clarkii* and *C. viridis* were found. When only the two anemonefish were compared, results pointed to the existence of a negative correlation between membrane PIn value and life expectancy, as it has previously been shown in mammals, birds and fish species of genus *Nothobranchius*. Nevertheless, when the two clownfish were compared to the shorter-lived *C. viridis*, data contradicted what the LHA theory of aging posits. Although new studies including a wider number of anemonefish and other phylogenetically-related species with different MLSP should be carried out to reinforce what was found in the present work, this data along with those obtained in previous studies on fish denote that the magnitude (and sometimes the direction) of the differences observed in membrane lipid composition and peroxidation index with maximum life expectancy cannot explain alone the diversity in longevity found among fishes.

Abbreviations

BHT, butylated hydroxytoluene; CL, cardiolipin; FAME, fatty acid methyl esters; LHA, longevity-homeoviscous adaptation theory of aging; mtROSp, mitochondrial ROS production; MLSP, maximum lifespan potential; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PIn, peroxidation index; PL, phospholipids; PS, phosphatidylserine; PUFA, polyunsaturated fatty acids; ROS, reactive oxygen species; SM, sphingomyelin; TLC, thin layer chromatography.

Declarations

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Conflicts of interest

The authors declare that they have no conflict of interest.

Ethics approval

Fish were treated in accordance with the current Spanish law regarding animal’s experiments and the experimental protocol performed for this work was approved by the Bioethics Committee of the University of Murcia (A13160603, from the Consejeria de Agua, Agricultura, Ganaderia y Pesca, Comunidad Autonoma de la Region de Murcia, Spain).

Consent to participate

Not applicable

Availability of data and material

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Code availability

Not applicable

Author contributions

Conceptualization: P.F.A.-P., A.C., P.M. and J.d.C.; Methodology: P.F.A.-P. and A.L.; Formal analysis: P.F.A.-P.; Investigation: P.F.A.-P.; Resources: P.F.A.-P.; Writing - original draft: P.F.A.-P.; Writing - review & editing: A.M.N., E.T., M.A.R.d.L., A.C. and J.d.C.; Supervision: A.C. and J.d.C.; Funding acquisition: P.F.A.-P., M.A.R.d.L. and J.d.C.

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Figures

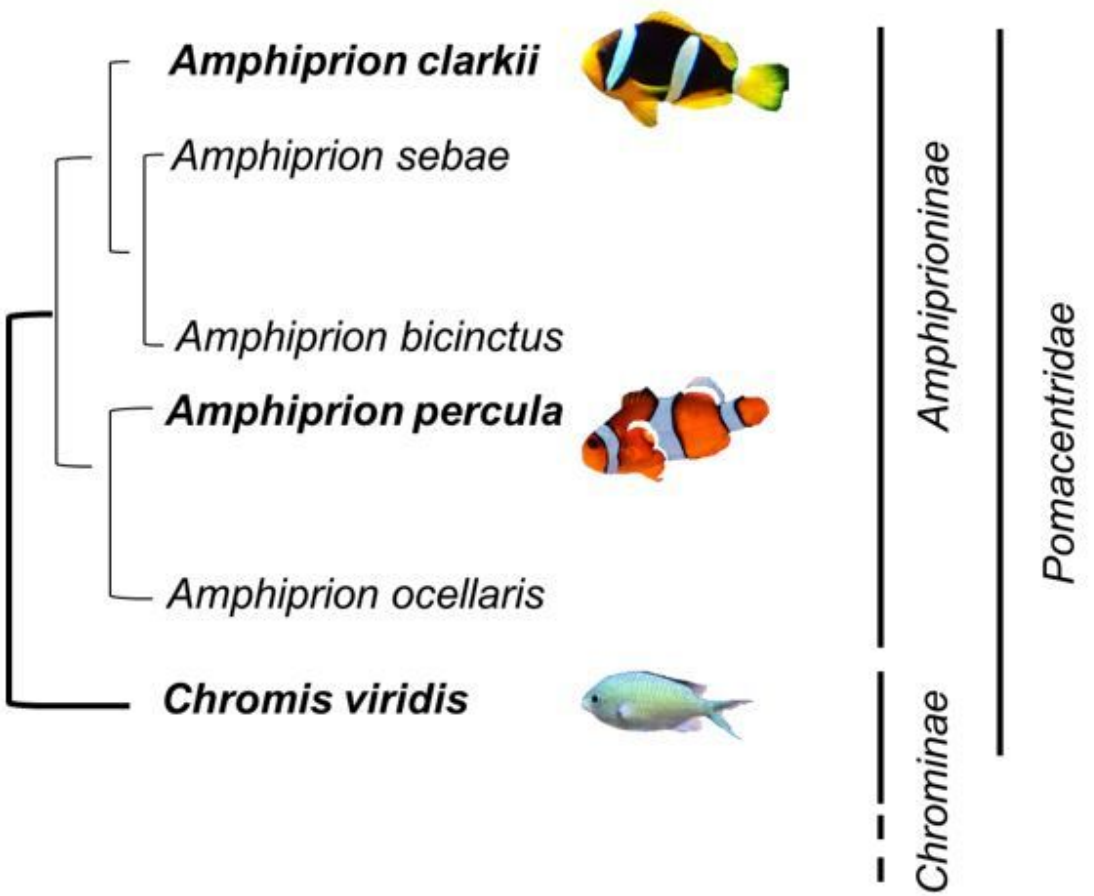

Figure 1

Nucleotide-based phylogeny of the analysed fish species as estimated in a previous study (Sahm et al. 2019). Chromis viridis represents the non-symbiotic sister-taxon to the Amphiprion genus. A. percula photo by Dylan McLeod on Unsplash. A. clarkii picture by Citron and C. viridis photo by Ben Lancaster (both taken from Wikipedia).

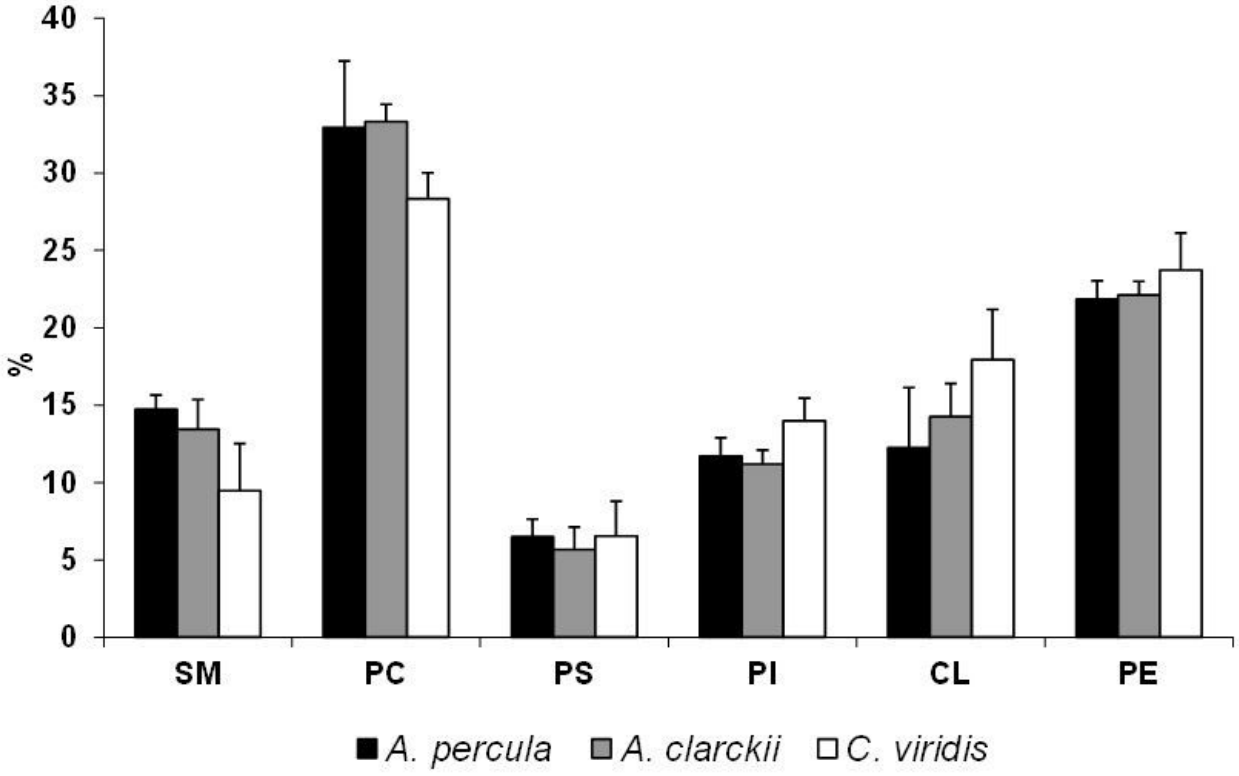

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Figure 2

Phospholipid class composition (percentage of total phospholipids) of membranes isolated from liver of young adult Amphiprion percula, Amphiprion. clarkii and Chromis viridis kept under equal temperature and rearing conditions and fed the same diet. Results are mean ± SD (n= 4). The absence of superscript letters means that no statistical differences among fish species for each phospholipid class were found as determined by a one-way ANOVA and Tukey's post hoc test ($p<0.05$). CL cardiolipin, PC phosphatidylcholine, PE phosphatidylethanolamine, PI phosphatidylinositol, PS phosphatidylserine, SM sphingomyelin.

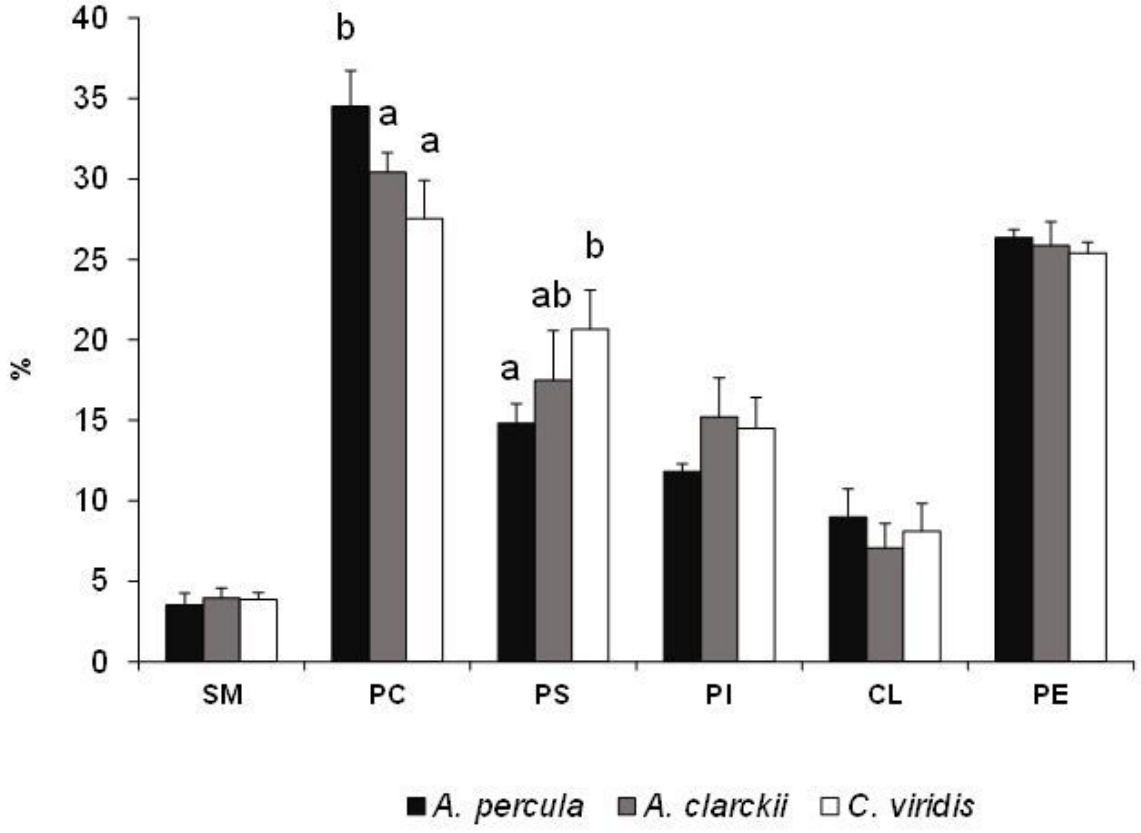

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Figure 3

Phospholipid class composition (percentage of total phospholipids) of membranes isolated from skeletal muscle of young adult Amphiprion percula, Amphiprion. clarkii and Chromis viridis kept under equal temperature and rearing conditions and fed the same diet. Results are mean ± SD (n= 4). Superscript letters mean statistical differences among fish species for each phospholipid class as determined by a one-way ANOVA and Tukey's post hoc test ("b" indicates a statically higher value than "a" for the same PL class; $p<0.05$). CL cardiolipin, PC phosphatidylcholine, PE phosphatidylethanolamine, PI phosphatidylinositol, PS phosphatidylserine, SM sphingomyelin.

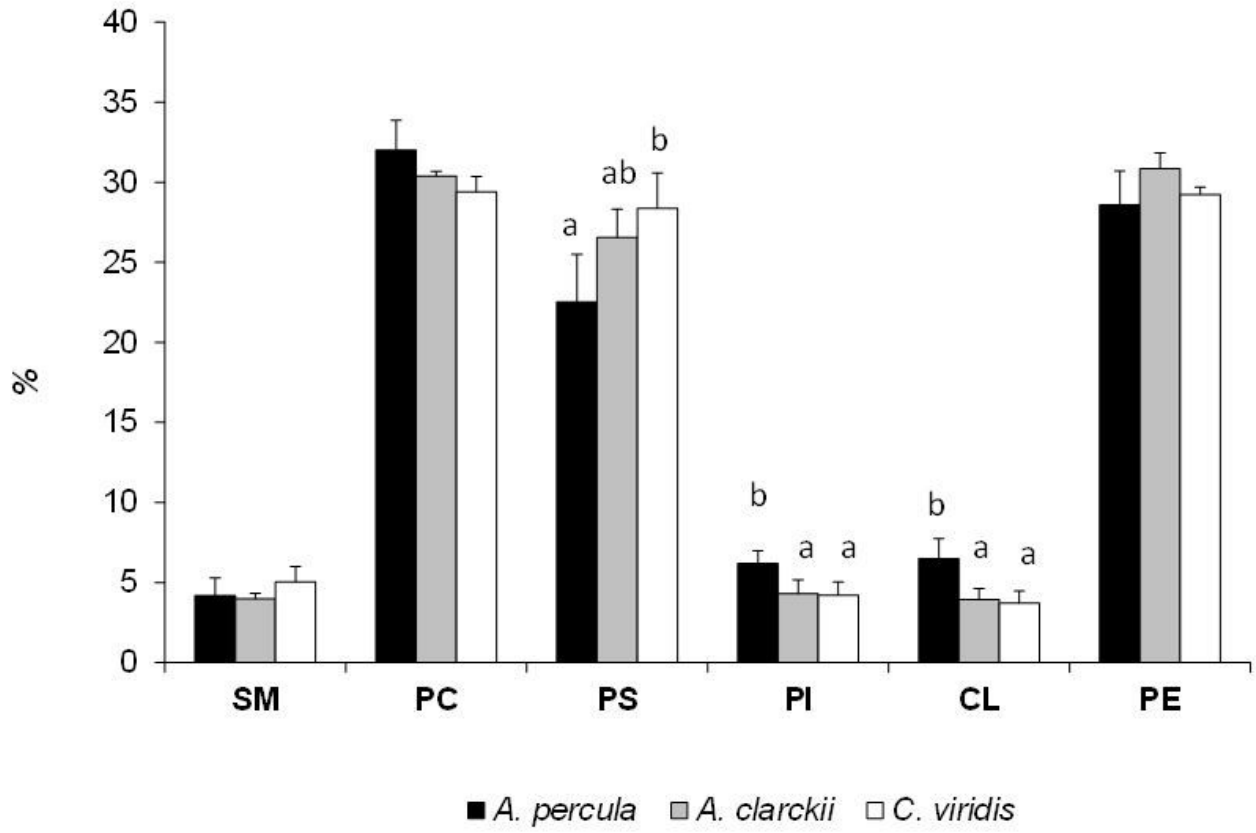

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Figure 4

Phospholipid class composition (percentage of total phospholipids) of membranes isolated from brain of young adult Amphiprion percula, Amphiprion. clarkii and Chromis viridis kept under equal temperature and rearing conditions and fed the same diet. Results are mean ± SD (n= 4). Superscript letters mean statistical differences among fish species for each phospholipid class as determined by a one-way ANOVA and Tukey's post hoc test ("b" indicates a statically higher value than "a" for the same PL class; $p<0.05$). CL cardiolipin, PC phosphatidylcholine, PE phosphatidylethanolamine, PI phosphatidylinositol, PS phosphatidylserine, SM sphingomyelin.

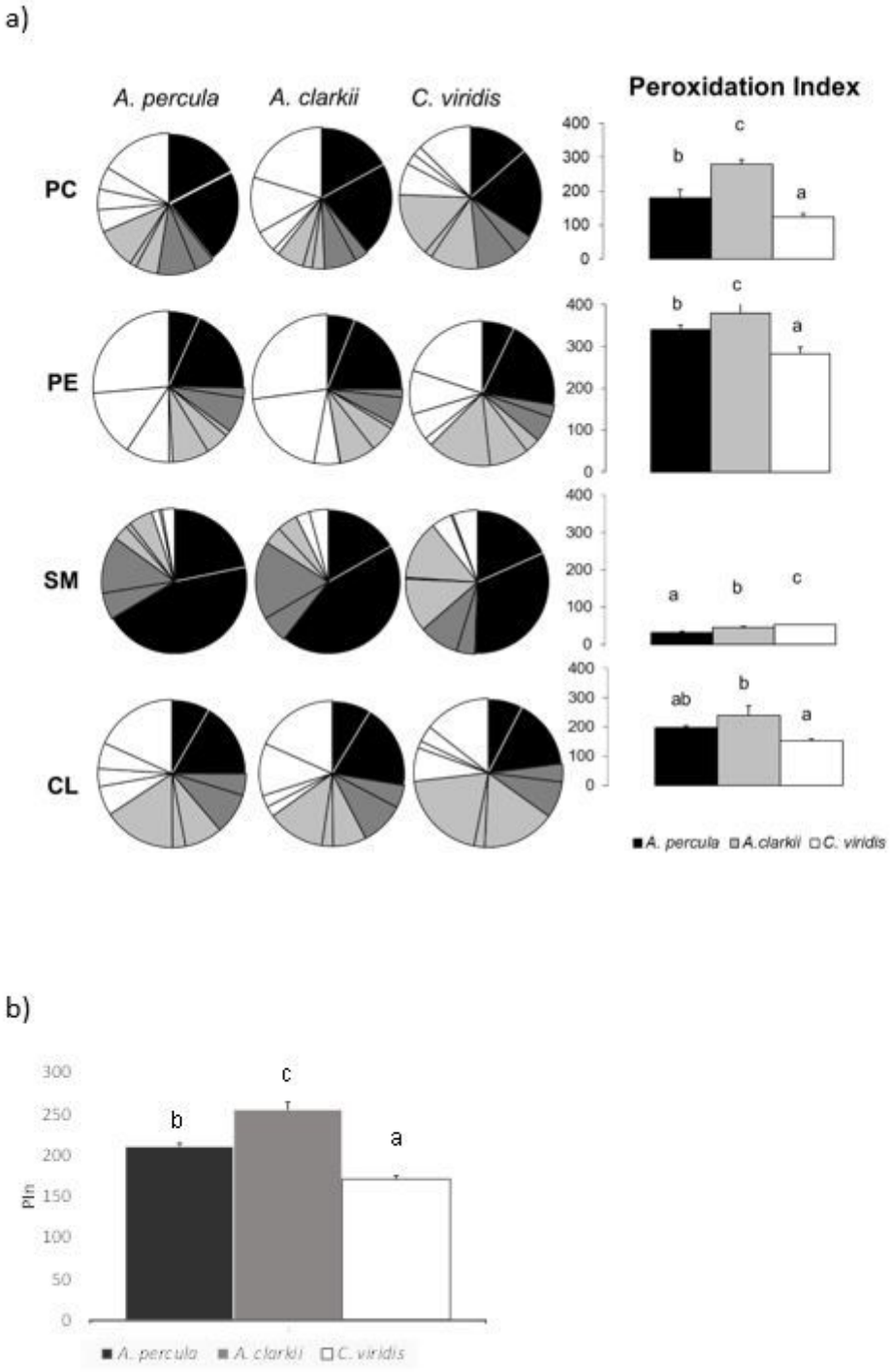

Figure 5

a) Phospholipid fatty acid composition of liver membranes from young adult Amphiprion percula, Amphiprion clarkii and Chromis viridis kept under equal temperature and rearing conditions and fed the same diet. Each segment of the pie chart represents the following fatty acids (clockwise order): saturated (black: 16:0 and Σsaturated), monounsaturated (dark grey: 18:1n-9 and Σmonounsaturated), n-6 polyunsaturated (light grey: 18:2, 20:4 and Σn-6 PUFA), n-3 polyunsaturated (white: 18:3, 20:5, 22:6 and

Σn-3 PUFA). Right column graphs present peroxidation index (PIn) values of each PL class for the three fish species. b) PIn values for membrane total PL from liver of the three fish species. Results shown in PIn graphs are mean ± SD (n= 4). Superscript letters mean statistical differences among fish species for PIn values as determined by a one-way ANOVA and Tukey t-test (“b” indicates a statically higher value than “a” for the same PL class; $p<0.05$). CL, cardiolipin; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; SM, sphingomyelin.

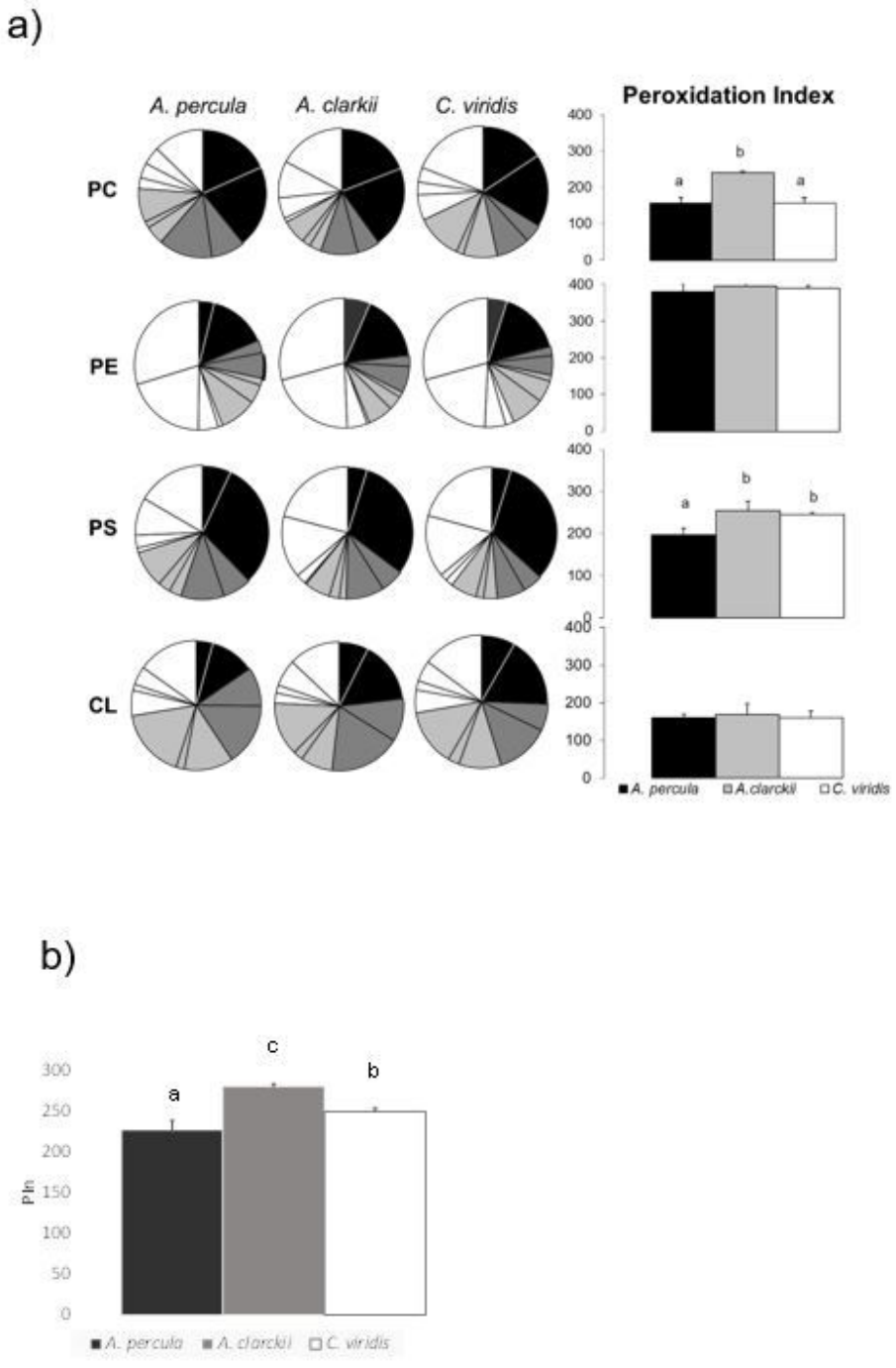

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Figure 6

a) Phospholipid fatty acid composition of skeletal muscle membranes from young adult Amphiprion percula, Amphiprion clarkii and Chromis viridis kept under equal temperature and rearing conditions and fed the same diet. Each segment of the pie chart represents the following fatty acids (clockwise order): saturated (black: 16:0 and Σsaturated), monounsaturated (dark grey: 18:1n-9 and Σmonounsaturated), n-6 polyunsaturated (light grey: 18:2, 20:4 and Σn-6 PUFA), n-3 polyunsaturated (white: 18:3, 20:5, 22:6 and Σn-3 PUFA). Right column graphs present peroxidation index (PIn) values of each PL class for the three fish species. b) PIn values for membrane total PL from skeletal muscle of the three fish species. Results shown in PIn graphs are mean ± SD (n= 4). Superscript letters mean statistical differences among fish species for PIn values as determined by a one-way ANOVA and Tukey t-test (“b” indicates a statically higher value than “a” for the same PL class; $p<0.05$). CL, cardiolipin; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; SM, sphingomyelin.

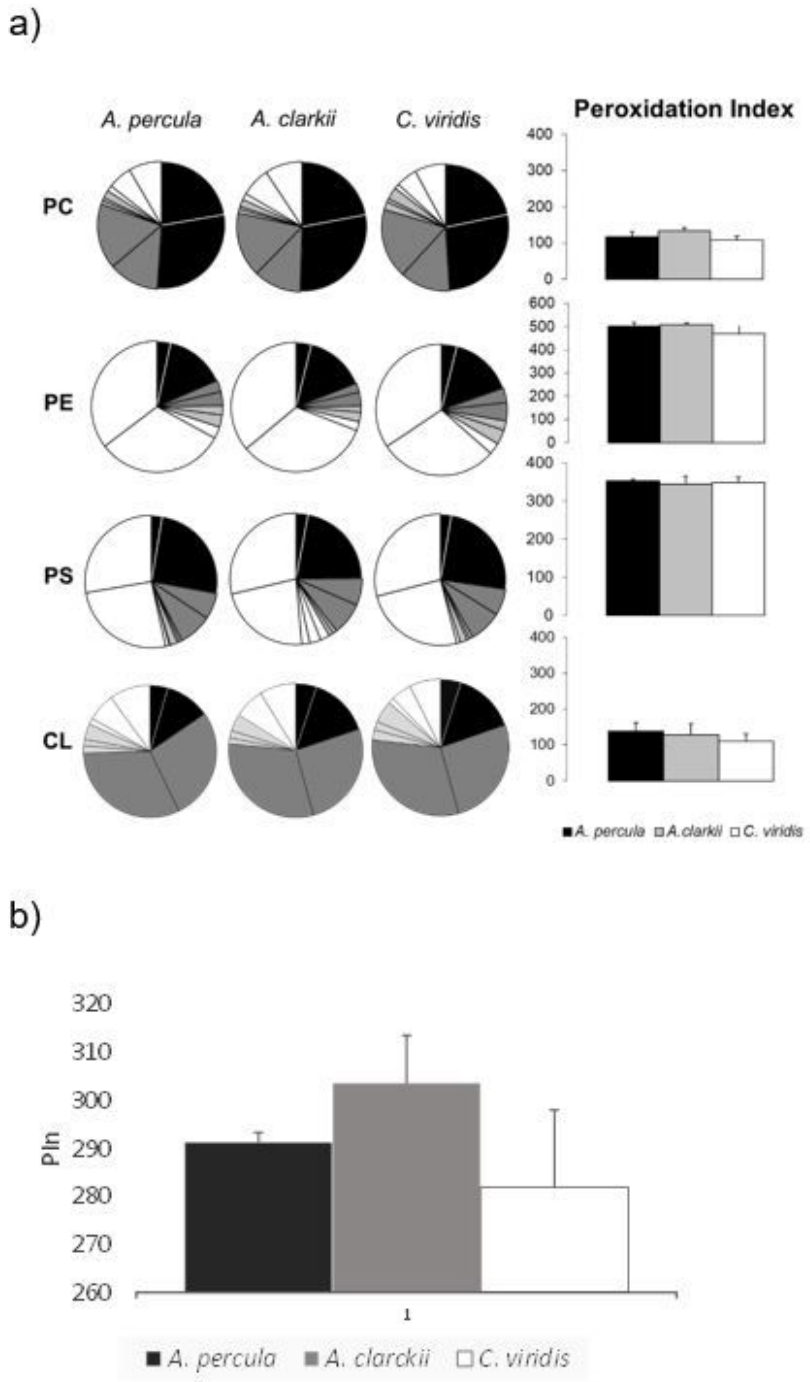

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Figure 7

a) Phospholipid fatty acid composition of brain membranes from young adult Amphiprion percula, Amphiprion clarkii and Chromis viridis kept under equal temperature and rearing conditions and fed the same diet. Each segment of the pie chart represents the following fatty acids (clockwise order): saturated (black: 16:0 and Σsaturated), monounsaturated (dark grey: 18:1n-9 and Σmonounsaturated), n-6 polyunsaturated (light grey: 18:2, 20:4 and Σn-6 PUFA), n-3 polyunsaturated (white: 18:3, 20:5, 22:6 and

Σn-3 PUFA). Right column graphs present peroxidation index (PIn) values of each PL class for the three fish species. b) PIn values for membrane total PL from brain of the three fish species. Results shown in PIn graphs are mean ± SD (n= 4). Superscript letters mean statistical differences among fish species for PIn values as determined by a one-way ANOVA and Tukey t-test (“b” indicates a statically higher value than “a” for the same PL class; $p<0.05$). CL, cardiolipin; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; SM, sphingomyelin.

Supplementary Files

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