SEASONAL AND ONTOGENETIC CHANGES IN SUBTROPICAL KRILL LIPIDS: IMPLICATIONS FOR TEMPORARY BLUE WHALE AND RESIDENT FIN WHALE STOCKS THAT INHABIT THE GULF OF CALIFORNIA

by

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ABSTRACT

The subtropical-neritic euphausiid *Nyctiphanes simplex* is a key food source for several resident and migratory organisms in the Gulf of California and the West Coast of the Baja California peninsula. Possibly, their main predators are two large mammals: a resident population of fin whales (*Balaenoptera physalus*), and blue whales (*B. musculus*) during their winter migration. Evidence shows that both baleen whale species breed and feed in the Gulf of California. In order to estimate the relevance of the region in terms of biomass intake by predators, it is necessary to understand and estimate the effect of development, reproduction, season and location on the energy content of krill. The development and maturity processes of this euphausiid were studied along with changes in size, weight and biochemical composition of the different life stages. Ovigerous females carrying eggs in pouches provide significantly higher energy than other life stages. Contribution of total lipids to dry weight was higher during the most productive months (February and May) in clear association with the spring reproductive peak previously reported for the species. Nonetheless, total energy content and body condition were higher during warmer months as a consequence of an increase in protein content, suggesting an increase in carnivory during warm months. Fasting experiments revealed that it was possible to detect unfavourable feeding conditions between 2.5 to 5 days as lipid content and the hepatosomatic index had a large negative significant correlation with fasting time. Wild euphausiids collected during spring 2010 never fasted >2.5 days and fatty acid analysis revealed that diatoms were the main food source in upwelling areas. However, cyanobacteria, Chlorophyceae or even copepods seemed to be a relevant food source for *N. simplex* in warmer areas. Energy content of euphausiids, together with seasonal patterns, metabolic rates and estimated abundances of blue and fin whales (*Balaenoptera musculus* and *B. physalus*) were

used to calculate prey biomass demands of both top predators. Individual estimates of daily energy requirements during winter were 10–19% higher for blue whales than for fin whales. However, individual estimates of prey biomass demand during spring were ~6% higher for fin whales. In fact, the density of krill required to satisfy the estimated biomass demand by female fin whales at the end of spring is very close to the maximum estimated density of euphausiids in the region. This suggests that alternative food sources are needed to sustain the resident population of fin whales in the area. On the other hand, the density of krill required to satisfy the estimated biomass demands by blue whales is within the limits of the estimated density of winter swarms. This is important as the North Pacific population of blue whales appears to be clearly recovering from exploitation. However, the closeness between the densities of euphausiids required in spring and those estimated in the field suggest that a decrease on euphausiid population density might be key in the migratory movement of blue whales to more profitable feeding areas.

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GLOSSARY

Active metabolic rate (AMR): Energy consumption per unit time (J h^{-1}) required for normal body activity. It is the maximum sustained aerobic metabolic rate measured in non-limiting conditions, i.e. those that do not interfere with oxygen supply (hypoxia, hypercapnia). Normal activity must be viewed in a broad sense and includes all physiological work such as swimming, feeding or mating.

Apparent digestible efficiency: Subtraction of faecal energy from gross energy intake (*GEI*) gives the digestible energy (*DE*). *DE* can also be expressed as apparent digestible efficiency (% *DE* or *AD*), which is the proportion of *GEI* which has been absorbed through the intestinal wall and entered the bloodstream. A method based on dietary Mn^{2+} as an inert marker can also be used to estimate it: % *DE* = (1 - (*Ci* x *Ef*/*Cf* x *Ei*)) x 100, where *C* is the concentration of Mn^{2+} and *E* is the *DE* of the food (*i*) and faeces (*f*) expressed on a dry matter basis.

Assimilation efficiency: Also known as conversion efficiency of ingested food to unit of body substance (CEI) or "growth efficiency". It is a rough scale of the amount of ingested food that is converted into growth in the animal's mass. It can be used to compare the growth efficiency as measured by the weight gain of different animals from consuming a given quantity of food relative to their body size. The formula of the conversion efficiency: CEI = AD (apparent digestibility) × CED (conversion efficiency of digested food) represents efficiencies of both digestion and metabolic efficiency, or how well digested food is converted to mass (CED).

Basal metabolic rate: Minimal rate of energy expenditure per unit time by endothermic animals at rest. It is reported in energy units per unit time ranging from watt (J s^{-1}) to joule per hour (J

h^{-1}) or even kcal day^{-1}. It also can be expressed in terms of O_2 volume units per unit time such as mL O_2 min^{-1} or mL O_2 h^{-1}. Here, the energy is used to perform the body's most basic (basal) life-sustaining functions. These basal functions include blood circulation, breathing, controlling body temperature, brain and nerve function, muscle contraction, cell growth, nutrient processing, protein synthesis and ion transport. BMR is most accurately measured under very restrictive conditions: in a physically and psychologically undisturbed state, in a thermally neutral environment, while in the post-absorptive state (*i.e.*, not actively digesting food). Unlike standard metabolic rate, reported estimates of BMR do not state the temperature at which it was estimated.

Branched fatty acids (BFA): Fatty acids synthesized by many microorganisms (most often with an *iso*- or an *anteiso*-methyl branch) and synthesized to a limited extent in higher organisms. They can also enter animal tissues via the diet, especially those of ruminants.

Coastal upwelling: The best-known type of upwelling, where wind-driven currents are diverted to the right of the winds in the Northern Hemisphere and to the left in the Southern Hemisphere due to the Coriolis effect. The result is a net movement of surface water at right angles to the direction of the wind, known as the Ekman transport. When Ekman transport is occurring away from the coast, surface waters moving away are replaced by deeper, colder, denser and usually nutrient-rich waters.

Development: A succession of stages, phases or changes occurring in organisms, each of which is preparatory for the next. It implies a natural process of increase in complexity, differentiation, or evolution by successive changes while gradually acquiring secondary sex characteristics. The development of many invertebrates (e.g. crustaceans) and amphibians involves the process of further metamorphosis after the eclosion (emergence) of larval forms.

Sometimes the term is applied to specific stages of the whole development process; for instance: embryo development, which in euphausiids includes six developmental stages: (1) Single cell stage (recently-spawned fertilized egg with minute perivitelline space, and no sign of cleavage), (2) Multiple cell stage (initial stage of cleavage towards multicellular blastula), (3) Gastrula (formation of two layers of cells enclosing a central cavity known as archenteron), (4) Early limb bud (the embryo is transformed into a nauplius, and the distal ends of the appendages are still connected with the body by a membrane. Limb primordia are visible as ridges in lateral view), (5) Late limb bud (the distal ends of the limbs become free, tube-like structures), and (6) Twitch (typical nauplius shape, without membrane surrounding it. In a live embryo, appendages move freely, and the heart has visible pulsating movements).

Doubly labeled water method: Is a method to estimate total carbon dioxide production using 'tagged' water in which both hydrogen and oxygen are traceable as they have been partly or completely replaced with ^{2}H (deuterium) and ^{18}O. When cellular respiration breaks down carbon-containing molecules to release energy, carbon dioxide is released as a by product, but carbon-containing molecules do not contain enough oxygen to provide both oxygen atoms found in CO_2. As a result; one of the two oxygen atoms in CO_2 is derived from body water. If the oxygen in water is labeled with ^{18}O, then CO_2 produced by respiration will contain labeled oxygen. As carbon dioxide is exhaled, ^{18}O is lost from the body. However, ^{18}O is also lost through body water loss in (mostly) urine, sweat, and breath. However, the ^{2}H is lost only when body water is lost. Thus, the loss of ^{2}H in body water over time can be used to mathematically compensate for the loss of ^{18}O by the water-loss route. The objective is to measure a subject's carbon dioxide production during the interval between first and last body

water samples. Field metabolic rate (*FMR*) can be estimated from the ratio of oxygen used in metabolism (and therefore heat generated), to carbon dioxide eliminated.

Ekman Transport: Term given for the 90° net transport of the surface layer of a fluid (the layer affected by wind) by wind forcing. At the surface, currents flow at a 45° angle to the wind due to a balance between the Coriolis force (an inertial force acting on moving objects relative to a rotating reference frame) and the drag generated by the wind and the water. If the ocean is divided vertically into thin layers, the magnitude of the velocity decreases from a maximum at the surface until it dissipates in deeper layers. The direction also shifts slightly across each subsequent layer. This is called the Ekman spiral. The layer of water from the surface to the point of dissipation of this spiral is known as the Ekman layer. If all flow over the Ekman layer is integrated, the net transportation is at 90° of the surface wind. The direction of transport is dependent on the hemisphere: in the northern hemisphere, transport occurs at 90° clockwise from wind direction, while in the southern hemisphere it occurs at a 90° counter clockwise. Technically it applies to the idealized situation involving only wind forces; however, Ekman motion describes well the wind-driven portion of ocean circulation seen in the surface layer.

Essential fatty acids (EFA): A large variety of polyunsaturated fatty acids which had the first double bond in the n-6 (ω6) or in the n-3 (ω3) position from the methyl end and with carbon numbers ranging from C14 to C36. These fatty acids are considered essential dietary components and have been termed ‘essential fatty acids’ as they can remove acute deficiency symptoms in animals that had been fed fat-free diets. When provided with sufficient ω3 and ω6 fatty acids in the diet, most animals can synthesize other ω3 and ω6 fatty acids by desaturation and elongation or by retroconversion to shorter-chain fatty acids. However, the

ω3 and ω6 series are not interconvertible in vertebrates and most other animals. The extent to which a given species can convert one ω3 fatty acid to another or one ω6 fatty acid to another leads to degrees of essentiality. In normal conditions, most cells synthesize saturated fatty acids from acetate, which is generated during catabolism of fats and carbohydrates. In all organisms, further synthesis into unsaturated fatty acids usually occurs via the insertion of the first double bond near the middle of the molecule, for example in the n-9 position in stearic acid (18:0) to create oleic acid (18:1n-9 or 18:1ω9). In plant cells, subsequent double bonds are introduced between an existing double bond and the terminal methyl group (the ω end), while animal cells normally introduce a second double bond between the existing position and the carboxyl end of the molecule (the α end). Plant cells produce two important enzymes: Δ12-desaturase and Δ15-desaturase. The former enzyme allows the synthesis of linoleic acid (LIN, or 18:2ω6, or cis-9, cis-12 octadienoic) from oleic acid (18:1ω9) by desaturation at C12. The later enzyme allows plant cells to produce α-linolenic acid (ALA, or 18:3ω3, or cis-9, cis-12, cis-15-octadecatrienoic acid) from linoleic acid by desaturation at C15. Linoleic (18:2ω6) and α-linolenic (18:3ω3) acids are the origin of the two main families of polyunsaturated fatty acids, ω6 and ω3. In animal tissues, those fatty acids are required for normal functioning but cannot be synthesized *de novo* or are synthesized in very low quantities. The inability of animals to insert a double bond between the first double bond and the methyl end, combined with a requirement for fatty acids with the first double bond in the n-3 or n-6 position for disease prevention, is the basis of the essentiality of ω6 and ω3 fatty acids.

Fatty acids (FA): Compounds synthesized in nature via condensation of malonyl coenzyme A units by a fatty acid synthase complex. They usually contain even numbers of carbon atoms

in straight chains (commonly C14 to C24), and may be saturated or unsaturated, and can contain a variety of substituent groups. Fatty acids consist of a hydrocarbon chain with a methyl group (CH_3) at one end and a carboxyl (COOH) group at the other end. Commonly, fatty acids are saturated (no double bonds = SFA), branched (with methyl or ethyl groups adjacent to the main carbon chain), monounsaturated (1 double bond = MUFA), and polyunsaturated (≥2 double bonds = PUFA). Long chain polyunsaturated fatty acids (LC-PUFA) are a specific category of fatty acids with ≥20 carbon atoms.

Fatty acid nomenclature: There are four common naming systems: trivial name, IUPAC, carboxyl-reference, and ω-reference. 1) Trivial names typically derive from a common source of the compound or the source from which it was first isolated. For example, palmitic acid is found in palm oil, oleic acid is a major constituent of olive oil (*oleum*) and stearic (from the Greek word meaning solid) acid is solid at room temperature. Trivial names contain no clues to the structures and the name must be associated with a separately learned structure. In contrast, all other systems attempt to denote the chain length and the number and positions of any double bonds. 2) In the IUPAC system, the chain length and the number of double bonds are denoted by a name derived from Greek (e.g., hexadecanoic acid or octadecanoic acid for 16C and 18C fatty acids respectively). Here, the carboxyl carbon is denoted by the number one, and positions in the chain are denoted with reference to it. For instance, a double bond is said to be at the 9-carbon if it originates at the ninth carbon and extends to the next (tenth) carbon in the chain, as in oleic acid (cis-9-octadecenoic acid or (Z)-octadec-9-enoic acid) or in linoleic acid ((9Z,12Z)-octadeca-9,12-dienoic acid). 3) The carboxyl-reference system denotes compounds using abbreviations. Again, the carboxyl carbon is denoted by the number one, but the number of carbons is indicated with a number.

The number of double bonds is indicated with another number after a colon, and the positions of the double bonds, with a superscript after the Greek capital letter delta (e.g. oleic acid will be denoted as 18:1 Δ^9 and linoleic acid as 18:2 $\Delta^{9,12}$). 4) The omega-reference system is the most succinct of all. It also uses numbers to indicate the number of carbons and the number after the colon specifies the number of double bonds. The main difference is that the methyl carbon (or ω carbon) is considered as the first carbon. The position of the terminal double bond is denoted in the form (n-x), where n is the chain-length of the fatty acid and x is the number of carbon atoms from the last double bond. The position of the double bond closest to the omega carbon is also represented after the Greek letter omega. However, no further double bonds are indicated as it is assumed that all the other double bonds are methylene-interrupted. Accordingly, oleic, linoleic and α-linoleic acids are represented as 18:1(n-9), 18:2(n-6) and 18:3(n-3), respectively; or 18:1ω9, 18:2ω6 and 18:3ω3. This system is useful not only because of its briefness, but because of the important physiological differences between ω3 and ω6 fatty acids, and the impossibility to interconvert them in the human body.

Geostrophic winds and geostrophic currents: Air naturally moves from areas of high pressure to areas of low pressure. Similarly, sea water naturally tends to move from a region of high pressure (or high sea level) to a region of low pressure (or low sea level). The force pushing the fluid (air or water) towards the low-pressure region is called the pressure gradient force. As soon as the fluid starts to move, however, the Coriolis "force" deflects it. As long as the force (pressure) continues to act, the fluid moves and its speed increases, and so does its Coriolis deflection. The deflection increases until the Coriolis and pressure gradient forces are in geostrophic balance. The resulting flow is known as geostrophic. At this point, the fluid is no longer moving from high to low pressure, but instead is directed parallel to isobars

(lines of constant pressure at a given height or depth). The direction of geostrophic flow is parallel to the isobars, with the high pressure to the right of the flow in the Northern Hemisphere, and the high pressure to the left in the Southern Hemisphere. The direction of the flow depends on the hemisphere, because the direction of the Coriolis force is opposite in the different hemispheres. The geostrophic wind (or current) is the theoretical wind (or oceanic flow) that would result from a balance between the Coriolis force and the pressure gradient force. The actual flow would equal the geostrophic flow only if there were no friction and the isobars were perfectly straight. For winds, this balance seldom holds exactly in nature, the true wind almost always differs from the geostrophic wind due to other forces such as the effect of friction between the air and the land. Friction slows the flow, lessening the effect of the Coriolis force. As a result, the pressure gradient force has a greater effect and the air still moves from high to low pressure, though with great deflection. Accordingly, non-geostrophic terms, although small, are essential for the time evolution of the flow and are necessary for modelling the growth and decay of storms. Despite this, much of the atmosphere outside the tropics is close to geostrophic flow most of the time and it is a valuable first approximation for the overall wind circulation. Similarly, in the ocean the principle of geostrophy is useful to infer currents from measurements of the sea surface height (by combined satellite altimetry and gravimetry) or from vertical profiles of seawater density taken by ships or autonomous buoys. The major currents of the world's oceans are all approximately in geostrophic balance and are examples of geostrophic currents.

Growth: The process of increase in size of an organism, commonly accompanied by increase in dry weight, Carbon weight and volume. It occurs through cell division and cell enlargement via assimilation of material. It has three distinct components; a) assimilation, b) cell

expansion or enlargement, and c) cell division. The basis of growth is cell division but in order to increase in size, cells must be able to synthesize new structures that are manufactured from raw materials assimilated from their immediate environment. In crustaceans, growth is typically discontinuous, occurring at each molting event.

Hydrographic regime: Dominant physical features of bodies of water.

Isopycnal: A line on a map connecting points of equal or constant density. In oceanography, isopycnals are used to display the vertical distribution of water density. In a body of water, varying degrees of salinity and temperature act to modify the density of water, and the denser water always lies below the less dense water. This creates distinguishable layers of water differing in physical properties. This phenomenon is called stratification. Turbulence can cause the layers to bend and the ways in which the isopycnals are transformed can be used by oceanographers to identify the force that caused the underwater disturbance.

Lipid classes: Hydrophobic or amphipathic (with both hydrophilic and lipophilic parts) small molecules present in lipid extracts which include hydrocarbons (HC), steryl esters (SE), wax esters (WE), ketones (KET), triacylglycerols (TAG), free fatty acids (FFA), alcohols (ALC), sterols (ST), acetone mobile polar lipids (AMPL), and phospholipids (PL).

Lipids: A diverse group of carbon and hydrogen-rich hydrophobic organic compounds. A structure-based classification of lipids considers two main classes: simple and complex lipids. Simple lipids are compounds that produce no more than two primary products per mole during hydrolysis. Normally they constitute energy reserves in food webs because they are constituted by fatty acids (FA) linked by an ester bond to monoalcohols as in the case of waxes, or fatty acids bound to a polyalcohol (glycerol), forming triacylglycerides or triglycerides (TAGs), diacylglycerides (DAGs) and monoacylglycerides (MAG). Some

simple lipids like sterols (ST), which are alcohols in ringed structure, fulfill structural functions in cell membranes. In contrast, complex lipids are compounds which produce three or more primary products per mole during hydrolysis. They normally form the structure of cell membranes and include phospholipids or glycerophospholipids consisting of a glycerol bound to one or two fatty acids and a polar phosphate group which in turn binds to other groups such as amines. Complex lipids also include glyceroglycolipids: a glycerol bound to two fatty acids and to a mono- or disaccharide, and sphingolipids: fatty acids bound by amide bonds to long chain bases (or sphingoid bases) and occasionally to other amines. Several biomolecules can be classified as lipids, including fatty acid derivatives such as esters and amides. Some authors include substances biosynthetically related to fatty acids such as prostanoids and aliphatic ethers or alcohols, or substances functionally related to fatty acids such as sterols and even bile acids. Despite this amplitude, such criteria still exclude compounds with little functional or structural relation with the previous ones like steroids, liposoluble vitamins, pigments, carotenoids and terpenes. However, such compounds are soluble in organic solvents like chloroform, benzene, ethers and alcohols, and are usually extracted and quantified as total lipids. For that reason, it is important to recognize that a fine separation of the different lipid classes allows a better interpretation of the processes under study.

Mass-specific basal metabolic rate: Rate of energy per unit time required to sustain a unit of mass on endothermic animals. It is expressed as: joule per hour per kg body mass ($J\ h^{-1} \cdot kg^{-1}$).

Maturity: The condition of being fully developed, especially in terms of sexual characteristics that allow reproduction. It is reached when a developing organism is capable of reproduction.

It is also applied to the formation of gametes (gametogenesis) following meiotic division of precursor cells and their development into fully functional gametes capable of fertilization.

Maturity Stage: Contrary to gonad development, which implies a detailed description of the different stages of germinal cells and the tissues involved, maturity stage is used in this work as a broad description of general stages such as juveniles, subadults (adult-sized krill) and adults, and among adults, different states of gonad development.

Monounsaturated fatty acids (MUFA): Straight- or normal-chain (often even-numbered), monoenoic components, i.e. with one double bond, which make up a high proportion of the total fatty acids in most natural lipids. Normally the double bond is of the cis- or Z-configuration, although some fatty acids with trans- or E-double bonds are known.

Neritic: Area constituting the belt or region of shallow water adjoining the seacoast over the continental shelf (typically <200 m depth).

Net Primary Productivity: Amount of organic carbon generated in the surface ocean by photosynthesis in planktonic organisms minus the amount of organic carbon used by these organisms in respiration. NOAA CoastWatch provides an estimate of primary productivity based on the following satellite measurements: Chlorophyll *a* concentration and photosynthetically available radiation (PAR) measurements from the SeaWiFS sensor aboard the GeoEye spacecraft, SST measurements from the NOAA Pathfinder Project and from the Reynolds Optimally-Interpolated SST (OISST) v2 product from NOAA's National Climatic Data Center (NCDC).

Odd-chain fatty acids (OFA): fatty acids with odd numbers of carbons biosynthesized from propionyl-CoA and acetyl-CoA, rather than to two acetyl-CoA at the final step. The propionyl-CoA is not a substrate for the TCA cycle or other simple pathways.

Ontogeny: The developmental course of an organism from the fertilized egg through to maturity.

Phospholipids (PL): Class of lipids containing a diacylglycerol, a phosphate group, and a simple organic molecule such as choline. They are essential components of membranes where they share a structural function with sterols, and can be used to indicate freshly biosynthesized material.

Polyunsaturated fatty acids (PUFA): Fatty acids with two or more double bonds which are usually in the cis-configuration separated by a single methylene group (methylene-interrupted). In higher plants, the number of double bonds in fatty acids only rarely exceeds three, but in algae and animals there can be up to six and sometimes even more. Two principal families of polyunsaturated fatty acids occur in nature that are derived biosynthetically from linoleic (9-cis,12-cis-octadecadienoic) and α-linolenic (9-cis,12-cis,15-cis-octadecatrienoic) acids; those families are named omega-6 (n-6 or ω6) and omega-3 (n-3 or ω3) respectively.

Previtellogenesis: During egg production and after differentiation of oocytes in lecithotrophic organisms (in which the only source of nutrition for the embryo is yolk originally contained within its egg), previtellogenesis is a phase of increase in the volume of nucleus and cytoplasm of primary oocytes where no synthesis or accumulation of food reserve material occurs within the oocyte. There is qualitative and quantitative increase in the amount of cytoplasm. The mitochondria increase in number, the network of the endoplasmic reticulum with ribosomes becomes more complicated, and the Golgi bodies manufacture cortical granules, besides performing their normal function.

Proximate analysis: Estimate of the relative amounts of proteins, lipids, water, ash and carbohydrates in an organism. Proteins, lipids and carbohydrates contribute to the total energy content of an organism while water and ash only contribute to mass. Consequently, the total energy content of a specimen can be reconstructed from its proximate composition using calorific equivalents from proximate compositions. Normally, energy content determined directly from calorimetry (bomb) is similar to energy content calculated indirectly from proximate composition. Energy content is frequently used as an expression of a specimen's condition or health status. However, energy varies in response to specific life history demands and environmental conditions. Therefore, the relative contribution of lipids, proteins and carbohydrates to an organism's mass provides powerful information for a better interpretation of the variations in energy content.

Saturated fatty acids (SFA): Straight- or normal-chain, saturated components (usually even-numbered). The most abundant saturated fatty acids in animal and plant tissues are straight-chain compounds with 14, 16 and 18 carbon atoms, but all the possible odd- and even-numbered homologues with 2 to 36 carbon atoms have been found in nature in esterified form. They are named systematically from the saturated hydrocarbon with the same number of carbon atoms, the final 'e' being changed to 'oic'.

Subtropical Ecosystem: The interacting system of a biological community and its non-living environmental surroundings in regions bordering on the tropics or the regions between tropical and temperate biogeographic regions. The subtropics are geographic and climate zones located roughly between the tropics and temperate latitudes in both hemispheres. In the North it is generally defined by the region between 23°26' N (the Tropic of Cancer) and the limit of temperate zones (~35° N), whereas in the Southern hemisphere is generally defined

by the region between 23°26' S (the Tropic of Capricorn) and ~35° S. Subtropical climates are often characterized by warm to hot summers and cool to mild winters with infrequent frost. Most subtropical climates fall into two basic types: 1- Humid Subtropical, where rainfall is often concentrated in the warmest months, and 2- Dry summer (or Mediterranean) where seasonal rainfall is concentrated in the cooler months.

Triacylglycerols (TAG): Consist of a glycerol moiety with each hydroxyl group esterified to a fatty acid. In nature, they are synthesised by enzyme systems, which determine that a centre of asymmetry is created about carbon-2 of the glycerol backbone, so they exist in enantiomeric forms, i.e. with different fatty acids in each position. Their primary biological function is to serve as a store of energy.

Upwelling ecosystems: Upwelling regions can broadly be divided into coastal Eastern boundary currents and open ocean systems, both characterized by surface layer Ekman divergence. Major commercial fisheries associated with Eastern boundary currents are based on shoals of pelagic fish that thrive on abundant phytoplankton and zooplankton food resources. Behavioral adaptations in planktonic algae and animals allow them to avoid being swept away from upwelling centers to less favorable habitats, but the survival of small pelagic fish is particularly affected by physical circulation patterns. Community structure in upwelling ecosystems is therefore sensitive to climate variability and change, with fluctuations in fish numbers impacting both higher and lower trophic levels.

Vitellogenesis: Process of yolk formation and deposition via nutrients being deposited in the oocyte, or female germ cells involved in reproduction of lecithotrophic organisms. In some insects and apparently some crustaceans, yolk formation occurs in fat bodies (in most vertebrates, yolk is formed in the liver of the mother). From there it is carried to the

cytoplasm of the oocyte. A major part of the material forming the yolk is exogenous (formed outside the oocyte). Yolk is a general term that covers the major storage material such as glycogen, certain other carbohydrates, proteins and lipids. Vitellogenesis is the period of rapid growth of the oocyte.

Wax esters (WE): In their most common form, wax esters consist of fatty acids esterified to long-chain alcohols with similar chain-lengths. The latter tend to be saturated or have one double bond only. Such compounds are found in animal and plant tissues, and in microbes. They have a variety of functions, such as acting as energy stores, waterproofing and lubrication.

Chapter 1. **Introduction**

1.1. *Nyctiphanes simplex* in the Gulf of California

In marine ecosystems, euphausiids (Crustacea: Euphausiacea), also known as "krill" play a key trophic role linking organic detritus, phytoplankton, micro- and mesozooplankton with organisms of higher trophic levels. Euphausiids are marine malacostracan crustaceans that occupy all slopes and basins of the World Ocean, and they are distributed from the surface layer to at least 4000 m depth and from Arctic to Antarctic waters (Brinton *et al*., 2000).

The euphausiid *Nyctiphanes simplex* Hansen, 1910, proliferates in regions near coastal upwelling systems along the north and south temperate margins of the Eastern Tropical Pacific (ETP), but is absent in the core of the oxygen deficient ETP (Tremblay *et al*., 2010). In the southeast Pacific it is common in the northern sector of the Humboldt Current System (north of Chile and Peru) (Antezana, 1970). In the Northern Hemisphere, it is particularly abundant in the southern shoreward region of the California Current System along both sides of the Baja California Peninsula (Boden *et al*., 1955; Brinton 1960, 1979). Here, it may extend variable distances to the north and south or be carried to the west in tongues of distribution. This relatively small euphausiid (*ca* 2 cm total length) represents the most abundant coastal (neritic) euphausiid along the eastern and western coasts of the Baja California Peninsula (Boden, 1951; Brinton, 1962; Brinton & Townsend, 1980; Brinton *et al*., 1986; Gómez-Gutiérrez & Hernández-Trujillo, 1994; Lavaniegos, 1994), where it periodically reaches elevated densities near upwelling regions (Gómez-Gutiérrez, 1995; Gómez-Gutiérrez *et al*., 1996, 2012; De Silva-Dávila & Palomares-García, 1998). Figure 1-1 shows the main areas referred to in this thesis.

1.2. Trophic role of *Nyctiphanes simplex* around the Baja California peninsula

The ecological role of *N. simplex* in this subtropical area is widely documented (Figure 1-2). It is a common prey of the large fish species such as the whale shark (*Rhincodon typus*: Clark & Nelson, 1997) and mantas (*Mobula japanica* and *M. thustoni*: Notarbartolo-Di-Sciara, 1988; Lavaniegos-Espejo, 1995; Fischer *et al*., 1995). It is also prey of commercial fish species such as the lumptail searobin (*Prionotus stephanophyrys*); the high-fin sand perch (*Diplectrum labarum*); and the naked-belly searobin (*Bellator gymnostethus*) (Schmitter-Soto & Castro-Aguirre, 1996). Other predators include: the benthic ocean whitefish (*Caulolatilus princeps*: Elorduy-Garay & Caraveo-Patiño, 1994) and the Panama hake (*Merluccius angustimanus*: Balart & Castro-Aguirre, 1995).

Swarms of this euphausiid species are consumed by different species of seabirds including the eared grebe (*Podiceps nigricollis*), Bonaparte's gulls (*Larus philadelphia*), black storm-petrels (*Oceanodroma melania*) and least storm-petrels (*Oceanodroma microsoma*: Tershy *et al.,* 1993b). The resident population of fin whales (*Balaenoptera physalus*) in the Gulf of California (Bérubé *et al*., 1998) is frequently reported as feeding on euphausiids throughout the year (Gendron, 1993; Tershy *et al*., 1993a; Croll *et al*., 1998; Ladrón de Guevara *et al*., 2008). During winter, the North Pacific population of blue whales (*Balaenoptera musculus*) migrates from California into Pacific Mexican waters. Here, their distribution has been associated with surface swarms of *N. simplex* (Gendron, 1992). Fecal analysis of both whale species confirms that they feed mainly on adult and juveniles of this euphausiid species (Del Angel-Rodríguez, 1997; Jiménez-Pinedo, 2010).

Nyctiphanes simplex is a prominent link between primary producers and higher trophic level organisms. One possible reason is that it is the most widespread euphausiid species year-round in

the Gulf of California (Brinton & Townsend, 1980; Brinton *et al*., 1986; Gendron, 1992). There, as in most tropical and subtropical marine ecosystems, primary productivity is not irradiance-limited, and reproduction for most krill species, occurs throughout the year (Lavaniegos, 1995; De Silva Dávila & Palomares-García, 1998; Ambriz-Arreola *et al.,* 2012). Evidence suggests that omnivory may account, at least partially, for the ecological success of *N. simplex* during non-upwelling events (Kanaeva & Pavlov, 1976; Theilacker *et al.,* 1993), or during warm conditions such as El Niño events (Lavaniegos-Espejo *et al*., 1989; Gómez-Gutiérrez *et al*., 1995).

1.3. Lipid biomarkers

The first goal of this thesis was to investigate how changes throughout life stages affect the lipid content and fatty acid composition in this subtropical krill species. Lipids comprise a group of naturally occurring hydrophobic or amphiphilic small molecules with biological functions such as energy storage, signaling, and structural components of cell membranes. The term lipid is sometimes used as a synonym for fats (triacylglycerols or TAG) which are a subgroup of lipids. In fact, lipids not only encompass fatty acids and their derivatives (including waxes, mono-, di-, triacylglycerols, and phospholipids), but also fat-soluble vitamins (such as vitamins A, D, E, and K), and other sterol-containing metabolites such as cholesterol, among other molecules. Some lipids are considered essential for specific organisms when they cannot be synthesized or produced in enough quantities trough their biosynthetic pathways and must be obtained from the diet (Figure 1-3).

Lipids have the potential to be biomarkers of nutritional condition in euphausiids (Ju & Harvey, 2004), and together with proteins, can be related to body condition. In tropical and subtropical coastal regions, omnivorous euphausiids successfully adapt to different food sources and are expected to have a permanent food supply. Therefore, lipid and protein content may be

expected to be relatively constant throughout the year (Postel *et al*., 2000). However, some evidence suggests that if food sources change seasonally, or if metabolic demands change throughout life stages, then the biochemical composition of euphausiids will likely change.

In the Gulf of California, primary production increases during spring upwelling pulses, which occur when coastal winds promote a shallow nutricline (Lluch-Cota, 2000; Hidalgo-González & Alvarez-Borrego, 2004). The direct effects of such local mesoscale processes on food quality and availability for krill have not yet been investigated. In this sense, lipid biomarkers such as fatty acids and sterols can help trace seasonal and ontogenic changes in diet. Lipid biomarkers provide a tool to reveal local spatial changes and short-term differences (days to weeks) in the diet for zooplankton because they integrate fatty acid proportions over time-scales between those indicated by prey found in stomach contents (minutes to hours) and by $\delta^{15}N$ and $\delta^{13}C$ stable isotopes (months). Fatty acids have been successfully used as trophic biomarkers in krill species from temperate and polar ecosystems (Cripps *et al.,* 1999; Mayzaud *et al.,* 1999; Virtue *et al*., 2000; Stübing *et al*., 2003; Hagen *et al.,* 2007). For instance, diatoms tend to have high proportions of 14:0 and 16:1ω7 as well as polyunsaturated 16-carbon fatty acids (C_{16} PUFAs), whereas dinoflagellates tend to have high proportions of 18:4ω3, 18:5ω3, and especially 22:6ω3, but small proportions of 16:1ω7 (Falk-Petersen *et al*., 2000; Virtue *et al*., 2000). Odd-numbered and/or branched fatty acids are considered typical of bacteria (Stevens *et al*., 2004), and together with multibranched fatty acids (such as phytanic and pristanic fatty acids) are used to define detritivorous habits in zooplankton. High levels of 18:1ω9 are common in carnivorous euphausiids (Falk-Petersen *et al*., 2000; Cripps & Atkinson, 2000), and the 18:1ω9/18:1ω7 ratio has been used as an index of carnivory in copepods (Stevens *et al*., 2004). Sterols tend to be more taxonomically specific. For instance, 24-methylenecholesterol can be used as a marker for

diatoms, dinosterol can be used as a marker for dinoflagellates, fucosterol and fucostanol are found in macroalgae, and 24-nordehydrocholesterol is found in zooplankton. Terrestrial plants also produce 24-ethylcholesterol, ethylcholest-5,22-dienol and 24-methylcholesterol, and fresh domestic wastewater can be identified by high coprostanol/cholesterol and 24-ethylcoprostanol/β-sitosterol ratios (Parrish *et al.,* 2000 and references therein).

Successful use of fatty acids as trophic biomarkers can be constrained for some krill species if the trophic spectrum diversifies during ontogeny (Ritz *et al.,* 1990), or if the diet switches from herbivory to carnivory (Atkinson *et al.,* 2002). Previous studies indicate that *N. simplex* larval stages grew better when their zooplankton diet included microalgae (Lavaniegos, 1992). Adults not only ingest microalgae (phytodetritus > 80%), but also a wide variety of animals (crustaceans > 9%, radiolarians 1%, and tintinnids > 1%), as shown in stomach content of *N. simplex* (Kanaeva & Pavlov, 1976). Apparently, on some krill species, facultative carnivory is a common trait, and eggs and larvae of pelagic fish can also be part of their diet (Theilacker *et al*., 1993).

Additionally, differential assimilation of fatty acids might occur in adult krill irrespective of the diet (Stübing *et al.,* 2003), and reproductive physiology can modify both the lipid content and the fatty acid composition (Pond *et al.,* 1995; Kattner & Hagen, 1998; Mayzaud *et al.,* 2003). Accordingly, differences in population structure at different locations can bias the interpretation of fatty acid biomarkers. This is relevant for an omnivorous subtropical species such as *N. simplex* which reproduces throughout the year (Lavaniegos, 1995; Gómez-Gutiérrez, 1995; De Silva-Dávila & Palomares-García, 1998).

1.4. Biochemical composition and energy content

The energy content of zooplankton at different stages can be derived from the proximate composition of the main organic components, accounting only for digestible molecules (Postel *et al*., 2000). As with lipid biomarkers, changes in life stages can drive strong differences in the biochemical composition and, ultimately, the energy content of krill swarms. Biochemical composition of euphausiids depends largely on the life phase (Clarke, 1980; Mayzaud, 1997; Mayzaud *et al*., 1999). For instance, during previtellogenesis and vitellogenesis, females of Antarctic krill *Euphausia superba* and North Atlantic krill *Meganyctiphanes norvegica* store long-term reserves in the form of lipids and carbohydrates (Cuzin-Roudy, 1993). In *M. norvegica* females, lipids are further mobilized to the eggs before spawning, allowing embryogenesis (Albessard *et al*., 2001), whereas spawned females of *E. superba* also exhibit lower protein content than gravid females (Clarke, 1980). In the case of *N. simplex* swarms, the population structure varies in sex-ratio and in the relative contribution of mature stages (Gendron, 1992), and therefore the energy content of a swarm will depend not only on quantity and quality of food sources, but on the life stages represented and their reproductive condition.

There is no comprehensive study of the biochemical composition and the energy content of *N. simplex* during the transition from late larval stages into juvenile and adult forms. It is known that throughout the embryonic development, *N. simplex* embryos and early larval stages rapidly consume their storage lipids and carbohydrates, just before reaching the first feeding stage (calyptopis I) (Montuy-Gómez *et al*., 2012). In a study on a parasitoid ciliate, *Pseudocollinia brintoni*, I examined the lipid composition of a small number of larval and adult stages of *N. simplex* (Gómez-Gutiérrez *et al*., 2015). I also performed a preliminary analysis of adult female and male lipid composition of *N. simplex* (Gómez-Gutiérrez *et al*., 2010). However, none of the

previous studies explicitly estimates energy content or the amount of energy devoted to reproduction.

Seasonal variations in energy content of this subtropical euphausiid have also not been studied. Theoretically it is possible to anticipate higher energy content of *N. simplex* during winter–spring months, when overall phytoplankton abundance and biomass increase. Lower energy content is expected in summer, when phytoplankton reach the lowest abundance and biomass. Nevertheless, consumption of alternative food sources (omnivory and carnivory) during summer, and ecological adaptations to a subtropical environment must also be considered.

1.5. Food eaten by large predators

In the past, feeding studies of fin whales (*Balaenoptera physalus*) and blue whale (*B. musculus*) were restricted to temperate or polar regions, where food abundance is high during summer months (Mackintosh, 1966; Jonsgard, 1966; Lockyer & Brown, 1981). In contrast with summer feeding grounds, winter migration of Balaenopterid whales towards subtropical and tropical regions was accompanied with lower frequency of feeding events; small amounts of food in their stomachs; and reports of lower yields of fat and oil by whaling vessels (Mackintosh, 1966; Lockyer & Brown, 1981). The evidence led to the notion of lower consumption rates occur during winter migration to lower latitudes, where breeding and mating take a central role.

During the four months of intensive feeding (summer), overall body weight of blue and fin whales increases ~50%, mostly as fat. In contrast, pregnant blue and fin whales increase their body weights at least 60–65%, and their blubber is ~25% thicker than that of resting (anoestrous) females (Lockyer, 1981). However, if summer fattening is not efficient, low body weight and low lipid reserves can reduce reproductive performance, resulting in larger reproductive intervals, lower pregnancy rates, and reduced calf survival (Lockyer, 1987).

Lockyer (1981) estimated that if southern-hemisphere whales were only feeding heavily for ~4 months and on nothing for the remaining 8 months of the year, then their total weight loss during their migration span would be about 45%. Such weight loss was calculated assuming daily body weight losses similar to those in other long fasting animals (0.2–0.3% day^{-1}). However, observed weight lost for blue and fin whales was lower (22–30% of body weight) than the estimated value, and therefore suggests sporadic and opportunistic feeding at lower latitudes during winter (Lockyer, 1981). Therefore, winter feeding grounds may contribute significantly to the survival of migratory Balaenopterid whales. If sporadic and opportunistic feeding occurs during winter, whales might overcome at least some of the energy and weight loss (Lockyer, 1987), which seems particularly important for lactating females, increasing the chances of a full recovery before the next reproductive cycle.

An important winter-feeding ground for baleen whales is the Gulf of California. Here, these two large whale species feed on the subtropical euphausiid *N. simplex* (Del Angel-Rodríguez, 1997; Jiménez-Pinedo, 2010), and might be their most important predators in the area. Sightings of fin whales occur yearlong (Rojas-Bracho, 1984), suggesting a resident population (Gilmore, 1957; Gambell, 1985; Leatherwood *et al*., 1988), with limited genetic flux with other areas of the Pacific Ocean (Bérubé *et al*., 2002). Such a population requires local and year-round food-sources, or temporary food-sources that satisfy yearly requirements. Studies have shown that fin whales feed on euphausiids in several areas of the gulf in both winter and summer (Gendron, 1992; Tershy *et al*., 1993a*;* Croll *et al*., 1998; Ladrón de Guevara *et al*., 2008).

In the case of blue whales, a fraction of the North Pacific population moves southward from California, traveling to Pacific Mexican waters during winter (Figure 1-4), reaching the Gulf of California as early as January (or even late December), but more intensively during March and

April (Leatherwood *et al*., 1988; Calambokidis *et al*., 1990; Gendron, 1990). It is assumed that many mysticetes generally do not feed while on their winter-spring breeding grounds (Corkeron & Connor, 1999). However, blue whale distribution in the south-western portion of the Gulf of California has been associated with surface swarms of the euphausiid *Nyctiphanes simplex* (Gendron, 1990, 1992), and feces of blue and fin whales from the Loreto and Bahía de La Paz regions, confirm that both species feed on adult and juvenile euphausiid *N. simplex* (Del Angel-Rodríguez, 1997; Jiménez-Pinedo, 2010).

Whale skin isotopic incorporation rates and baleen growth rates suggest that female blue whales are more likely to migrate to warmer waters in winter-spring to nurse their calves. (Busquet-Vass *et al*., 2017). Those data also show that females had a high fidelity returning to specific winter-spring foraging grounds year after year. High site fidelity of females has also been confirmed via photo-identification and genetic analysis (Gendron, 2002; Sears *et al*., 2013; Costa-Urrutia *et al*., 2013). In the case of blue whale males, Busquet-Vass *et al*. (2017) hypothesize that there are two migratory strategies in the northeast Pacific. Some individuals migrate to winter-breeding grounds in the Gulf of California or the Costa Rican Dome, while others remain within the California Current System. Whale skin isotopic analysis suggests that only a portion of the males in the northeast Pacific are using the Gulf of California or the Costa Rican Dome in winter-spring. However, vocalizations specific to male blue whales have been recorded year-round in the California Current System (Stafford *et al*., 2001; Oleson *et al*., 2007a; 2007b; 2007c). Photo-identification data have also shown that some males have a high site fidelity to the Gulf of California (Gendron, 2002).

Knowing the biochemical composition and energy content of local key species has proven to be valuable for salmon, cod, trout and halibut aquaculture (Storebakken, 1988; Virtue *et al*.,

1995; Moren *et al*., 2007), and for understanding trophodynamics in ecosystems (Clarke, 1980; Falk-Petersen *et al*., 2000; Färber-Lorda *et al*., 2004). It is equally important to understand the effect of somatic growth, sexual maturity or environmental conditions on the biochemical composition of subtropical krill, and how those changes might impact upper trophic levels. Major changes in energy content of prey can have a significant impact on food intake rates or even overall prey preferences in upper trophic levels (Roby, 1991; Mårtensson *et al*., 1996; Benoit-Bird, 2004) and can be used as an index of food quality or nutritional value (Rosen & Trites, 2004).

In this thesis I measured how internal and external factors change the energy content of *N. simplex*, and estimated its further effect on energy requirements, assimilation and biomass demands of blue and fin whales in the Gulf of California. For those long-lived species, two important aspects of population demographics are key to whale conservation: birth rates and survival to reproductive maturity. Modeling their biomass demands is a necessary first step to estimate the contribution of wintering grounds to whale's energetics and to evaluate the effect of decadal scale changes in food supply on population demographics.

1.6. Rationale

To understand how energy is mobilized from primary producers to apex predators, we need to understand seasonal and ontogenic changes in key prey species. Tracking environmental changes and their effect in food availability for krill will help explain seasonal and ontogenic changes in the energy content of *N. simplex* available to whales. The Gulf of California also offers unique conditions to investigate the trophic relationships between krill and two apex predators that share similar feeding habits. Knowledge of the degree of dependence and the

impact of these whales over the pelagic ecosystem can be used for conservation and management.

1.7. Goals and Objectives

1.7.1. General

This thesis aimed to evaluate the seasonal and ontogenetic dietary changes of *Nyctiphanes simplex* through lipid biomarkers and to estimate the energy content for this subtropical euphausiid species. The working hypotheses were that 1) mature postvitellogenic females and females carrying eggs will be the stages with higher energy content due to lipid reserves, and 2) that energy content should be higher during colder months as consequence of higher primary productivity of diatoms. It also aimed to investigate how population structure affects estimates of energy content in *N. simplex* swarms. Here the working hypothesis was that swarms composed mostly of mature females will have higher energy content than those composed mostly of juvenile or males. Finally, the last goal was to evaluate the implications of swarm composition on biomass demands of their main whale predators: *Balaenoptera musculus* and *Balaenoptera physalus*.

1.7.2. Specific Objectives

1) To analyze the biochemical composition of *Nyctiphanes simplex* in the southwest part of Gulf of California as a function of life stages (sex, somatic growth and gonadic development) and seasonal changes.

2) To analyze the effect of short-time fasting and life stage on lipid biomarkers, and to provide reliable experimental and observational methods to account for those effects in trophic studies.

3) To relate both life stage and seasonal changes to the body condition and energy content of *Nyctiphanes simplex* in the Gulf of California.

4) To model the energy content of *N. simplex* swarms during the winter-spring season, considering variation in population structure in swarms.

5) To estimate minimal winter-spring energy requirements and biomass demands of blue and fin whales in the Gulf of California and analyze their feeding strategies during that period.

1.8. References

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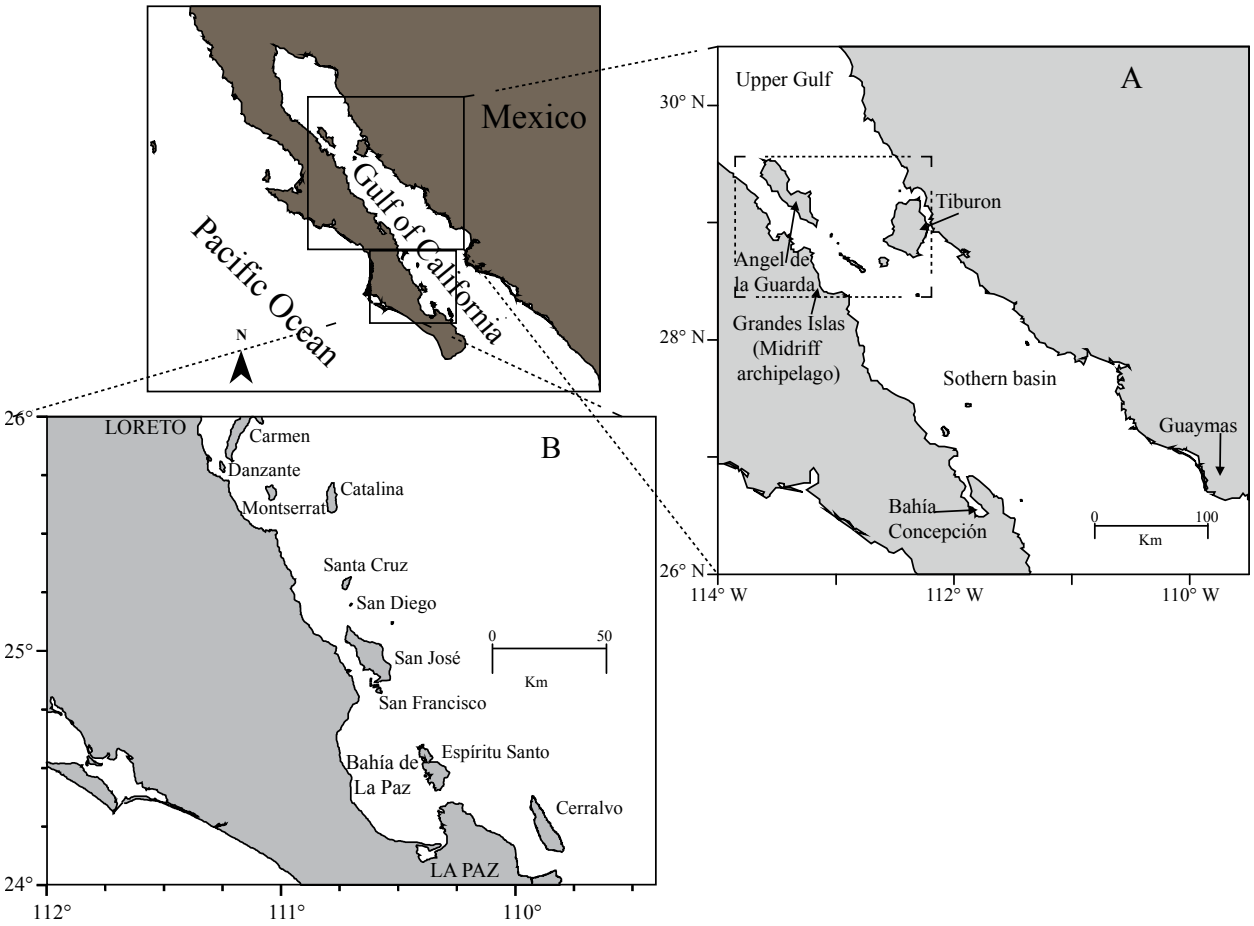

Figure 1-1 The Gulf of California: studied areas and toponymics used in this thesis. A) Central part of the gulf, highlighting the presence of a midriff archipelago. B) South-west portion of the gulf, highlighting a large bay (Bahía de La Paz) and the presence of several islands near the coast.

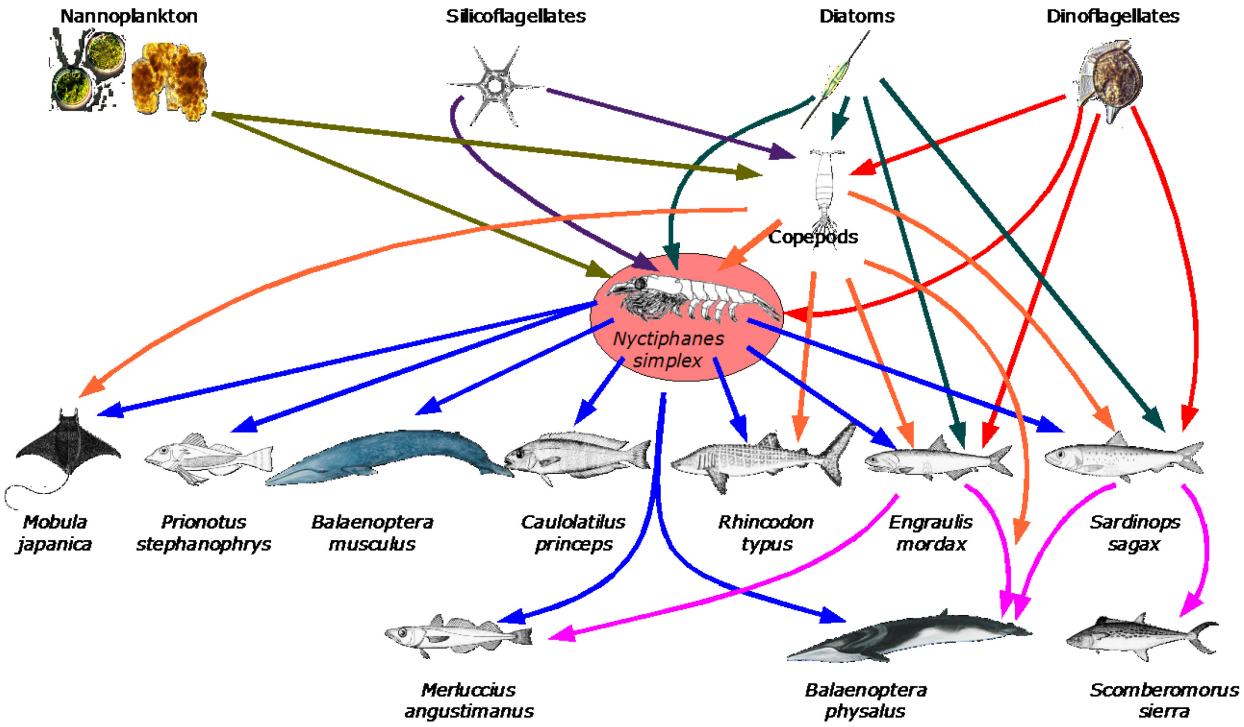

Figure 1-2 Schematic food web highlighting the key role of *Nyctiphanes simplex*. Primary producers are displayed at the top Figure and higher trophic levels are displayed at bottom. Arrows represent predator-prey interactions, colors represent a particular prey item (Fish images from fishbase.org).

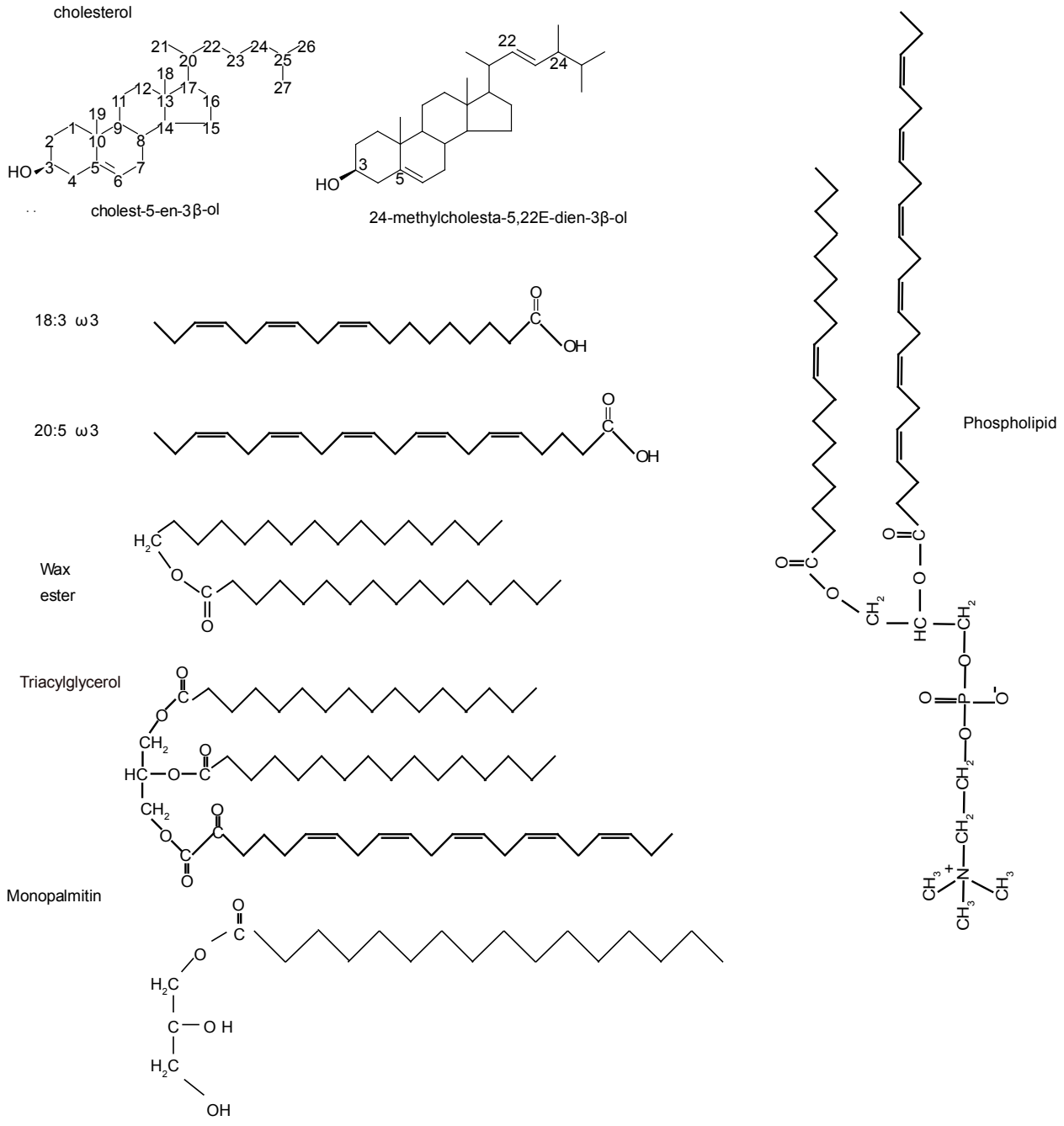

Figure 1-3 Examples of structural formula of different lipids. Carbon atoms are represented as the vertices between lines and chemical bonds within the molecule are depicted explicitly with straight lines, or implicitly (not shown) with hydrogen

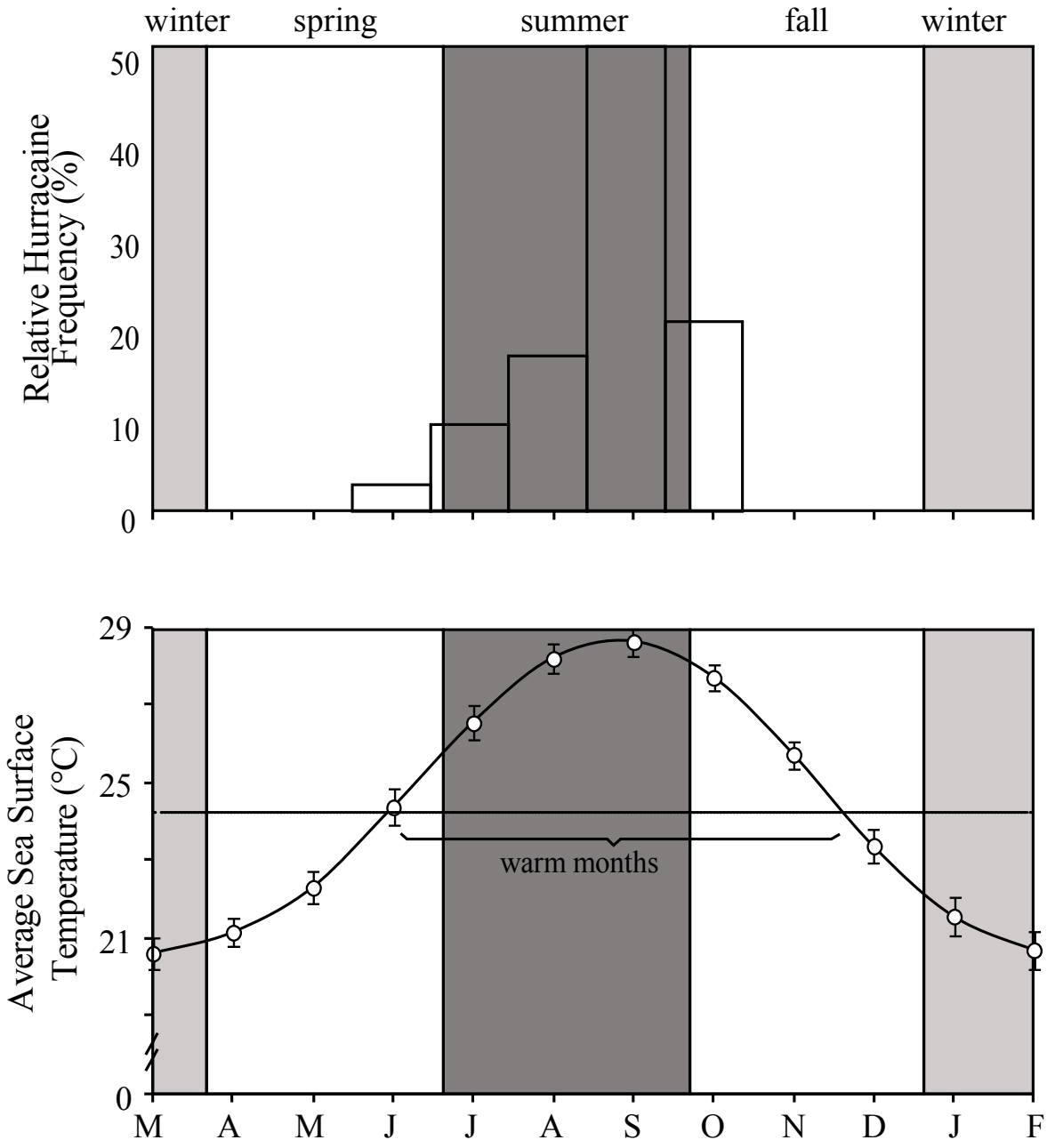

Figure 1-4 Schematic representation of seasonality within the Gulf of California. The hurricane season provides most of the rain to the area and encompasses mostly the summer months. In terms of temperature, winter is mild but with strong winds.

Chapter 2. **Developmental and maturity changes in biochemical composition and energy content of *Nyctiphanes simplex* (Crustacea: Euphausiacea) from the Gulf of California during winter**

2.1. Abstract

The neritic euphausiid *Nyctiphanes simplex* plays a key role in marine food webs in the Eastern Central Pacific Ocean. Energy content of euphausiid swarms may vary with developmental stage and maturity of individuals, affecting energy requirements and consumption rates of predators. Total length (*TL*), wet weight (*WW*), dry weight (*DW*), and proximate composition were determined in juvenile, adult-sized (>8 mm), and adult forms (close to full gonadic development) of krill in a subtropical bay (Bahía de La Paz) during spring (February 2002). Lipids and fatty acids in adult forms were also determined in the central Gulf of California at the end of spring (March 2010). The relationship between *TL* and *DW* followed a power function and significant differences in the slopes confirmed sexual dimorphism. For both sexes, somatic growth was driven by proteins followed by polar and neutral lipids. In fact, polar lipids increased at higher rates than neutral lipids as *TL* increased, as expected for membrane lipids during cell division. Saturated fatty acids dominated the neutral lipid fraction, but declined with sexual maturity, whereas essential polyunsaturated fatty acid proportions (20:4ω6, 20:5ω3 and 22:6ω3) increased. High lipid content in eggs carried in ovigerous ovisacs increased both total lipids and the energy content of mature females (1.2–1.8 times). Energy content was modified through somatic development and gonadic maturity. Variation in energy content associated with sex on adult krill swarms (kJ mg^{-1}) ranged by a factor of 1.5. However, overall

variation in energy content due to swarm structure ranged by a factor of 1.7. Consequently, variation in food intake (kg) by predators can range by a factor of 1.7.

Keywords: Biochemical composition, energy density, krill development, sexual dimorphism, Gulf of California.

2.2. Introduction

Major changes in energy content of a prey can have a significant effect on food intake rates and overall prey preferences in upper trophic levels (Roby, 1991; Mårtensson *et al*., 1996; Benoit-Bird, 2004). Energy content can be used as an index of food quality or nutritional value (Rosen & Trites, 2004). For example, knowing the biochemical composition and energy content of local key prey species has proven to be valuable for salmon, cod, trout and halibut aquaculture (Storebakken, 1988; Virtue *et al*., 1995; Moren *et al*., 2007), and for understanding trophodynamics in marine ecosystems (Clarke, 1980; Falk-Petersen *et al*., 2000; Färber-Lorda *et al*., 2004).

In euphausiids, as in many invertebrates, changes in metabolic requirements during somatic growth and gonad development (including energy storage for reproduction) are frequently associated with changes in biochemical composition (Cuzin-Roudy, 1993; Mayzaud *et al*., 1998; Falk-Petersen *et al*., 2000; Ross & Quetin, 2000; Albessard *et al*., 2001), and in turn, modify their total energy content. However, to date, most biochemical studies have examined krill species from polar and temperate environments, where physiological requirements and reproductive strategies are different than those observed in subtropical species (Gómez-Gutiérrez *et al*., 2010a, 2015, Montuy-Gómez *et al*., 2012). For instance, a multiannual study of the biochemical composition of the subtropical *Nyctiphanes australis* observed that physiological condition appears to be similar throughout the year (Virtue *et al*., 1995). As expected, the lipid

content is low (5–10%) compared with polar species, although the relative contributions of two essential fatty acids (EPA at 37% and DHA at 25%) were higher than those reported for polar species such as *Meganyctiphanes norvegica*, *Thysanoessa raschii*, *T. inermis*, and *Euphausia superba*. In a preliminary analysis of lipid content for *Nyctiphanes simplex* (Gómez-Gutiérrez *et al*., 2010a), I observed similar levels of lipid content (4–13%) and a quasi linear positive relationship between total lipid content and euphausiid total length.

Swarms of *N. simplex* vary widely in the female: male ratio, the proportions of larvae, juveniles, and adults, and in the degree of gonad development among adult forms (Gendron, 1992; Gómez-Gutiérrez *et al*., 2012). Accordingly, variable swarm composition might result in variable energy content per unit weight, hence the importance of assessing the effect of somatic growth and sexual maturity on the biochemical composition of subtropical krill, and how those changes might impact upper trophic levels.

Although the energy content at different life stages can be derived from the proximate composition of the main organic components (Postel *et al*., 2000), at this time neither the basic biochemical composition nor the energy content are known for this key subtropical euphausiid. The objectives of this study were: 1) to characterize the biochemical composition of *N. simplex* from Bahía de La Paz and central region of the Gulf of California as a function of life stages (sex, somatic growth and gonadic development), and 2) to evaluate if population structure affects estimates of energy content in *N. simplex* swarms. The underlaying hypothesis was that energy content of euphausiid swarms was higher when swarms included a higher proportion of mature postvitellogenic females or females carrying eggs.

2.3. Materials and methods

2.3.1. Sampling

During February 2002, an oceanographic survey was carried out over the continental shelf of Bahía de La Paz, Baja California Sur, Mexico, using a 51-station grid (Figure 2-1). Zooplankton hauls were made at night by oblique tows with a vessel speed of 2 knots (~3.7 km h^{-1}). A Bongo net (0.6 m diameter; 505 μm mesh size) was towed for 30 minutes from 280 m to the surface. Mature adults, adult-sized individuals, and juveniles of *N. simplex* were sorted, placed in sealed containers, frozen in liquid nitrogen (-196°C), and stored at -80°C until biochemical analysis. To verify if observed trends in total fatty acids were similar between stations and throughout time, a second set of samples was collected during March 2010 from two coastal locations on the east side of the Gulf of California: S06 (29°17.21' N, 112°33.18' W) and S47(28°37.87' N, 112°18.07' W). Here, the combined effect of gonad status and location was also analyzed, as both stations had a broad spectrum of adult krill with different gonad status (males with intruded and extruded spermatophore, and females at previtellogenic or postvitellogenic stages, with or without ovisacs). Collecting methods for those samples are fully described in Chapter 3. Briefly, a 1 m diameter, 5 m long, and 300 μm mesh net equipped with an underwater lamp was used to attract zooplankton. The net had a closed PVC cod end (0.215-m diameter and 0.70-m length), and when deployed, the ship was allowed to drift to avoid krill damage during collection. Samples from March 2010 were only used for fatty acid comparisons, without separation between neutral and polar fractions.

Sea surface temperature (°C), net primary productivity (mg C m^{-2} day^{-1}), wind data at 10 meters (m s^{-1}) and Ekman Transport data (kg m^{-1} s^{-1}) were obtained from the Global Earth

Observation Integrated Data Environment (GEO-IDE) via their live access server (https://geo-ide.noaa.gov/access-las.php).

2.3.2. Morphological features

Specimens of *N. simplex* were separated according to life stage (sex and developmental stage). Table 2-1 shows the morphological features and total length values used for identification of each life stage. Eggs contained in ovigerous sacs were counted and analyzed separately from ovigerous females. Single specimens were washed with distilled water, measured to the nearest mm from the end of the rostrum to the tip of the telson (*TL*), briefly blotted with Whatman No. 1 filter paper, and weighed wet (*WW*) to the nearest 0.1 mg.

Samples were freeze-dried for 24 h and weighed dry (*DW*) to the nearest 0.001 mg with a Cahn C-33 microbalance. Body condition (*BC*) was estimated as the ratio between observed and expected dry weight given the sex. To compute it, I accounted for the variation in weight due to total length and sex. The size-dependent equations used to calculate the expected dry weight (*DW*) adjusted well to a power regression model ($DW = \gamma\ TL^{\beta}$). An ANCOVA test was used to confirm differences in the allometric parameter β. Individual regression analyses by sex of $\log_{10}$ transformed variables were used to compute confidence intervals for γ and β parameters.

St-Pierre *et al.* (2018) demonstrated that coefficient estimates differ substantially when comparing those obtained from data transformation *vs* those obtained using non-normal error structures within the framework of the generalized linear model (GLM). Still, the same authors show that data transformation gives better control over Type I error and almost all estimates of power law exponents in the published literature are computed via $\log_{10}$ transformation. In the present work, allometric parameters were obtained via $\log_{10}$ transformation in order to make

them comparable to literature estimates. Additionally, parameters were also estimated using an iterative non-linear curve fitting routine to fit to a power law, with a least squares criterion.

2.3.3. Brood size

To calculate the amount of energy devoted to egg production, eggs were analyzed separately. Evidence of missing eggs in ovigerous females (broken sacs and large differences in the number of eggs between both sacs) suggested that some eggs were lost during sampling. For that reason, brood size was estimated from a regression equation calculated from data provided by Gómez-Gutiérrez *et al*. (2010b) between total length and brood size of females collected in the central Gulf of California during January 2007. An ANCOVA test of their original data (November 2005, January and July 2007), shows evidence of seasonal differences in brood size, i. e. a significantly smaller brood size for females of similar size during January 2007 ($F_{2,76}$=4.07, p = 0.021). Once samples from November and July were excluded, the coefficient of determination improved (from R^2= 0.16 in the original data to R^2= 0.42 in January 2007).

According to this equation, there is a significant positive relationship between brood size and female size ($F_{1,32}$=15.49 p<0.001).

BS=7.296 TL - 46.07, R^2=0.42

where:

BS= Brood size: total number of eggs in both ovisacs.

TL= Total length (in mm) of ovigerous females

2.3.4. Biochemical composition

Carbohydrates were determined using Dreywood's anthrone reagent (Morris, 1948) and D-glucose as a standard. For protein concentration (Bradford, 1976), freeze-dried samples were

digested with 5 mL of 0.1 N NaOH for 30 min at 80°C (Mayzaud & Martín, 1975). Aliquots of 50 to 200 µL of this solution were adjusted to 800 µL with distilled water. No neutralization was required after use of NaOH because final pH was equal to that obtained in solutions without NaOH (0.75) after addition of 200 µL of dye-binding reagent (Bio-Rad, Hercules, CA).

For lipid analysis, freeze-dried specimens were homogenized, re-hydrated with distilled water, and homogenized in a $CHCl_3$:CH_3OH solvent system to obtain an 8:4:3 mixture of $CHCl_3$:CH_3OH:H_2O (Folch *et al*., 1957). After extraction and evaporation down to 0.5 mL, lipids were separated into polar and neutral fractions on a 60–200 µm silica gel microcolumn (Palacios *et al*., 2002; Christie, 2003), and collected in tubes containing an internal standard (23:0 FAME) plus butylated hydroxytoluene (BHT) 0.01% as an antioxidant. A set of standards (dipalmitin, triestearin, cholesterol and lecithin) was run in parallel to account for losses during separation and elution. Each lipid fraction was divided into two equal volumes and dried under a nitrogen stream. Lipids were determined in one replicate of each polar and neutral fraction following a charring method (Marsh & Weinstein, 1966).

2.3.5. Fatty acids

The fatty acids in the other replicate were transmethylated (Sato & Murata, 1988) and methyl esters were analyzed by gas chromatography on a 30 m × 0.25 mm fused silica capillary column (Supelco Omegawax, Bellefonte, PA) with polyethylenglycol as the stationary phase with a thickness of 0.25 µm, and helium as the carrier gas. The column was mounted in a gas chromatograph coupled to a mass spectrometric detector (GCD 1800B Hewlett-Packard, Palo Alto, CA). Chromatographic conditions were set as follows: 0.9 mL min^{-1} helium flow and 250°C injector temperature. After injection, the temperature of the column was maintained at 110°C for 3 min, then increased to 165°C at a rate of 30°C min^{-1}, and maintained at 165°C for 2

min. For the final ramp, temperature was increased to 220°C at a rate of 2°C min^{-1}, and maintained at 220°C for 16 min. The detector was set at 260°C, and the ion source was set at 70 eV.

Identification of fatty acids was based on interpretation of mass spectra and on comparisons of mass spectra with those generated from commercial standards of 37 fatty acid methyl esters (FAMEs) commonly found in food products (Supelco 47885-U). When fatty acid isomers were found, retention times of at least one of the isomers in the commercial standards allowed double bond positioning of the other isomer. Analyses of fatty acid picolinyl esters (Destaillats & Angers, 2002) were used to differentiate between isomers when required.

Differences in MS-detector response (slope) among fatty acids were calculated by plotting the areas (mAU × sec) of integrated chromatographic peaks[1] against known concentrations of commercial standards of 37 FAMEs (µg mL^{-1}) within their dynamic linear range (5–80 µg mL^{-1}; the interval where signal:noise ratio > 10, and where area and concentration correlate linearly). Linear regression analysis on each plot yielded the response factor (slope) for each fatty acid ([mAU sec]/ [µg mL^{-1}]). Quantification (µg mL^{-1}) of samples was achieved by division of areas from integrated chromatographic peaks by their respective response factor. Proportion of fatty

[1] Peak area (A) is defined as $A = h \times W½$, where h is the peak height, and $W½$ is peak width at half-height. Therefore, the corresponding units for h are the units of the y-axis in the chromatogram (signal units = mV) whereas the corresponding units for $W½$ are the units for the x-axis in the chromatogram (time units = minutes). Accordingly, peak area units are equal to mV × minute. Most packages for data analysis convert the raw electrical signal (mV) into a more meaningful spectral signal defined as absorbance units (AU) or mili absorbance units (mAU). That way the absorbance value on the chromatogram matches what it's seen on the detector. Similarly, minutes are commonly transformed into seconds. Once converted, area would be expressed as mAU × sec.

acids (%) was calculated as the relative contribution of each fatty acid to the overall fatty acid composition.

2.3.6. Energy content

Energy was calculated as the mean amount of energy provided by partial oxidation of carbohydrates (17.2 J mg^{-1}), proteins (21.4 J mg^{-1}) and lipids (35.6 J mg^{-1}) as suggested by Postel *et al*. (2000).

2.3.7. Statistical Analysis

Biochemical and morphometric differences among developmental stages were assessed with univariate ANOVA tests, using the Fisher statistic (F). Assumptions of normality, homogeneity and independence of residuals were examined with residual analyses. Homogeneity was examined with plots of the residuals *vs* fitted values for the response variable in relation to the predictive variable, searching for an acceptable band in the error dispersion, with no strong patterns of cones expanding either to the right or to the left. Independence was examined plotting each residual against a neighboring value, searching for distributions with no trends (up or down). Normality was examined with histograms of the residuals, searching for symmetrical shapes and clustering around the zero value, and searching for no obvious differences between the cumulative distribution of the residuals and the cumulative distribution of the normal distribution. When those assumptions were not met and the p values were close to the α value (0.05), a p-randomization was conducted 1,000 times to test the data empirically. For variables with residuals not meeting ANOVA assumptions, 95% confidence intervals were calculated by randomization. Allometric parameters and confidence intervals of power regression models were estimated via $\log_{10}$ transformation and using an iterative non-linear curve fitting routine.

Shifts in fatty acid profiles of structural and storage lipids among life stages was tested with multivariate analyses using the PRIMER software 6.1.16 & PERMANOVA + version 1.0.6 (PRIMER-E, Plymouth, UK). Similarities among life stages and lipid classes were investigated using the similarity percentages (SIMPER) function. This function provides the percentage of similarity within the studied factors; among levels of the factors; and for specific levels of the factors. It also provides the relative contribution of each variable to the observed similarity and shows the cumulative contribution so that it is possible to know how many variables explain a given percentage of the similarity.

Relative contribution (%) of fatty acids to the overall fatty acid profile was also analyzed using permutational multivariate ANOVA based on distances (PERMANOVA). The percent fatty acid values were transformed using the centered $\log_{10}$ ratio transformation (division by the geometric mean of the sample followed by $\log_{10}$ transformation) before testing (Aitchison, 1986; Loseto *et al*., 2009). Analysis of transformed data was applied only to those FA > 1% to avoid artificial weighting of trace FA.

2.4. Results

2.4.1. Environmental conditions

During February 2002, coexisting juvenile and adult forms of krill were detected and collected in only 8 of the 52 oceanographic stations (Figure 2-1A). Krill occurred on coastal areas <10 km from the coast and mostly at <170 m seafloor depth (Figure 2-1B), with sea surface temperatures ranging from 18.6 to 20.0°C (Figure 2-1C). Chlorophyll *a* concentration for stations with krill ranged from 0.964 to 2.380 mg m^{-3} (Figure 2-1D). Those concentrations were relatively high compared to the rest of the area and were observed on the west side of the bay

and around the islands. This process was driven by the dominating winds (5–7 m s^{-1}) from north-north east that produced a westward Ekman transport ranging from 550 to 750 kg m^{-1} s^{-1} towards a pronounced slope of the continental shelf (Figure 2-1E).

2.4.2. Sampled stages

From the nine groups of expected postlarval stages (Table 2-2), only eight were well represented in winter samples. Mature females (M_f) with pink gonads ready to spawn (Gómez-Gutiérrez & Robinson, 2005), *i.e.* females with type IV oöcytes in meiosis phase (Ross *et al*., 1982), were not found. It is not rare for *N. simplex* M_f to rapidly lay eggs under sampling conditions and become ovigerous females (O_f) with eggs at single-cell stage, a well-represented stage in this study.

2.4.3. Brood size

Using the modified regression equation that relates brood size with *TL* (Figure 2-4A), it was possible to incorporate the weight and energy provided by eggs (Table 2-3). On average, an egg of *N. simplex* was assumed to weigh 8.1 µg similar to *Nyctiphanes australis* (Hosie & Ritz, 1983; Virtue *et al*., 1993; 1995), and nauplii 8.6 µg. Accordingly, a 10.7-mm female will produce on average ~32 eggs and will increase its weight an average of ~260 µg. However, this 6% increase in weight was not statistically significant, likely as consequence of a wide range in size and weight of O_f.

Once an average brood size was estimated, the number of eggs in the ovisacs of O_f stage were used to infer the most relevant biochemical changes and the required energy for M_f stage to complete vitellogenesis, *i.e.* to estimate the amount of energy redirected to eggs.

2.4.4. Total length and weight

Undifferentiated juveniles (J_u) had the shortest *TL* (< 6.3 mm) (ANOVA: $F_{9,178} = 87.45$, $p < 0.001$); followed by sexually differentiated juveniles of both sexes (J_f and J_m) ranging from > 6.3 to < 8 mm. Among post-juvenile females (A_f, O_f and S_f stages), differences in *TL* were not significant. However, most adult sized females (A_f) occurred at sizes between 8–9 mm (80%), and the remaining 20% exhibited sizes between 10–11 mm. In contrast, all ovigerous females and all spent females (S_f) exhibited sizes ≥9 mm and more than 70% reached sizes ≥11 mm, suggesting a somewhat larger size of first maturity (>9 mm) for February 2002 (Table 2-2). A bimodal distribution of frequencies for adult-size males (A_m) suggest a group composed of two size-classes: a size >8 mm and ≤10 mm (35%), and sizes >10 mm (65%). Mature males with extruded spermatophores (M_m), were all small (≤10 mm) during the sampling period. Accordingly, to control for the effect of size, M_m stage was mostly compared with the small class of adult-size males (AS_m).

No significant differences in *WW* or *DW* were observed among juvenile stages although there were differences between juvenile and postjuvenile stages (Table 2-2). Body water comprised 75% of the wet weight in J_u, and up to 82% in reproductively mature specimens (*WW*: $F_{9, 178} = 9.5$, $p < 0.001$). On a dry weight basis, post-juvenile females were almost four times heavier than juvenile stages (both J_f and J_m). In contrast, small post-juvenile males (SA_m and M_m) were only three times heavier than juveniles, but larger males (A_m) were 6.5 times heavier than juveniles (ANOVA: *DW*: $F_{9, 178} = 58.46$, $p < 0.001$) (Table 2-2).

Sexual dimorphism in *DW* occurred after undifferentiated juveniles differentiated into females and males. Analysis of covariance confirmed higher weight gains for males *vs* females as a function of *TL* ($F_{1, 129} = 6.78$, $p = 0.01$). As no significant difference in intercepts was found

between males and females, data of different furciliae stages (I to VI) were included to improve both equations (Figure 2-2). The $\log_{10}$-$\log_{10}$ regressions between *DW* and *TL* had high coefficients of determination for both females ($R^2 = 0.97$) and males ($R^2 = 0.97$), but the allometric exponent was lower for females (2.6 ± 0.1 C. I. of 95%) than for males (2.8 ± 0.1 C. I. of 95%) (Figure 2-2). In both sexes, the allometric exponents observed in February 2002 were below the annual average observed in animals collected in other surveys during the whole year (2.8 ± 0.1 for females and 3.0 ± 0.1 for males; Chapter 4).

For comparison purposes, unbiased estimates of the same parameters were estimated using non-linear curve fitting (non-log transformed). The iterative curve fitting routine to a power law with a least-squares criterion yielded different parameter estimates. For males and females, the intercept was estimated at 0.00135 (±0.00026 C. I. of 95%), whereas, the allometric exponent for females was 2.9949 (±0.2407 C. I. of 95%), and for males was 3.3184 (±0.2598 C. I. of 95%).

Equations 1 to 4 illustrate the relationship between *DW* and *TL* for *N. simplex* females and males using both methods.

$\text{Log } DW_F = 2.6\ (\text{Log } TL_F) - 2.6$	or	$DW_F = 0.0025\ TL_F^{2.6}$	Eq. 1 $\log_{10}$-$\log_{10}$ regression
$\text{Log } DW_M = 2.8\ (\text{Log } TL_M) - 2.6$	or	$DW_M = 0.0025\ TL_M^{2.8}$	Eq. 2 $\log_{10}$-$\log_{10}$ regression
		$DW_F = 0.0012\ TL_F^{3.0}$	Eq. 3 non-linear fit
		$DW_M = 0.0007\ TL_M^{3.3}$	Eq. 4 non-linear fit

Despite the use of a curvilinear function, the inspection of the residuals for equations 3 and 4 shows some degree of heterogeneity. This suggests that if a normal error is assumed, there may be some bias in estimates of confidence limits for both the γ and the β parameters (Appendix 2-1). However, Figure 2-2b shows that parameter estimates do not appear to be biased by outliers, indicating that they are good estimates of the real parameters. Accordingly, for the

present work, the degree of heterogeneity does not warrant new research to find a better error model, although confidence limits should be taken with caution.

Following the traditional approach, estimated parameters from $\log_{10}$-$\log_{10}$ regressions were used for the subsequent analysis. Equations 1 and 2 diverged at a *TL* of 6.8 mm and *DW* of 0.41 mg, suggesting that sexual dimorphism in *DW* relative to *TL* occurred after J_u differentiated into J_f and J_m stages.

A consequence of differential weight gain between sexes is that weight differences between males and females become larger as animals increase their size, and can be confirmed algebraically: $(DW_M / DW_F) = (TL_M / TL_F)^{(2.8–2.6)}$. Accordingly, large-sized males seem to contribute considerably to the differences observed in those power equations (Table 2-2 and Figure 2-2).

Body condition was higher in post-juvenile females (1.2) than in males (0.8), perhaps as a consequence of less variable protein content. When the appropriate number of eggs was added to the female (from the brood-size equation), females carrying eggs or nauplii exhibited the highest body condition (1.4) (Table 2-2).

2.4.5. Biochemical composition and energy content

The most notable increase in *DW* occurred during development from differentiated juvenile stages into postjuvenile stages (3 fold for males and almost 4-fold for females) (Table 2-2). Here proteins increased at higher rates, followed by neutral lipids, and a moderate increase in polar lipids. Carbohydrates increased also at high rates with *TL*, but the overall contribution to the *DW* was minimal (Table 2-3).

In general, the biochemical composition of *N. simplex* was dominated by proteins (~45%), followed by neutral lipids (~20% in post juvenile forms); polar lipids (~10%) and carbohydrates

(<3%) (Table 2-3). Non-digestible materials were only analyzed in post juvenile forms and their contribution to total energy was assumed to be negligible. The contribution of non-digestible materials to *DW* was smaller in females than in males: most of the exoskeleton and chitin accounted for ~18% of dry weight in mature females and males ($F_{1,84}$=0.06, p = 0.810); whereas ash was close to 2% in females but was almost 4% in mature males ($F_{1,77}$=11.63, p = 0.001).

In 2002 samples (Bahía de La Paz), M_f stage, *i.e.* gravid females with a pink ovary in the meiosis stage (Gómez-Gutiérrez & Robinson, 2005), were poorly represented. In a strict sense, recently spawned females (O_f) and females collected after the total release of embryos in the metanauplii stage (S_f), are both spent females, and as expected, when eggs were removed from the former, the biochemical composition did not differ between the two stages. Furthermore, covariance analyses (ANCOVA) of $\log_{10}$-$\log_{10}$ transformed data showed that the increase rate of each biochemical fraction due to increases in size (*TL*) was not significantly different between males and females, neither for carbohydrates ($F_{1, 96}$=0.16, p = 0.691), proteins ($F_{1, 104}$=0.45, p = 0.502), neutral lipids ($F_{1, 80}$=2.14, p = 0.147), nor polar lipids ($F_{1, 92}$=0.03, p = 0.860). The analysis confirmed that as *TL* increased, all biochemical fractions increased following a power function (Figure 2-3): carbohydrates ($F_{1, 96}$=168, p<0.001), proteins ($F_{1, 104}$=688.04, p<0.001), neutral lipids ($F_{1, 80}$=560.18, p < 0.001) and polar lipids ($F_{1, 92}$=354.94, p < 0.001). Accordingly, when eggs were removed from females and sex and stages (including J_u) were considered together, *TL* (mm) had a significant (p<0.001) positive correlation with all biochemical fractions (µg ind^{-1}). Carbohydrates had the lowest coefficient of determination (n=112, R^2=0.685), followed by polar lipids (n=82, R^2=0.7372), neutral lipids (n=72, R^2=0.8024) and proteins (n=117, R^2 = 0.889). Figure 2-3 shows protein clearly played a key function during somatic growth for both sexes, with lipids increasing at lower rates during those stages.

The development from early juveniles into sex-differentiated juvenile stages was characterized by an increase in proteins and neutral lipids, although juvenile males reached slightly higher neutral lipid content. In both sexes, the transition into adult-size forms involved a notable increase in protein content (>2.5 times), and moderate increases in neutral and polar lipids. There was no significant increase in carbohydrates for males, but females increased their carbohydrate content substantially through their post-juvenile development (~2.5 times) (Figure 2-5).

Large adult sized males had very high protein contents, not only compared to small adult-sized males, but also to post-juvenile females. However, neutral and polar lipids, as well as carbohydrate content were all similar to that observed for non-reproductive post-juvenile females. This suggests that weight and size differences between large males and females were mainly due to an increase in protein content. Similarly, when small-sized males reached maturity (when spermatophores became evident) without a significant increase in size, changes in the protein content were negligible and the slight increase in polar lipid content during maturity was not significant. In mature males, carbohydrate content almost doubled, and there was a significant decrease in neutral lipid content (~21%), suggesting the use of lipid reserves to achieve maturity and spermatogenesis (Table 2-3, Figure 2-5).

Reproductive females (O_f plus eggs) did not show substantial differences in the carbohydrate content nor in the protein content, suggesting that most of the reproductive effort toward egg production occurred via lipid accumulation. Here, neutral lipids increased 1.5 times (~4.7 µg per egg), whereas polar lipids increased by more than twice after eggs were produced (~5.8 µg per egg) (Figure 2-5). This amount of neutral and polar lipids must necessarily be stored by the female in order to produce eggs, and apparently a large amount of it is metabolized during egg

development into nauplii, as neutral lipids decreased 1.7 times, or ~1.5 μg per egg, whereas polar lipids decreased down to 170 μg (a reduction of 4.84 μg per egg or 85% of the previous gain). As expected, once the eggs hatched and females released the metanauplii larvae, both neutral and polar lipids in post-ovigerous females (S_f) returned to levels similar to those observed in adult-sized females.

Based on these data, a mature female ranging from 10.3–11.1 mm would require between 4–24 J to produce a brood size of 29–35 eggs female^{-1}. Whereas this value can be considered an acceptable estimate for comparison purposes, a cautious approach is required in order to use it as a direct indicator of energy transferred to predators. Heat production at complete combustion tends to be higher than food oxidation by living organisms as they cannot oxidize some compounds completely (Beukema & De Bruin, 1979). Considering partial oxidation during catabolism, the M_f stage close to the spawning process and the O_f stage plus eggs should provide ~3–21 J extra energy to their predators. A higher lipid content in these two stages provides a higher amount of energy than any other life stage considered here.

2.4.6. Fatty acids

A matrix of 35 cases (composites of animals collected in February 2002) and 37 variables (major fatty acids found in the neutral fraction) was analyzed with multivariate methods. Gonad maturity contributed to higher similarity among neutral-fraction profiles, as O_f was the stage with higher within-group similarity (SIMPER: 95.6%) followed by M_m stage (87.1%). In contrast, A_f and J_m stages had the lowest within-group similarity in their fatty acid profile (77.1% and 77.3% respectively), suggesting a broad range in gonad maturity for A_f stage, and some mixing with prior or subsequent stages for J_m. A permutational multivariate analysis of variance (PERMANOVA) of the fatty acid profile of krill collected in February 2002 showed that there

was a significant interaction between life stage and lipid fractions (Pseudo-$F_{1,10} = 1.85$, $p_{(perm)} = 0.02$): *i.e.* the relative contribution of fatty acids to life stages was different between neutral and polar fractions.

A distance-based test for homogeneity of multivariate dispersions (PERMDISP) did not show significant deviations from the centroid after 1,000 permutations, either for fatty acids of the polar fraction ($F_{7,20} = 0.736$ $p = 0.994$), or for those of the neutral fraction ($F_{7,21} = 4.8197$ $p = 0.069$). As the p value for the neutral fraction was close to 0.05, a second set of 5,000 permutations was performed with similar results ($F_{7,21} = 4.8197$ $p = 0.0633$). This shows that the different fatty acids analyzed do not differ in their relative dispersions, and therefore, a dispersion effect can be ruled out on a distance-based PERMANOVA test.

According to the PERMANOVA main test, there were significant differences in the fatty acid profile of neutral lipids among developmental and maturity stages (Pseudo-$F_{7,21} = 2.979$ $p = 0.001$). However, those changes were gradual throughout development, as most contiguous life stages did not exhibit significant differences in their fatty acid profile (Pair-wise test $t \leq 2.007$, $p > 0.05$, n≥2). The transition from undifferentiated juveniles into differentiated juveniles of both sexes was accompanied by significant increases in saturated (13:0, 14:0, 15:0, 19:0 and 20:0), monounsaturated (16:1ω5, 20:1ω7), and polyunsaturated fatty acids (16:3ω3), and in the SFA/PUFA and SFA/LC-PUFA ratios (Table 2-4 and Table 2-6). There were significant decreases in ω3 LC-PUFA such as 20:5ω3 (EPA) and 22:6ω3 (DHA), as well as in the ω3/ω6 and 18:1ω 7/18:1ω 9 ratios (Table 2-4 and Table 2-6).

The transition from differentiated juvenile forms into mature forms was marked by decreases in SFA in the neutral fraction of both sexes and increases in the relative contribution of PUFA, particularly long-chain PUFA (20:4ω6, 20:5ω3 and 22:6ω3). To corroborate this trend, fatty acid

concentrations (mg g^{-1} *DW*) were plotted for each life stage. As krill reached their adult size, a notable increase in 20:5ω3 and 22:6ω3 during growth and maturity was evident in males (Figure 2-6). Females exhibited a similar trend but not always with statistically significant increases (Figure 2-6). Adult-sized and spent females exhibited higher variability of long-chain PUFA than males, most likely because oöcytes in the ovary comprised a wide range of stages (I—III).

The gonad-maturity stage was characterized by a significant decrease in the C_{16} PUFA/C_{18} PUFA ratio in both sexes. In males, gonad development was associated with an increase in 16:1ω7 and a decrease in 16:2ω4 and C_{16} PUFA. In females gonad development was associated with an increase in the relative contribution of 18:1ω9, 18:2ω6, 18:3ω3 and 22:6ω3. The latter corroborated by an increase in concentration of 22:6ω3 (Figure 2-6). Once the eggs were produced and nauplii released, concentration of DHA in S_f stage was more variable (Figure 2-6).

For the fatty acid profile of the polar fraction, a matrix of 34 cases (samples collected in February 2002) and 37 variables (main fatty acids) was analyzed. Here, no significant differences were found among developmental and maturity stages (Pseudo-$F_{7,20}$ = 1.604 p = 0.123). Although A_f was the stage with the lowest within-group similarity (80.7%), the fact that in all stages similarity values were >80% suggests that structural lipids were more stable throughout development and maturity (Table 2-5, Table 2-6).

The combined effect of gonad status and location on fatty acids was analyzed using swarms collected in two eastern stations in the Gulf of California (S06 and S47) during March 2010. Both stations had a broad spectrum of adult krill with different gonad status (males with intruded and extruded spermatophore, and females at previtellogenic or postvitellogenic stages, with or without ovisacs). Here, lipids were not separated into neutral and polar fractions, and SFA, PUFA, LC-PUFA, and most ω3 fatty acids did not differ significantly between stations or among

life stages. Still, significant increases in 22:6ω3 and in the LC-PUFA/saturated and 20:5ω3/22:6ω3 ratios throughout gonad development were more relevant than differences produced by location. Those result were similar to those observed for February 2002, suggesting a generalized pattern not affected by location or year.

With some fatty acids and fatty acid ratios, significant differences occurred ($p < 0.05$) both between sampling locations and according to gonad status (16:1ω7, 16:2ω4, 16:3ω4, 16:4ω1, 18:4ω3, branched, 16:1ω7/16:0, and C_{16} PUFA), although the interactive effect of location and gonad status was not significant. Here, despite local differences, differences among individuals due to gonad status were similar between the two stations. A third set of fatty acids and fatty acid ratios fulfilled one important requirement to be used as trophic markers for *N. simplex*, as differences can be attributed solely to locations but not to gonad status or sex (two-way ANOVA). These included bacterial biomarkers (*i*16:0, phytanic acid, 15:0 and odd chain fatty acids); phytoplankton biomarkers (18:1ω7); diatom biomarkers (16:1ω7/18:4ω3 ratio); dinoflagellate biomarkers (18:5ω3/18:3ω3 ratio, and C18 PUFA); and copepod biomarkers (20:1ω9 and 24:1ω9).

2.5. Discussion

2.5.1. Sexual dimorphism

Previous studies of *N. simplex* and *N. capensis* found that males are frequently larger than females (Barange & Stuart, 1991; Gómez-Gutiérrez, 1995; Lavaniegos, 1995), suggesting faster growth rates for males of those species. This feature seems to be species specific, as females of *Euphausia eximia* and *E. hanseni* are commonly larger than males of the same species (Barange & Stuart, 1991; Gómez-Gutiérrez, 1995). For Antarctic krill (*E. superba*), lower abundances of

large males suggest that although males might grow faster, they also have a shorter lifespan than females in the natural environment (Kawaguchi *et al*., 2007).

Maximum-recorded *TL* for *N. simplex* is 19 mm (Gómez-Gutiérrez *et al*., 2012), and maximum observed *TL* in this study was 13.3 mm in males and 12.5 mm in females. In the present study, mature males with spermatophores (M_m) were small and did not show significant differences in size when compared with mature female stages (O_f and A_f). In contrast, large A_m –males without spermatophores– were larger than any other group. This lack of spermatophores in large adult-sized males prevented their identification as mature individuals and forced their separation from small A_m stage in order to compare with small sized M_m stage. Two contradictory and mutually exclusive circumstances can explain this result: either spermatophore detachment (and probably reproduction) are more frequent in larger males, or rates of spermatophore regeneration (and therefore reproduction) are slower in larger individuals. Size-related differences in reproductive rates can be important for secondary production estimates and therefore deserve a more detailed study.

Although male euphausiids can be easily distinguished from females by the presence of spermatophores and petasma, and within small individuals the lappet's shape, more subtle morphometric differences between sexes are seldom documented. For instance, mature females (> 40 mm) of Antarctic krill (*E. superba*) had an enlarged ovary that produces a thicker cephalothorax than that observed on males of similar size (Amakasu *et al*., 2011). Also, males of *Euphausia vallentini* collected in the Southern Indian Ocean exhibited significantly higher weights than females of similar size as shown by log-log regressions between length and weight (Mayzaud *et al*., 2003), a result similar to the present study.

Here, *DW* differences between sexes were clear. On average, large A_m stage was 42% heavier than A_f, O_f and S_f stages; consistent with a higher allometric exponent. Previous allometric relationships (of the form $DW = \gamma\ TL^{\beta}$) for *N. simplex* were proposed without sex distinction and no confidence intervals were estimated. In the allometric relationship $DW = 0.002162\ TL^{2.856}$ obtained from specimens collected at Bahía Vizcaíno (Lavaniegos, 1995), coefficients γ and β fall between the confidence intervals calculated for equations 1 and 2 in the present study. On the other hand, the equation: $DW = 0.005371\ TL^{2.316}$ obtained from specimens collected off Bahía Magdalena without sex distinction (Gómez-Gutiérrez & Robinson, 1997), suggests heavier individuals at early stages, and lower weight gain through their life span than those found in the present study.

Whereas regional and seasonal differences might explain different allometric relationships in each case, the effect of particular methodologies and equipment precision cannot be discounted. For instance, the observed differences in exponents may be due to log transforms, which are known to produce biased estimates (St-Pierre *et al.,* 2018). Additionally, given the properties of the equation $DW = \gamma\ TL^{\beta}$, small sample sizes can result in high leverage by a few values far from the mean (very large or very small *TL*), tipping the slope up or down. Still, lack of information at early stages has larger impacts on allometric relationships than underrepresented mature stages. The present study provided greater and more reliable data for early stages (2 to 4 mm *TL*) than previous studies that barely considered them. Also, ovigerous females were considered as a particular case, whereas individuals above 12 mm *TL* were not well represented. Similarly, lack of sex separation and differences in preservation techniques and measurement of animal *DW* probably contributed to the estimation of different relationships in previous studies (Lavaniegos, 1995; Gómez-Gutiérrez & Robinson, 1997).

Such differences in allometric equations are not negligible. Estimations of production rates and biomass of this euphausiid often depend on the combined effect of population structure and allometric coefficients (Lavaniegos, 1995; Gómez-Gutiérrez *et al*., 1996; 2012; De Silva-Dávila & Palomares-García, 1998). Table 2-7 shows that for a population with a high contribution of larvae, and depending on the selected parameters, differences in productivity (*P*) and biomass (*B*) can be as high as 58 and 49% respectively, whereas variations in the productivity: biomass ratio (*P:B*) can be as high as 82%. In fact, *B* shows a positive correlation with γ and a negative correlation with β, whereas the *P:B* ratio shows a negative correlation with γ and a positive correlation with β. In contrast, when the population includes larger animals and larvae contribute moderately, then differences in *P* and *B* can be as high as 67 and 64% respectively, whereas variations in the *P:B* ratio can be as high as 93%. Here, this ratio has a positive correlation with γ, and a negative correlation with β (Table 2-7). Accordingly, in order to decrease bias on production rates and biomass, future research attempting to look at regional and seasonal differences should: 1) preferably separate sexes, 2) preserve by freeze-drying, 3) avoid log transformation to estimate the allometric coefficients (β and γ), and 4) utilize large sample sizes with emphasis on early stages.

2.5.2. Changes in biochemical composition

Somatic growth and increase in *DW* of *N. simplex* were driven mainly by an increase in protein content followed by lipids and to a lesser extent by carbohydrates. During embryogenesis, neutral lipids in O_f stage increased ~1.75 times, but the added weight of eggs buffered their relative contribution. In contrast, the increase in polar lipid content surpassed the increase in weight, increasing its relative contribution to dry weight. During egg development into nauplii, total lipids in eggs of *N. simplex* decreased ~60%, a lower figure than that estimated

with the histological method used by Montuy-Gómez *et al.* (2012) on the same species. These authors found that the area covered with lipid reserves decreased ~80% from the single cell stage to the hatching nauplius stage. While the estimated values cannot be directly compared (% of area *vs* % of lipids), evidence in both cases supports the idea that a large proportion of lipids are rapidly metabolized during ontogeny from single cells into metanauplii. It is precisely at the metanauplius stage (when lipid reserves reached a minimum) that they are released from the ovigerous sac to start their energy-expensive free-swimming phase. Lipid depletion highlights importance of release of metanauplii and further calyptopis I first feeding larval stages into food-rich environment to avoid starvation and death (Montuy-Gómez *et al*., 2012).

The biochemical composition of subtropical krill undergoes substantial changes during somatic growth and gonad maturity processes. For instance, the protein content of non-reproductive postlarval stages of *N. simplex* (43–60%) was similar to that observed for the tropical krill *E. lamelligera* (54–57%) from waters of Mexico (Färber-Lorda *et al.* 2004). According to Ambriz-Arreola *et al.* (2015) *E. lamelligera* is a broadcast spawner with short hatching times (<18 h) and fast embryonic development (~12 h). In contrast, protein content of ovigerous *N. simplex* females was similar (37–48%) to that reported for adult forms of the sibling species *N. australis* (40.6%) from temperate waters of Tasmania (Virtue *et al*., 1995).

A multiannual study of the biochemical composition of *Nyctiphanes australis* (Virtue *et al*., 1995) showed that physiological condition appears to be maintained with relatively low variability throughout the year. For instance, lipid content of adult forms was low (5–10%) compared with polar species which can reach >40% during highly productive conditions (austral spring-summer). In contrast, the present study shows that total lipid content changed during development and maturity, being as high as 47–57% for juvenile forms; decreasing down to 12–

28% in post-juvenile non-mature forms, and increasing up to 28–33% in mature forms. We can assume that lipid contents observed in February for *N. simplex* are among the highest the species can reach seasonally, as surface chlorophyll *a* and primary productivity are higher during colder months (Chapter 4. Section 4.4.1 this thesis, Hidalgo-González & Alvarez-Borrego, 2004).

Changes in protein and lipid content had a substantial effect on the energy content at different life stages: On average, females increased *DW* 3.8 times before reaching adult size, but their energy content only increased 2.7 times on average. However, during gonad maturity, energy content increased 1.45 times, despite no statistically significant increase in *DW*. As this increase was calculated from the lipid content of eggs, the small, non-significant change in weight (~6%) seems advantageous for females carrying large numbers of eggs while continuously feeding.

As a consequence of somatic growth, males increased *DW* an average 2.8– 6.1 times (from J_m stage to SA_m and A_m stages respectively), but energy content only increased 2.1–2.4 times. No significant biochemical changes occurred during gonadic maturity of males, neither in size nor in weight. Consequently, there were no substantial changes in the energy content or *DW* during gonadic maturity of males.

The total energy content of a swarm will depend upon its population structure. A hypothetical swarm with an assumed 1,000 individuals and with various male: female ratio similar to those observed in nature by Gendron, (1992), will alter its total energy content up to 1.7 times depending on the relative abundance of mature individuals of each sex (Table 2-8).

2.5.3. Changes in lipid classes and fatty acids

In many organisms, polar lipids comprise mostly structural (membrane) lipids, and neutral lipids comprise mostly storage lipids (*e.g.* triacylglycerols). However, in some krill species

phospholipids can be the dominant lipid class and the primary lipid storage model (Pleuthner *et al.*, 2016). If the role of phospholipids in subtropical krill is mainly structural, then it is expected that polar lipids will increase during somatic growth or gonadic maturity (cell division and cell growth), decreasing the neutral to polar lipid ratio (N: P). Juvenile forms of *N. simplex* had an N: P ratio >4 but decreased to ~2 in post juvenile forms. Only reproductive stages exhibited a neutral: polar ratio close to 1 (1.1 for O_f and 1.3 for M_m stages). This suggests that an N: P ratio ≤1.1 can be indicative of reproductive maturity for this species.

Fatty acid profiles of polar lipids did not exhibit strong changes throughout development and maturity. This suggests that *N. simplex* homeostasis relies on the stability of the fatty acid profile of polar lipids, and therefore those profiles might provide important non-trophic information. For instance, the fatty acid profile of polar lipids has been used to discriminate two reared stocks of cod (*Gadus morhua*) fed with the same food (Joensen *et al*., 2000). Similarly, total lipid extracts of polar-lipid rich organs (such as heart tissue), have been successfully used to identify stocks of reared salmon and several populations of tropical fish (Grahl-Nielsen, 2014).

In contrast, neutral lipids of *Nyctiphanes simplex* increased at lower rates than polar lipids during somatic growth, and while they also increased substantially during gonadal maturity of females, they experienced significant changes in fatty acid composition. The transition from differentiated juvenile forms into adult-sized forms was marked by a notable increase in the relative contribution of 20:4ω6 in both sexes. For males, strong increases in 20:5ω3 and gradual increases of 22:6ω3 during development accounted for most of the variation in essential fatty acids, whereas for females, the relative content of 22:6ω3 increased almost two-fold during gonad maturity.

A similar result was observed in follow up samples (March 2010) from two coastal location on the east side of the Gulf of California. Here, only adult sizes were compared and the increases in 22:6ω3, and the LC-PUFA/saturated and 20:5ω3/22:6ω3 ratios throughout gonad development were more relevant than differences produced by location. The contribution of essential fatty acids via adult stages of *N. simplex* to upper trophic levels could be significant, as ω3 fatty acids constituted ~30% in neutral lipids and ~40% in polar lipids. These findings are consistent with high levels of PUFA observed in *N. australis*, where almost half of the total fatty acids were ω3 fatty acids, dominated by the two essential fatty acids, 20:5ω3 and 22:6ω3 (Virtue *et al*., 1995). The prevalence of ω3 PUFA is widespread among crustaceans; their role in ovarian development has been demonstrated in other crustaceans (Alava *et al*., 1993), and the increase in PUFA during vitellogenesis has been associated with increases in prostaglandins, which are also involved in reproductive processes (Spaziani & Hinsch, 1997).

2.5.4. Energy content and reproductive costs

In the present study, females with type IV oöcytes were not collected. In order to estimate the increase in lipid content and the energy devoted to egg production, O_f stage was compared against both A_f or S_f stages. In comparison with a preliminary study (Gómez-Gutiérrez *et al*., 2010a), the overall conclusions are similar, although the results differ slightly. For instance, in the present study, the relationship between total lipid content and euphausiid total length were better described by a power function than a linear one, and average total lipid content was significantly higher in female mature forms (~350 μg ind^{-1}), increasing from 27% of *DW* in A_f stage or 22% in S_f stage, up to 41% in O_f stage for females of similar size. Consequently, in order to produce eggs, females increase their total lipids 1.5–1.8 times during oogenesis (vitellogenesis to meiosis). If 77.6% of lipids is assumed to be carbon (Gnaiger & Bittcrlich,

1984), then the carbon content will increase 13–14%. Similar results were reported by Gómez-Gutiérrez *et al*. (2010a). However, because females differed in size and lipid content between both studies, the novelty of the present data is that such increases seem to occur irrespective of the female total length or lipid content. Moreover, in the present study it is clear that most of the increase in total lipids was driven by a ~2-fold increase in polar lipids. Such a change is even more impressive when considering that females can transition from previtellogenesis into vitellogenesis in as little as 1–4 days (Gómez-Gutierrez *et al*., 2010b).

Significant decreases in lipid reserves have been observed in *E. superba* males after reproduction (Virtue *et al*., 1996), indicating a relatively high energy cost involved in the fertilization process. In a previous study I estimated a 5.4% difference in carbon content between males with intruded and extruded spermatophore (Gómez-Gutiérrez *et al*., 2010a). Likewise, in the present study I estimated a decrease of ~5% in carbon content during spermatophore production (produced by an increase of ~1% via polar lipids, and a decrease of ~6% via neutral lipids). However, in the present work the decrease was not statistically significant. Moreover, whereas lipid contribution to dry weight of males was high, differences in total lipid content were not significant between small adult-sized males (21–29%) and males with spermatophore (23–43%). Apparently, when small-sized individuals are involved, their higher lipid content and deviations from the mean result in large variations in their relative contribution to dry weight. A larger sample size is recommended when using small-sized individuals to estimate the energy cost involved in male reproduction.

In the present study, the mean weight of a 10.7 mm-length gravid *N. simplex* female plus eggs was 1.8 mg, *i.e.* ~0.2 mg heavier than O_f stage with their eggs removed. With an average of 32 eggs for each female (via the modified equation of Gómez-Gutiérrez *et al*., 2012), each egg of

N. simplex was estimated to weigh 6.25 µg; less than the 7.4–18.0 µg estimated for just spawned embryos of *N. simplex* (Gómez-Gutierrez *et al*., 2012), and slightly less than the 8.1 µg egg^{-1} reported for *N. australis* (Hosie & Ritz, 1983). A *N. simplex* female can provide ~24 J mg^{-1} in energy to predators, whereas a spent female can provide ~19 J mg^{-1}. Although the results were similar to the mean energy content of the Antarctic krill *Euphausia superba*, with ~23 J mg^{-1} in gravid females and ~21 J mg^{-1} in spent females (Nicol *et al*., 1995), such differences in the present study were not statistically significant, mainly due to the high variability in female *N. simplex TL* (8.5–12.1 mm). In terms of individual energy devoted to reproduction, a gravid *N. simplex* female will have ~33% more energy than a spent female (40 *vs* 30 J ind^{-1}) while increasing its *DW* only 18%. In contrast, a 349 mg gravid *E. superba* female will have >89% more energy than a 199 mg spent female (8.1 *vs* 4.3 kJ ind^{-1}) by increasing its dry weight by 75%. Large increases in weight coupled with large increases in energy content for reproduction of polar species are expected where high production events (spring and summer) occur for short periods, with no reproduction the rest of the year. In contrast, a low weight increase with moderate increase in energy for reproduction is consistent with an environment with a continuous food supply where *N. simplex* can reproduce throughout the entire year (Lavaniegos, 1995; De Silva-Dávila & Palomares-García, 1998; Gómez-Gutiérrez *et al*., 2012).

According to these energy estimations, once the M_f stage is achieved, further egg production in S_f stage will require less energy than that used by A_f stage to develop and produce eggs. This idea is consistent with the relatively short interbrood period (7 to 15 days) observed in *N. simplex* (Gómez-Gutiérrez & Robinson, 2005; Gómez-Gutiérrez *et al*., 2012), which is about one quarter to half of that previously assumed for this species (Lavaniegos, 1995).

Based on these results, ~550 adults would yield 1 g of dry biomass during February, providing as little as 15–15.5 kJ if the whole biomass is composed of non-mature adult-sized forms. However, the energy content could be 1.45 times higher if the main proportion of such biomass is composed of lipid-rich O_f or M_f stages. If changes in *N. simplex* population structure are likely to produce changes in total energy content of swarms, it is likely that those changes might also modify the estimation of biomass demand by predators. For instance, the whole breeding population of adult seabird Cassin's Auklets (*Ptychoramphus aleuticus*) in the Farallon Islands is estimated to consume approximately 4,440 kg day^{-1} of krill, based on an average content of 4.65 kJ g^{-1} (wet basis) for euphausiid prey, and assuming an assimilation efficiency of 76% (Hodum *et al*., 1998). However, if a lower energy content of prey is considered (2.6 kJ g^{-1} wet basis), –as found in AS_f and S_f stages of *N. simplex*–, then their biomass demand will increase to 7,663 kg day^{-1} in order to obtain the same amount of energy. Similarly, Mårtensson *et al*. (1996), show that even a small decrease (11%) in the energy content of the food of minke whales in the North Atlantic would increase previous estimates by 300,000 tons.

In the present work, lipid and energy content of *Nyctiphanes simplex* were closely associated with developmental and reproductive processes. Therefore, swarm population structure can constitute a relatively good proxy for energy content. The occurrence of continuous reproduction with subsequent peaks and frequent swarming behavior in the Gulf of California during winter and spring (Gendron, 1992; De Silva-Dávila & Palomares-García, 1998), requires an extension of the present study to seasonal analysis of the energy content of *N. simplex*. The ecological function of this neritic, subtropical euphausiid as a pivotal food source for several resident and migratory pelagic and epibenthic predators in the Gulf of California, does not solely rely on high abundance or density, since adults in breeding condition appear to provide a substantial source of

essential fatty acids, and since ovigerous females carrying eggs can constitute a considerable energy supply to upper trophic levels.

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Table 2-1 Classification of developmental stage, sex, and gonad maturity of *Nyctiphanes simplex*

Code	Stage	Diagnostic features
J_u	Undifferentiated juveniles	Animals with one spine in the telson, five pleopods completely developed and no evident sexual dimorphism. Total length 4 to 6 mm. When placed on petri dishes, specimens lay on one side of their body unlike larger furciliae which lay on their ventral side.
J_f	Juvenile females sexually differentiated[1]	Animals that have not reached the age or size of first maturity but that can be distinguished from males by a shorter forward-canted lappet, lack of petasm in the first abdominal pair of pleopods and the presence of young oöcytes (stage I) in the gonad. Total length 6 to 8 mm.
A_f	Adult-sized females[1]	Females with further development although not ready for reproduction. Oöcytes in ovary in stage II to III. Total length >8 mm.
M_f	Mature females[1, 2]	Females with further gonad development ready for reproduction. Pink gonad. Oöcytes in ovary in stage IV. Total length >8 mm.
O_f	Ovigerous females[3]	Spawned females carrying external ovisacs containing eggs. Total length >8 mm.
S_f	Empty or spent females[3]	Recently spawned females with a hollow space between the thoracic limbs and the first pair of pleopods, no longer filled with embryos. Total length >8 mm
J_m	Juvenile males sexually differentiated	Animals that have not reached the age or size of first maturity but can be distinguished from females by an incipient petasm in the first abdominal pair of pleopods, and longer-flat lappet. Total length 6 to 8 mm.
A_m	Adult-sized males	Males with further development although not ready for reproduction, discerned from mature males by the lack of spermatophore. Total length >8 mm.
M_m	Mature males	Males bearing an internal brown or dark spermatophore, visible through the cuticle, or a protruding spermatophore. Total length >8 mm.

[1]Maturity stages of oöcytes after Ross *et al.* (1982)
[2]Not observed in this study.
[3]After Lavaniegos (1995); Gómez-Gutiérrez & Robinson (2005).

Table 2-2 Total length, wet and dry weight, and body condition of *Nyctiphanes simplex* in relation to sex and maturity stage. Mean ± 95% C.I.

	(n)	Wet Weight (mg)	Dry Weight (mg)	Total Length (mm)	Body Condition	Water %
Juveniles	10	1.0 ± 0.3^{a}	0.3 ± 0.02^{a}	5.7 ± 0.3^{a}	1.1 ± 0.06^{c}	72 ± 9^{a}
Juvenile Females	18	2.0 ± 0.4^{b}	0.4 ± 0.04^{b}	7.0 ± 0.3^{b}	1.0 ± 0.11^{c}	80 ± 4^{ab}
Adult-sized Females	26	10.4 ± 0.7^{d}	1.6 ± 0.18^{cde}	10.7 ± 0.4^{d}	1.2 ± 0.06^{c}	85 ± 1^{c}
Ovigerous Females* (with eggs)	26	10.3 ± 0.6^{d}	1.8 ± 0.21^{de}	10.7 ± 0.4^{d}	1.4 ± 0.05^{d}	82 ± 1^{b}
Ovigerous Females* (with nauplii)	26	10.3 ± 0.6^{d}	1.8 ± 0.20^{e}	10.7 ± 0.4^{d}	1.4 ± 0.06^{d}	82 ± 1^{b}
Spent Females	21	11.6 ± 0.8^{d}	1.7 ± 0.14^{de}	11.2 ± 0.3^{d}	1.2 ± 0.06^{c}	85 ± 1^{c}
Juvenile Males	16	2.2 ± 0.2^{b}	0.4 ± 0.03^{b}	6.9 ± 0.2^{b}	0.8 ± 0.03^{a}	81 ± 2^{ab}
Small Adult-sized Males	14	7.7 ± 0.5^{c}	1.2 ± 0.20^{c}	9.5 ± 0.5^{c}	0.8 ± 0.05^{a}	85 ± 1^{c}
Males with Spermatophore	9	8.3 ± 0.5^{c}	1.3 ± 0.30^{cd}	10.0 ± 0.8^{cd}	0.8 ± 0.07^{a}	84 ± 1^{c}
Adult-sized Males	22	16.7 ± 1.1^{e}	2.6 ± 0.19^{f}	12.0 ± 0.2^{e}	0.9 ± 0.04^{b}	84 ± 0.4^{c}

Univariate ANOVA tests were performed to analyze differences between groups. Significant differences ($p<0.05$) among means are indicated with a different superscript in the same column. *WW* = wet weight; *DW* = dry weight; *TL* = Total length; (n) = sample size; *Eggs were weighted separately from Ovigerous females.

Table 2-3 Biochemical composition of *Nyctiphanes simplex* in relation to sex and maturity stages. Mean ± 95% C.I.

	(n)	Carbohydrates	(n)	Proteins	(n)	Neutral Lipids	(n)	Polar Lipids	Energy[1] (TO)	Energy[2] (PO)	N:P
		(µg ind^{-1})		(µg ind^{-1})		(µg ind^{-1})		(µg ind^{-1})	(J ind^{-1})	(J ind^{-1})	
Juveniles	18	6± 1^{a}	9	141± 42^{a}	4	107± 2^{a}	6	20± 7^{a}	8± 1^{a}	8± 1^{a}	5.2
Juvenile Females	18	6± 1^{a}	18	223± 28^{b}	9	117± 8^{b}	9	30± 3^{a}	11± 1^{b}	10± 1^{b}	3.9
Adult-sized Females	18	19± 4^{c}	27	727± 107^{c}	12	196± 25^{cd}	15	126± 35^{cd}	30± 5^{c}	27± 4^{c}	1.6
Ovigerous Females (+ eggs)	18	19± 4^{c}	27	727± 108^{cd}	17	351± 21^{f}	20	318± 43^{e}	44± 5^{d}	40± 5^{d}	1.1
Ovigerous Females (+ nauplii)	18	19± 4^{c}	27	731± 101^{c}	17	286± 20^{e}	20	173± 29^{d}	36± 4^{cd}	32± 4^{cd}	1.7
Spent Females	15	22± 4^{c}	21	842± 86^{cd}	14	208± 25^{cd}	17	126± 33^{cd}	33± 4^{c}	30± 4^{c}	1.6
Juvenile Males	15	8± 3^{ab}	18	245± 23^{b}	9	182± 32^{c}	9	44± 9^{b}	15± 2^{b}	13± 2^{b}	4.1
Small Adult-sized Males	14	10± 1^{ab}	9	655± 120^{c}	11	277± 25^{e}	12	115± 38^{cd}	31± 5^{c}	28± 5^{c}	2.4
Males with Spermatophore	9	21± 1^{c}	9	665± 133^{c}	5	218± 17^{cd}	6	161± 64^{cd}	31± 6^{c}	28± 6^{c}	1.3
Adult-sized Males	18	16± 4^{cb}	27	903± 70^{d}	9	258± 42de	12	105± 30^{c}	36± 5^{cd}	33± 4^{cd}	2.5
		% of *DW*		% of *DW*		% of *DW*		% of DW	(J mg^{-1})	(J mg^{-1})	
Juveniles		1.8± 0.18^{cd}		37± 11^{ab}		40± 8^{c}		7± 1.7^{b}	27± 6^{bcd}	25± 6^{bcd}	6.1
Juvenile Females		1.5± 0.34^{bcd}		55± 4^{c}		39± 4^{c}		9± 0.4^{c}	32± 3^{cd}	29± 2^{cd}	4.3
Adult-sized Females		1.1± 0.14^{ab}		50± 6^{bc}		18± 6^{b}		9± 1.8^{bc}	22± 5^{b}	20± 4^{b}	1.9
Ovigerous Females (+ eggs)		0.9± 0.13^{ab}		42± 5^{b}		22± 3^{b}		19± 1.5^{d}	26± 3^{b}	24± 3^{b}	1.2
Ovigerous Females (+ nauplii)		0.9± 0.12^{ab}		41± 5^{b}		18± 3^{b}		10± 1.4^{c}	21± 3^{b}	19± 3^{b}	1.8
Spent Females		1.1± 0.14^{ab}		52± 8^{bc}		14± 3^{b}		8± 1.8^{bc}	21± 4^{b}	19± 3^{b}	1.7
Juvenile Males		2.0± 0.77^{d}		55± 6^{c}		46± 6^{c}		11± 1.3^{c}	36± 4^{d}	32± 4^{d}	4.2
Small Adult-sized Males		1.2± 0.12^{abc}		48± 4^{bc}		18± 1^{b}		7± 2.3^{bc}	22± 2^{b}	20± 2^{b}	2.5
Males with Spermatophore		0.8± 0.05^{a}		55± 5^{c}		21± 7^{b}		11± 2.5^{c}	26± 5^{bc}	23± 4^{bc}	1.9
Adult-sized Males		1.1± 0.17^{abc}		34± 1^{a}		8± 1^{a}		4± 0.9^{a}	13± 1^{a}	12± 1^{a}	2.3

Different superscript in the same column indicate significant differences among means. *DW* = dry weight; n = sample size; [1]TO=Assuming total oxidation; [2]PO=Assuming 'Physiological Oxidation' (see text for details), N:P= Neutral lipid to polar lipid ratio.

Table 2-4 Fatty acid proportions (% total fatty acids) in the neutral lipid fraction of *Nyctiphanes simplex* at different life stages, Mean ± 95% C.I.

FAME Neutral	Juvenile n=2	JuvFem n=3	ASFem n=3	OvFem n=6	SpFem n=4	JuvMal n=4	ASMal n=3	SpMal n=4	
12:0	0.13±0.02	0.67±0.52	0.89±0.75	0.41±0.30	0.27±0.14	1.44±0.52	1.49±0.62	0.58±0.19	sig
14:0	2.4±0.3	3.3±0.4	4.5±1.8	3.9±0.3	5.0±1.1	3.7±0.4	3.6±0.8	3.6±0.3	sig
15:0	0.87±0.06	1.64±0.43	1.42±0.64	0.96±0.04	0.96±0.17	2.65±1.18	1.25±0.47	1.00±0.18	sig
16:0	28.7±0.5	29.7±3.3	29.9±5.0	28.6±0.4	26.6±1.7	30.7±6.4	29.7±5.0	27.1±3.4	n.s.
17:0	0.89±0.01	1.05±0.01	1.07±0.28	1.17±0.04	1.03±0.07	1.01±0.22	1.06±0.30	1.05±0.04	sig
18:0	10.9±5.5	9.2±2.4	7.2±2.2	5.4±0.3	9.9±3.5	12.9±1.4	7.4±1.0	6.4±1.1	sig
20:0	0.47±0.05	0.63±0.05	0.43±0.21	0.33±0.04	1.18±0.94	0.85±0.15	0.79±0.28	0.66±0.28	sig
Σ Saturated	45±5	48±6	47±9	42±1	46±5	56±6	48±8	42±5	sig
Σ Branched	1.46±0.01	1.70±0.24	1.82±0.43	1.68±0.16	1.69±0.13	6.01±4.01	2.11±0.53	1.83±0.30	sig
ΣSFA+BFA	47±5	50±6	49±10	43±1	47±6	61±4	50±9	44±5	sig
16:1ω7	1.5±0.1	1.4±0.2	3.8±2.7	2.8±0.3	3.5±0.9	0.9±0.1	1.5±0.3	2.2±0.4	sig
16:1ω9	1.2±0.4	4.4±3.3	2.2±1.5	1.1±0.2	1.0±0.3	0.8±0.3	1.1±0.4	1.6±0.7	n.s.
18:1ω7	2.1±0.3	1.7±0.3	2.0±1.0	2.6±0.2	2.4±0.4	1.6±0.5	2.3±0.2	2.0±0.2	sig
18:1ω9	4.6±0.5	9.7±4.6	3.9±0.5	6.9±0.3	5.4±0.3	5.6±1.9	7.4±2.5	6.1±0.9	sig
20:1ω9	0.64±0.05	0.70±0.08	0.45±0.27	0.56±0.04	0.48±0.11	0.60±0.09	0.80±0.18	0.98±0.32	sig
22:1ω7	0.8±0.2	0.4±0.2	2.4±1.8	0.5±0.2	1.0±0.6	1.2±0.5	0.4±0.2	0.8±0.7	sig
Σ Monoenoic	11.3±0.8	19.0±7.6	13.4±3.0	14.8±0.4	14.4±0.9	11.2±1.9	13.7±2.7	14.1±0.7	sig
16:2ω4	0.4±0.1	1.5±1.1	1.2±0.7	0.5±0.2	3.3±3.0	1.6±1.2	4.4±2.5	0.7±0.3	sig
18:2ω6	1.2±0.1	3.7±2.5	1.5±0.3	2.3±0.1	1.8±0.3	2.3±1.4	2.2±1.1	2.0±0.1	sig
22:2ω6	1.2±0.6	1.3±1.0	1.3±0.6	0.5±0.1	0.5±0.2	2.6±0.2	0.9±0.4	1.4±0.8	sig
Σ Dienoic	2.9±0.9	6.1±2.5	3.8±0.7	3.5±0.3	5.8±2.8	6.2±1.6	8.1±3.8	4.2±1.0	sig
16:3ω3	0.17±0.04	0.83±0.22	0.67±0.53	0.18±0.04	0.32±0.10	0.95±0.43	0.81±0.27	0.47±0.30	sig
18:3ω3	1.0±0.1	0.8±0.1	0.9±0.1	1.6±0.1	1.5±0.3	0.9±0.3	1.2±0.3	1.5±0.5	sig
18:4ω3	1.2±0.7	1.1±0.6	1.2±1.0	0.4±0.1	0.6±0.3	2.3±0.4	1.8±0.4	1.3±0.4	sig
20:3ω6	0.2±0.1	0.3±0.2	0.3±0.1	0.2±0.1	0.3±0.1	2.6±2.1	0.4±0.1	0.2±0.1	sig
20:4ω6	1.7±0.3	1.3±0.3	2.3±0.7	2.4±0.1	2.2±0.6	1.3±0.3	2.4±0.2	2.2±0.5	sig
20:4ω3	1.2±0.7	1.1±0.6	1.2±1.0	0.4±0.1	0.6±0.3	2.3±0.4	1.8±0.4	1.3±0.4	sig
20:5ω3	14±1	9±3	15±6	12±1	13±4	5±2	11±2	13±1	sig
22:6ω3	20±2	13±2	12±3	21±1	15±4	8±3	14±4	19±3	sig
ΣLC-PUFA (≥20C)	37±4	25±4	30±10	36±1	31±7	17±6	28±7	34±5	sig
ΣPUFA	39±4	27±5	32±10	38±1	33±7	21±5	32±6	38±5	sig
ΣC_{16}PUFA	0.5±0.1	2.3±1.3	1.4±0.7	0.7±0.3	3.6±3.1	2.5±1.6	5.1±2.4	1.1±0.5	sig
ΣC_{18}PUFA	3.3±0.5	5.5±2.2	3.6±0.9	4.3±0.2	3.9±0.6	5.5±2.0	5.2±1.1	4.8±0.9	sig
Σω3	37±3	26±4	29±9	36±1	31±7	18±5	29±6	35±5	sig
Σω6	4.5±1.2	6.4±1.4	5.3±0.5	5.7±0.3	5.1±0.8	9.2±3.7	6.3±1.6	5.9±0.6	n.s.

Univariate ANOVA tests were performed to analyze differences between groups. When ANOVA assumptions were not met a *p*-randomization was conducted 1,000 times to test the data empirically. A similar approach was used for 95% confidence intervals for variables with residuals not meeting ANOVA assumptions. ΣSFA, ΣMUFA, and ΣPUFA: Sum of saturated, monounsaturated, and polyunsaturated fatty acids, respectively. Minor fatty acids (<1%) such as 13:0, 19:0, 21:0, 22:0, 24:0, *ai*14:0, *i*15:0, *i*16:0 , *i*17:0, 16:1ω5, 20:1ω7, 20:2ω6 are not included in the table. Rounding of significant figures was based on the magnitude of the error. A further analysis revealed that the average contribution of the neutral fraction to total lipids was: triacylglycerols 12%, free fatty acids 11%, aliphatic hydrocarbons 9%, ethyl esters 6%, ethyl ketones 6%, diacylglycerols 4%, free aliphatic alcohols 3%, and wax esters 2%.

Table 2-5 Fatty acid proportions (% total fatty acids) in the polar lipid fraction of *Nyctiphanes simplex* at different life stages, Mean ± 95% C.I.

FAME Polar	Juvenile n=2	JuvFem n=3	ASFem n=3	OvFem n=6	SpFem n=4	JuvMal n=4	ASMal n=3	SpMal n=4	
13:0	0.07±0.03	1.65±1.62	0.36±0.31	0.06±0.02	0.48±0.44	1.22±0.68	0.88±0.10	0.29±0.27	sig
14:0	2.1±0.6	1.8±0.3	2.2±0.9	2.5±0.4	2.0±0.4	1.9±0.3	1.4±0.1	1.9±0.3	sig
15:0	1.01±0.50	0.71±0.13	0.65±0.14	0.85±0.16	0.56±0.06	0.87±0.13	0.51±0.02	0.57±0.13	sig
16:0	23±3	26±3	27±6	31±6	22±3	29±3	18±1	22±1	sig
17:0	0.97±0.11	0.92±0.12	1.13±0.33	1.62±0.41	1.10±0.06	1.09±0.06	0.79±0.14	0.98±0.03	sig
18:0	7.4±1.2	5.1±0.8	6.1±2.1	6.9±1.5	6.7±3.3	6.4±0.3	3.6±0.6	4.2±0.7	sig
22:0	0.8±0.3	0.9±0.1	0.9±0.4	1.1±0.2	0.5±0.1	1.0±0.1	0.8±0.2	0.6±0.1	sig
Σ Saturated	37±3	37±3	40±10	45±9	36±6	43±4	27±2	32±2	sig
Σ Branched	1.7±0.5	1.8±0.2	2.3±0.9	2.2±0.5	2.2±0.8	2.1±0.2	1.5±0.2	1.4±0.1	sig
ΣSFA+BFA	38±4	39±3	42±11	47±9	38±7	45±4	28±2	33±3	sig
16:1ω7	1.3±0.2	1.5±0.3	1.1±0.2	1.3±0.3	1.9±0.2	1.4±0.2	1.9±0.2	1.9±0.2	sig
16:1ω9	2.6±2.3	0.4±0.1	0.7±0.3	0.8±0.3	0.6±0.1	0.6±0.3	0.5±0.1	0.5±0.2	sig
18:1ω7	1.21±0.05	1.40±0.12	1.37±0.54	1.05±0.26	1.91±0.11	1.30±0.12	1.61±0.20	1.48±0.19	sig
18:1ω9	7.3±1.5	7.0±0.9	5.4±2.2	6.2±1.4	10.3±1.3	5.8±0.3	9.8±2.2	8.0±0.1	sig
22:1ω7	0.8±0.6	0.7±0.6	1.6±1.4	1.6±0.5	0.7±0.4	0.6±0.1	0.4±0.1	0.3±0.2	sig
Σ Monoenoic	13.9±3.3	11.9±0.8	11.1±1.1	11.7±1.2	16.7±2.0	10.8±0.4	15.1±2.7	12.7±0.2	sig
16:2ω4	0.6±0.3	0.8±0.6	0.4±0.1	0.5±0.2	1.7±1.6	0.7±0.3	2.3±2.0	0.4±0.2	n.s.
18:2ω6	1.7±0.3	1.2±0.1	1.3±0.1	1.7±0.3	1.8±0.4	1.2±0.1	1.7±0.3	1.8±0.2	sig
22:2ω6	0.8±0.3	1.7±0.9	1.0±0.1	0.7±0.2	0.5±0.1	1.7±0.5	1.4±0.5	1.1±0.6	sig
Σ Dienoic	3.3±0.4	4.0±1.3	3.2±0.3	3.2±0.4	4.3±1.3	4.1±0.7	5.5±2.3	3.5±0.7	n.s.
18:3ω3	0.63±0.01	0.75±0.06	0.99±0.24	1.07±0.19	1.30±0.40	1.08±0.37	1.20±0.21	1.35±0.41	sig
18:4ω3	0.22±0.01	0.58±0.26	0.50±0.15	0.31±0.05	0.44±0.31	0.68±0.19	0.97±0.20	0.54±0.16	sig
20:3ω6	0.16±0.08	1.14±0.92	0.40±0.19	0.20±0.05	0.26±0.17	0.76±0.09	1.48±1.10	0.24±0.14	sig
20:4ω6	2.0±0.1	1.8±0.1	2.3±0.4	2.6±0.4	2.4±0.6	1.5±0.3	2.3±0.1	2.7±0.5	sig
20:4ω3	0.6±0.3	0.5±0.1	0.6±0.2	0.6±0.1	0.6±0.3	0.7±0.2	0.3±0.1	0.4±0.2	sig
20:5ω3	13.4±0.2	12.1±0.8	12.5±1.6	10.2±2.6	11.3±3.4	10.9±1.2	12.0±2.9	13.5±0.8	sig
22:6ω3	27±1	27±2	26±8	23±6	25±6	24±2	33±5	32±3	sig
ΣLC-PUFA (≥20C)	43±1	42±2	42±9	36±8	39±10	38±4	49±7	48±4	sig
ΣPUFA	44±1	44±3	44±10	38±8	41±10	40±4	52±7	50±3	sig
ΣC_{16}PUFA	0.98±0.03	1.03±0.75	0.75±0.31	0.60±0.20	1.81±1.59	1.35±0.35	2.77±2.10	0.67±0.35	sig
ΣC_{18}PUFA	2.5±0.3	2.6±0.3	2.7±0.2	3.1±0.5	3.5±1.0	3.0±0.6	3.9±0.7	3.7±0.7	sig
Σω3	42±1	41±3	41±10	35±8	38±10	38±3	48±8	48±3	sig
Σω6	4.9±0.2	6.1±1.6	5.5±0.3	5.4±0.6	5.3±0.7	5.7±0.8	7.0±1.5	5.9±0.3	sig

Univariate ANOVA tests were performed to analyze differences between groups. When ANOVA assumptions were not met a *p*-randomization was conducted 1,000 times to test the data empirically. A similar approach was used for 95% confidence intervals for variables with residuals not meeting ANOVA assumptions. ΣSFA, ΣMUFA, and ΣPUFA: Sum of saturated, monounsaturated, and polyunsaturated fatty acids, respectively. Minor fatty acids (<1%) such as 12:0, 19:0, 20:0, 21:0, 24:0, *ai*14:0, *i*15:0, *i*16:0 , *i*17:0, 16:1ω5, 20:1ω7, 20:1ω9, 20:2ω6, 16:3ω3, are not included in the table. Rounding of significant figures was based on the magnitude of the error. A further analysis revealed that the average contribution of the polar fraction to total lipids was: acetone-mobile polar lipids 20%, phospholipids 17%, and sterols 11%.

Table 2-6 Changes in fatty acid indices of the neutral and polar fraction of lipids of *Nyctiphanes simplex* at different life stages, Mean ± 95% C.I.

FAME index Neutral	Juvenile n=2	JuvFem n=3	ASFem n=3	OvFem n=6	SpFem n=4	JuvMal n=4	ASMal n=3	SpMal n=4	
SFA/PUFA	1.18±0.24	1.90±0.29	1.74±0.82	1.09±0.04	1.55±0.53	3.03±0.96	1.71±0.53	1.18±0.30	sig
SFA/LC-PUFA	1.27±0.29	2.16±0.37	1.85±0.90	1.16±0.05	1.69±0.62	3.94±1.42	1.99±0.69	1.30±0.34	sig
ω3/ω6	8.8±1.6	4.4±1.7	5.3±1.2	6.3±0.4	5.9±0.5	2.7±1.4	5.1±1.6	6.0±1.4	sig
16:1ω7/16:0	0.054±0.003	0.045±0.009	0.147±0.112	0.098±0.011	0.132±0.034	0.032±0.010	0.046±0.012	0.085±0.025	sig
18:1ω7/18:1ω9	0.45±0.02	0.25±0.15	0.50±0.22	0.38±0.05	0.45±0.08	0.29±0.08	0.35±0.10	0.32±0.04	sig
EPA/DHA	0.73±0.01	0.65±0.13	1.14±0.32	0.57±0.02	0.85±0.13	0.67±0.05	0.84±0.12	0.67±0.07	sig
C16/C18 PUFA	0.2±0.1	0.4±0.3	0.5±0.3	0.2±0.1	1.1±0.9	0.5±0.3	0.9±0.3	0.3±0.1	sig
Σ Total (μg mg^{-1})	43±8	65±21	69±36	48±2	63±16	65±24	47±10	42±2	sig
% DW	4.3±0.8	6.5±2.1	6.9±3.6	4.8±0.3	6.3±1.6	6.5±2.4	4.7±1.0	4.2±0.3	sig
Σ Total (μg ind^{-1})	0.08±0.03	0.06±0.03	0.04±0.02	0.06±0.01	0.06±0.02	0.03±0.01	0.07±0.02	0.05±0.02	sig
Polar	**n=2**	**n=3**	**n=3**	**n=6**	**n=4**	**n=4**	**n=3**	**n=4**	
SFA/PUFA	0.8±0.1	0.9±0.1	1.0±0.5	1.9±1.1	1.0±0.4	1.1±0.2	0.5±0.1	0.6±0.1	sig
SFA/LC-PUFA	0.9±0.1	0.9±0.1	1.1±0.5	2.0±1.2	1.0±0.4	1.2±0.2	0.6±0.1	0.7±0.1	sig
ω3/ω6	9±1	7±2	7±2	6±1	7±1	7±1	7±3	8±1	sig
16:1ω7/16:0	0.057±0.016	0.060±0.018	0.049±0.016	0.050±0.016	0.091±0.023	0.050±0.008	0.105±0.002	0.083±0.008	sig
18:1ω7/18:1ω9	0.17±0.03	0.21±0.02	0.21±0.06	0.17±0.02	0.19±0.01	0.22±0.01	0.18±0.03	0.19±0.03	sig
EPA/DHA	0.50±0.03	0.44±0.02	0.51±0.11	0.45±0.03	0.46±0.03	0.45±0.03	0.37±0.04	0.43±0.01	sig
C16/C18 PUFA	0.39±0.03	0.43±0.31	0.27±0.09	0.21±0.09	0.72±0.67	0.43±0.05	0.63±0.44	0.22±0.13	sig
Σ Total (μg mg^{-1})	37±17	28±5	37±15	20±5	37±6	31±5	29±1	37±15	sig
% DW	3.7±1.7	2.8±0.5	3.7±1.5	2.0±0.4	3.7±0.6	3.1±0.5	2.9±0.1	3.9±1.7	sig
Σ Total (μg ind^{-1})	0.075±0.047	0.019±0.003	0.025±0.012	0.023±0.005	0.038±0.006	0.014±0.001	0.042±0.007	0.036±0.013	sig

Univariate ANOVA tests were performed to analyze differences between groups. When ANOVA assumptions were not met a *p*-randomization was conducted 1,000 times to test the data empirically. A similar approach was used for 95% confidence intervals for variables with residuals not meeting ANOVA assumptions. SFA, PUFA and LC-PUFA: Sum of saturated, monounsaturated, polyunsaturated and highly polyunsaturated fatty acids, respectively. EPA/DHA: average ratio of 20:5ω3 over 22:6ω3.

Table 2-7 Comparisons of mean growth production (Pg) [mg m^{-2} $year^{-1}$ or mg m^{-3} $year^{-1}$], mean annual biomass (B) [mg m^{-2} $year^{-1}$ or mg m^{-3} $year^{-1}$], and P:B ratios of *N. simplex* estimated from three different allometric relationships using data from i) A study with 99.4% larval abundance*, and ii) A study with 27.2–85.5% larval abundance.**

Allometric relationship $DW = \gamma\ TL^{\beta}$	**A**	**B**	**C**		**D**		**E**
γ constant	0.0054	0.0022	0.0025		0.0012	0.0007	0.0006
			Females	Males	Females	Males	
β coefficient	2.3	2.9	2.6	2.8	3.0	3.3	3.3
*i) Higher contribution of larvae**							
Productivity (mg m^{-3} y^{-1})	198.2	**200.1**	180.3		139.1		117.0
Biomass (mg $m^{-3}y^{-1}$)	5.9	**5.2**	4.9		3.5		2.9
P:B (y^{-1})	33.48	**38.16**	37.05		40.18		40.82
*ii) Moderate contribution of larvae***							
Productivity (mg m^{-2} y^{-1})	**273.4**	407.4	330.2		367.3		348.3
Biomass (mg m^{-2} y^{-1})	**39.1**	61.4	49.3		56.0		53.4
P:B (y^{-1})	**6.99**	6.64	6.70		6.55		6.53

In both data sets, production and biomass were calculated using the estimated values of allometric coefficients γ and β for the weight-length regression obtained by: A) Gómez-Gutiérrez *et al.* (1996) using a $\log_{10}$-$\log_{10}$ transformation; B) Lavaniegos-Espejo, 1995 using a $\log_{10}$-$\log_{10}$ transformation; C) Present study using a $\log_{10}$-$\log_{10}$ transformation; D) Present study using non-linear curve fitting, and E) Present study using non-linear curve fitting and assuming lack of sexual dimorphism.

When coefficients differed by sex, a proportion of males: females (1:1) was assumed, and productivity and biomass were computed separately for each sex before totaling.

*Estimates from data in De Silva-Dávila & Palomares-García (1988). Bold numbers represent original estimates.

**Estimates from data in Gómez-Gutiérrez *et al.* (1996). Bold numbers represent original estimates.

Table 2-8 Effect of population structure on energy content (kJ mg^{-1}) of a swarm *of N. simplex* with 1000 individuals

% Mature Males-Females	Female: Male ratio	Proportion of Immature Females (J_f+AS_f)	Proportion of Immature Males (J_m+AS_m)	Proportion of Mature Females (O_f+S_f)	Proportion of Mature Males (S_m)	Energy (kJ mg^{-1})	Energy Ratio
4–4	1:1	0.48	0.48	0.02	0.02	15.722	1
4–4	2:1	0.64	0.32	0.03	0.01	18.130	1.15
72–43	2:1	0.38	0.09	0.29	0.24	26.359	1.68
72–43	1:1	0.29	0.14	0.21	0.36	27.197	1.73

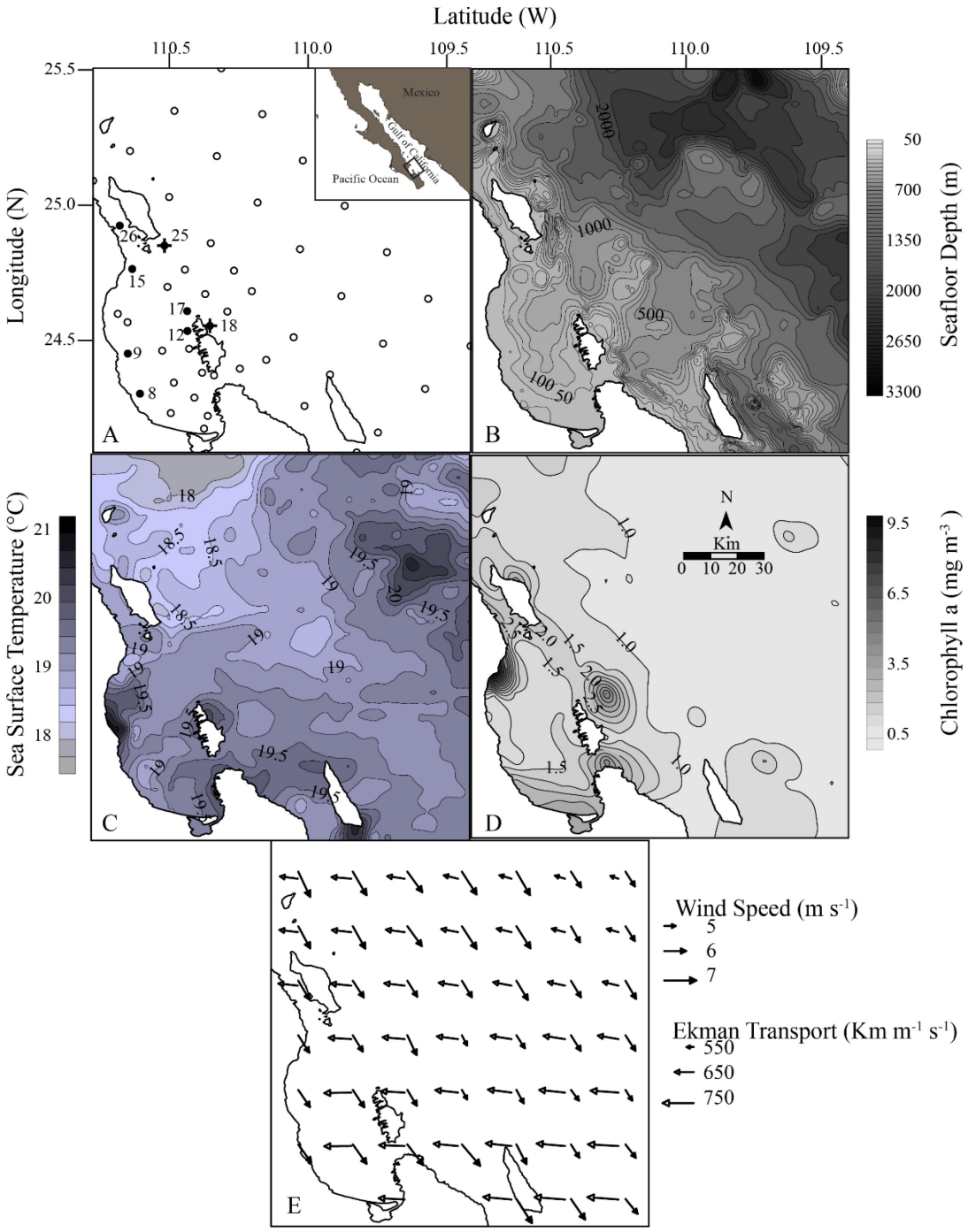


Figure 2-1 Study area in the Gulf of California, Mexico: A) Oceanographic stations (open circles), stations with either juveniles or adult stages of krill in February 2002 (filled circles) and stations with higher abundance of *Nyctiphanes simplex* (plus signs). B) Bathymetry of the area (m). C) Sea surface temperature (°C) in February 2002. D) Chlorophyll a (mg m^{-3}). Wind speed (m s^{-1}) from the North-north east, and the calculated westward Ekman transport (kg m^{-1} s^{-1}). Data source: GEO-IDE (https://geo-ide.noaa.gov/access-las.php).

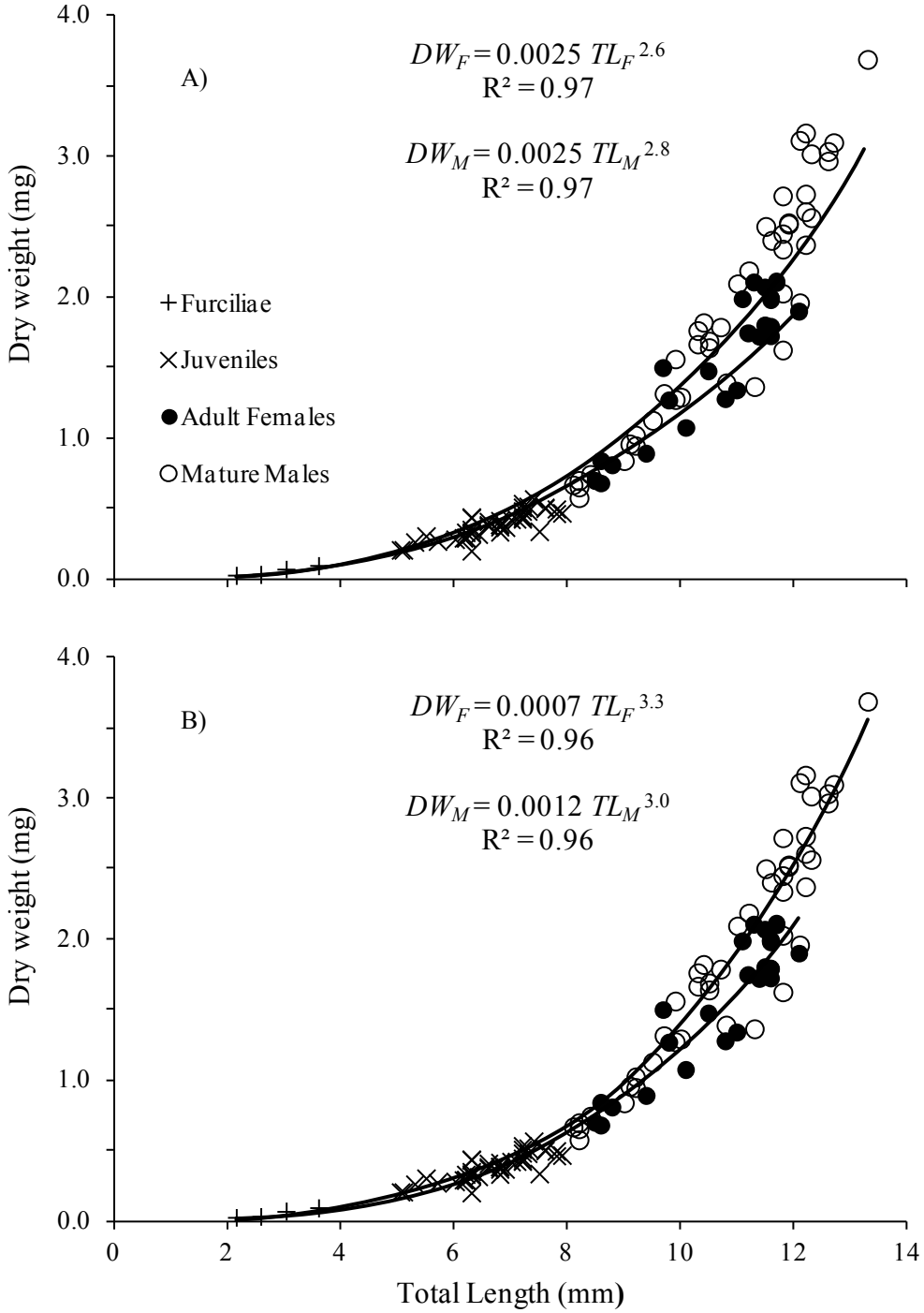


Figure 2-2 Increase in dry weight of *Nyctiphanes simplex* as a power function of total length during February 2002. A) Using log10-log10 transformation, and B) Using a non-linear fit.

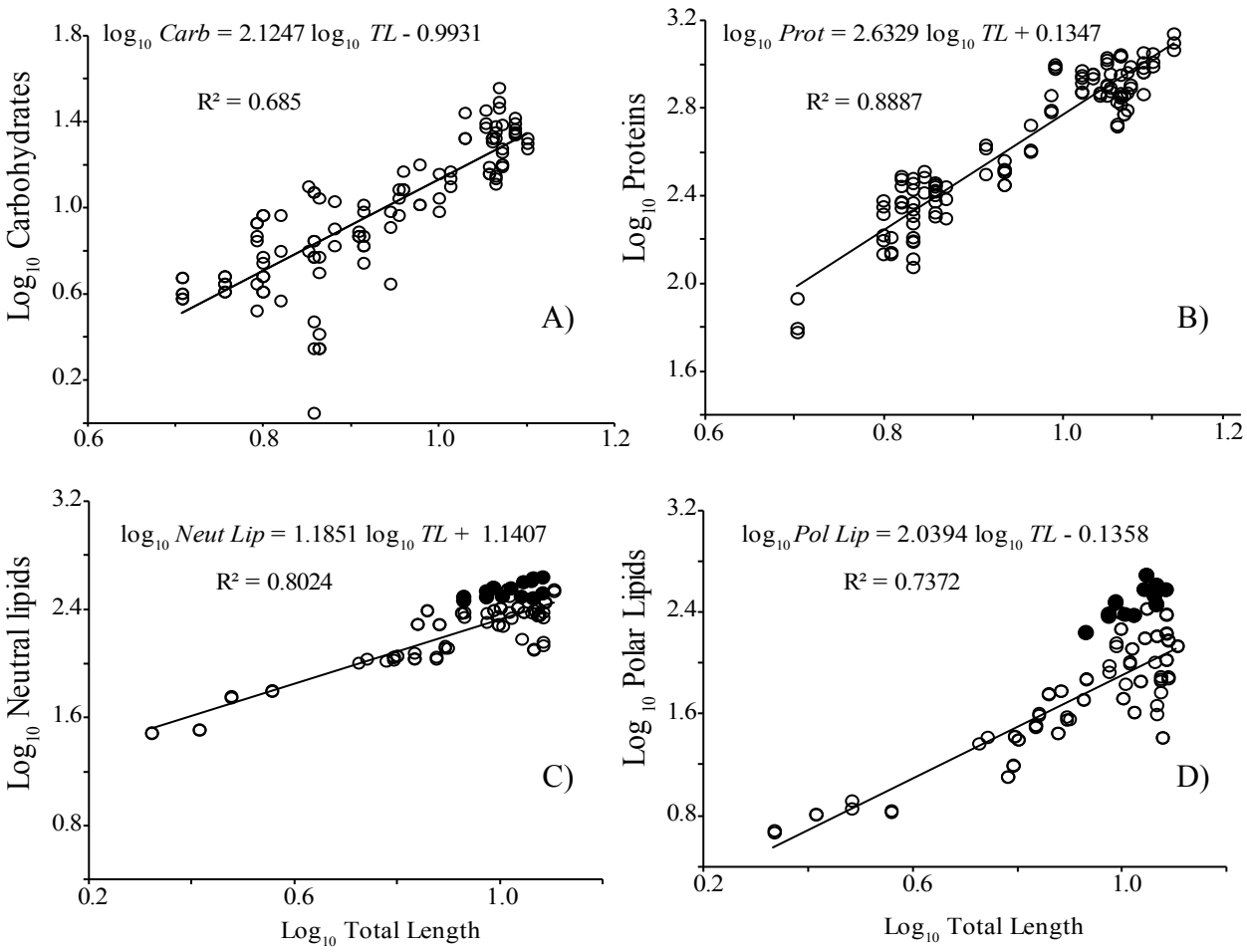


Figure 2-3 *Nyctiphanes simplex*. Allometric increase of biochemical fractions as function of *TL*: A) Carbohydrates; B) Proteins; C) Neutral Lipids and D) Polar lipids. Open circles: Log-log regressions using juvenile, adult-sized individuals, and adults without eggs; filled circles: lipid content of females carrying eggs in ovisacs.

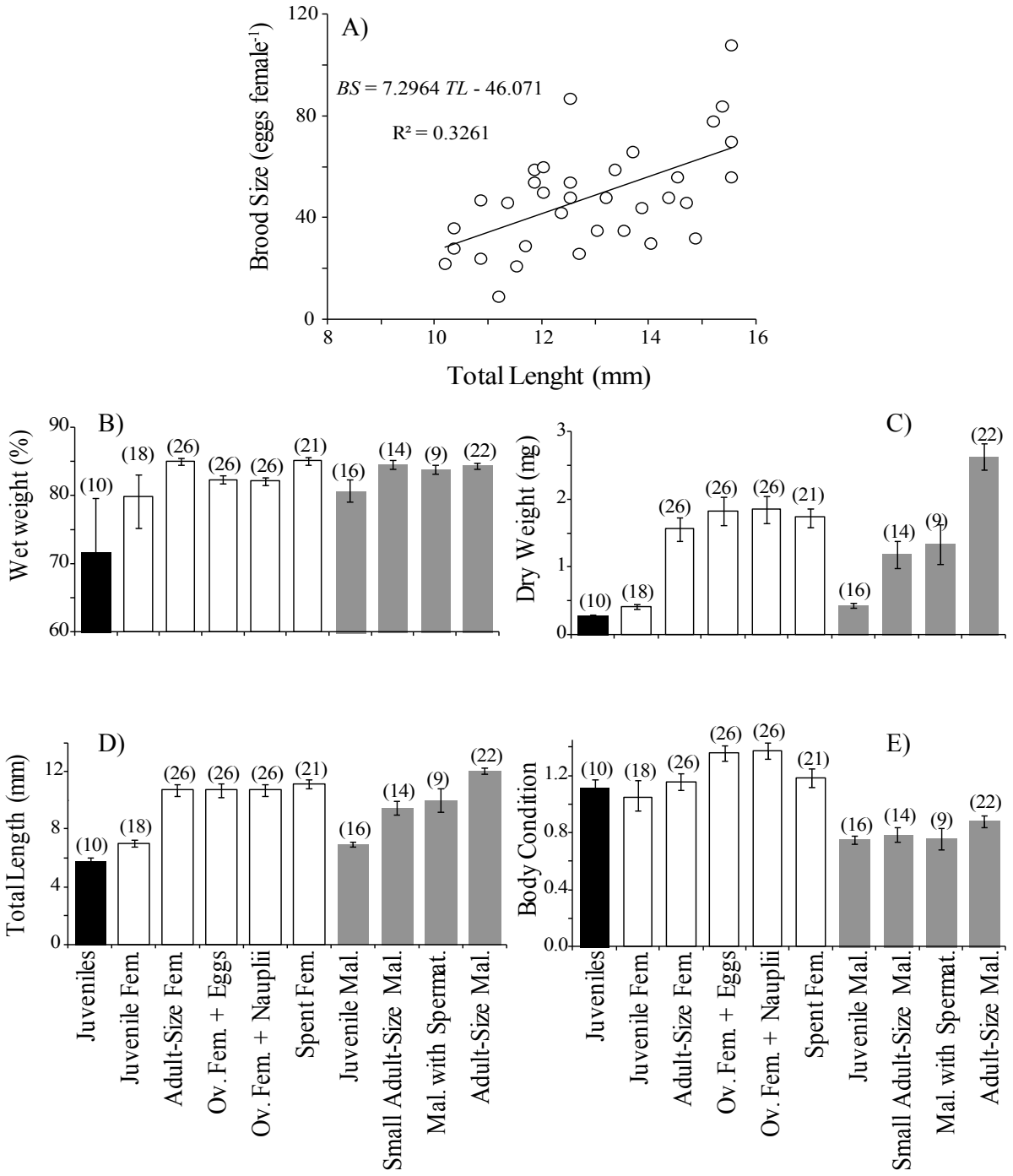


Figure 2-4 *Nyctiphanes simplex*: A) Adjusted *BS-TL* equation for ovigerous females (January 2007) modified from Gómez-Gutiérrez *et al.* (2012) , B) Wet weight (%); C) Dry weight (mg ind^{-1}); D) Total length (mm) & E) Body condition of krill (February 2002). Black bars: undifferentiated juveniles, white bars: female stages; gray bars: male stages; error bars:±95% confidence interval of the mean; numbers in parenthesis: sample size.

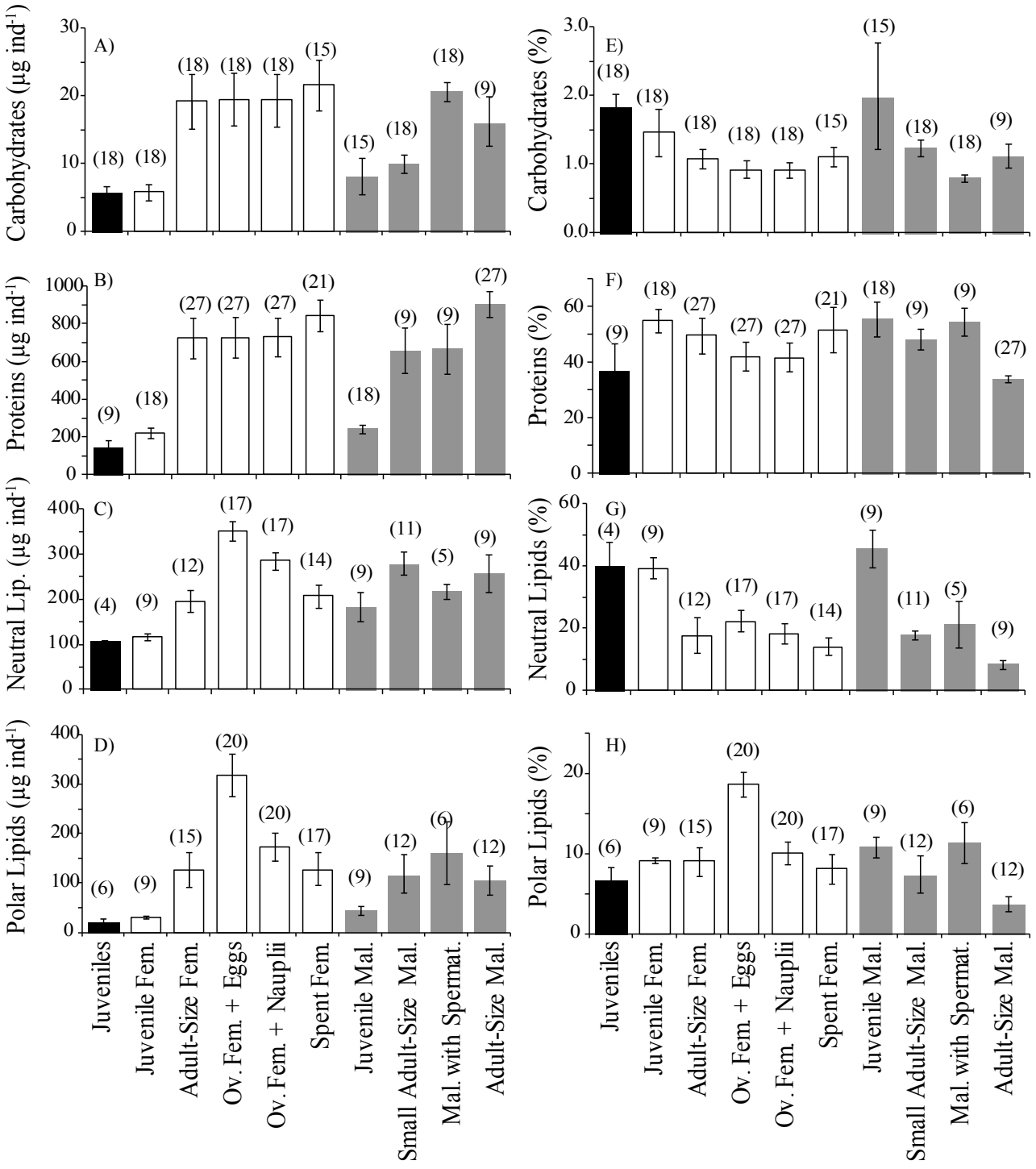


Figure 2-5 *Nyctiphanes simplex*: Main biochemical fractions as mg ind^{-1} or as percentages: A) and E) Carbohydrates, B) and F) Protein, C) and G) Neutral lipids; D) and H) Polar lipids. Black bars: undifferentiated juveniles, white bars: female stages; gray bars: male stages; error bars:±95% confidence interval of the mean; numbers in parenthesis: sample size.

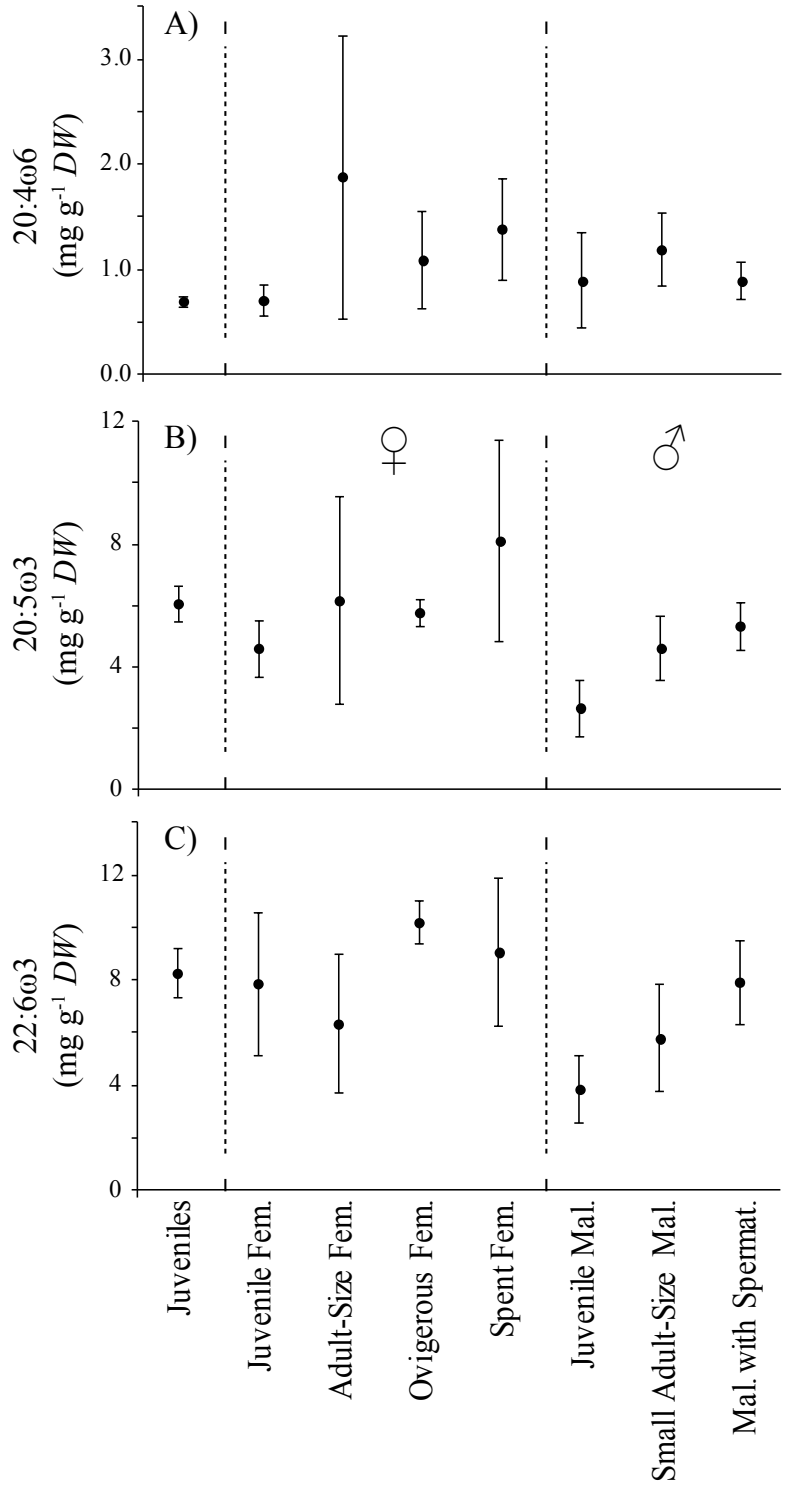
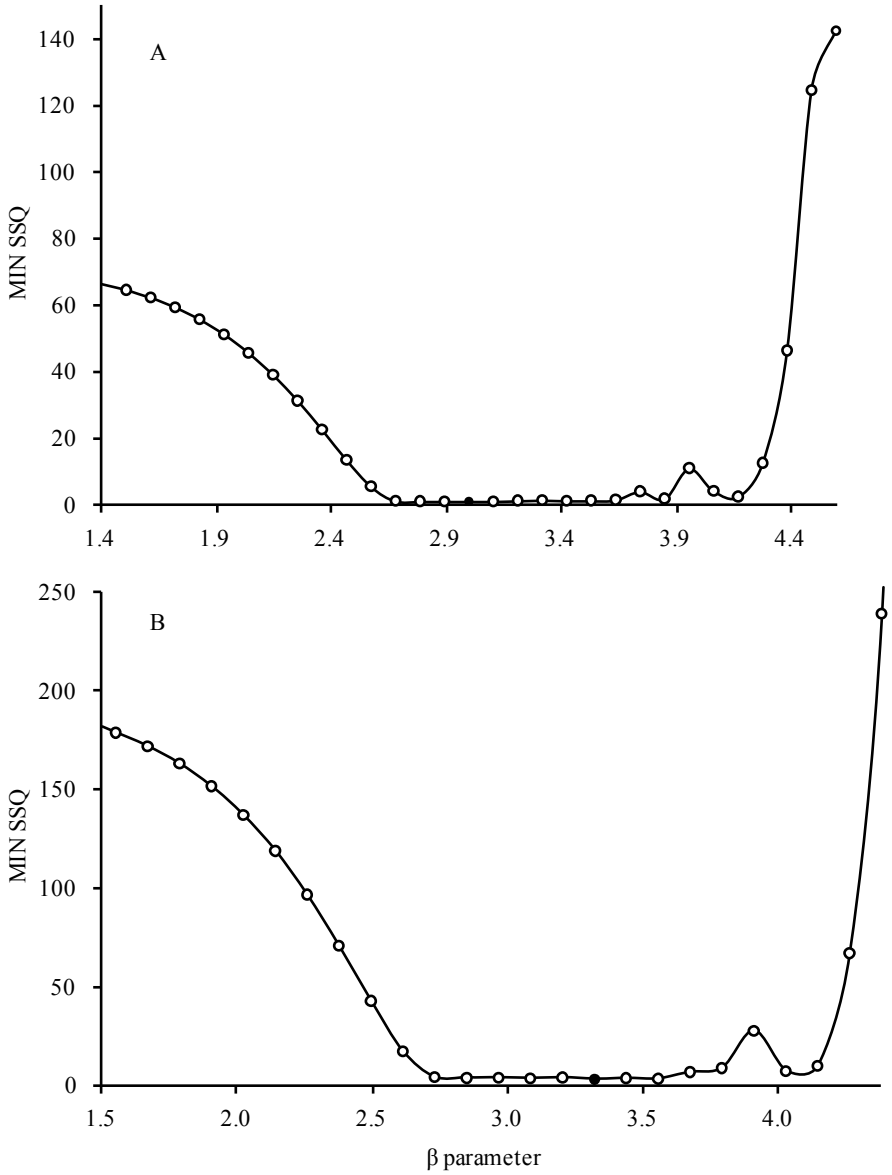


Figure 2-6 *Nyctiphanes simplex*: Average content (mg g^{-1} *DW*) of essential fatty acids in neutral lipids of different life stages: A) 20:4ω6 B) 20:5ω3 C) 22:6ω3. Left panel: Undifferentiated juveniles; central panel: females; right panel: males. Error bars: ±95% confidence interval of the mean.

Appendix 2-1 Graphs of the likelihood limits for the estimates of the parameter β as referred in equations 3 and 5.



For the non-linear curve fitting, values of the β parameter are plotted against the minimum sum of least squares (SSQ) for A) Females and B) Males. Darker dots represent the β value where the lowest SSQ was observed. In both cases, likelihood limits on the exponent are wider (2.67 to 4.16 for females and 2.73 to 4.14 for males) than the confidence limits (2.75 to 3.24 for females and 3.06 to 3.58 for males). This means that when assuming a normal error, the evidence supports a degree of uncertainty that exceeds that of the uncertainty in an inference from the sample to a population. In other words, the evidence supports the estimated confidence limits for β being perhaps unduly narrow given some heterogeneity in the residuals. Further research is recommended to identify a better error model (perhaps a gamma error with power law link) for estimating the power law relationship.

Chapter 3. **Subtropical krill experience short fasting periods and variable food quality in response to hydrographic conditions: Evidence from morphometric and fatty acid indices**

3.1. Abstract

In subtropical marine ecosystems, irradiance conditions and continuous food supply promote reproduction throughout the year in dominant krill species (Crustacea: Euphausiacea). Unlike temperate and polar krill species, it is assumed that long-term lipid accumulation is not required for survival. However, coastal subtropical regions exhibit intermittent wind-driven or tide-driven production throughout the year. Therefore, most of the energy uptake and feeding by krill depends on local episodic events. To investigate the effect of those events in the Gulf of California, temporal and spatial scales of trophic events were inferred using morphometric and biochemical indices. We hypothesized that short-term fasting (≤ 5 days) decreases lipid content and body condition of subtropical *Nyctiphanes simplex*, and tested its occurrence in the Gulf of California during spring (March, 2010). In fasting experiments, lipid and fatty acid concentrations, hepatosomatic indices, length-corrected dry-mass, and stomach fullness correlated negatively with fasting time. However, estimated fasting periods for wild krill were < 2.5 days, regardless of prevailing hydrographic conditions. After controlling for effects of sex and reproductive condition on fatty acid profiles of wild krill, multivariate and univariate methods identified high proportions of diatom biomarkers (20:5ω3, 18:1ω7, C_{16} PUFA) along the east coast associated with cooler upwelling conditions. Krill from the southernmost and warmest stations relied on both heterotrophic and autotrophic prey, as suggested by higher ratios of zooplankton (18:1ω9/18:1ω7) and dinoflagellate indices (22:6ω3/20:5ω3) in areas with high

chlorophyll-*a* concentrations, or high 18:3ω3 and 18:2ω6 originating in either cyanobacteria or Chlorophyceae. Development of basic criteria to detect areas with limiting food and the identification of temporal and spatial scales of trophic events highlights the influence of dynamic mesoscale processes on the feeding ecology of this subtropical krill in the Gulf of California. We conclude that while *N. simplex* is highly sensitive to short-term fasting, spring hydrographic conditions in the gulf ensure a sufficient and diverse food supply.

3.2. Introduction

In tropical and subtropical marine ecosystems, primary productivity is not irradiance-limited, and reproduction for most krill species (Crustacea: Euphausiacea), occurs throughout the year (Lavaniegos, 1995; Gómez-Gutiérrez *et al*., 1996; Ambriz-Arreola *et al*., 2012). Unlike in temperate and polar ecosystems, it is assumed that continuous food availability negates the physiological need for long-term lipid accumulation. However, coastal tropical and subtropical regions exhibit distinct wind-driven or tide-driven intermittent production at coastal divergences throughout the year (Longhurst, 1995). Therefore, most of the spatial and temporal variability of food for zooplankton is controlled by local primary production, together with advection processes and plankton and nekton consumption. In the Gulf of California, primary production increases during spring upwelling pulses, which occur when coastal winds promote a shallow nutricline (Lluch-Cota, 2000; Hidalgo-González & Alvarez-Borrego, 2004). As food supply to krill depends on local episodic events that further influence secondary productivity rates (Gómez-Gutiérrez *et al*., 2012), the scale of those events and the effects on food quality, energy uptake and feeding by krill need to be investigated.

The Gulf of California is a highly dynamic ecosystem, where currents, water column properties and biota exhibit strong spatial and temporal mesoscale changes (Marinone, 2012).

Here, *Nyctiphanes simplex* periodically reaches elevated densities (Boden *et al*., 1955; Brinton, 1979), and represents the most abundant coastal euphausiid along the eastern and western coasts of the Baja California Peninsula (Brinton & Townsend, 1980; Lavaniegos, 1994; Ambriz-Arreola *et al*., 2017).

Omnivory may account, at least partially, for the ecological success of *N. simplex* in the subtropics if like *Nyctiphanes australis*, it broadens its food spectrum as it grows (Ritz *et al*., 1990). *N. simplex* larval stages seem to grow better when 98–100% of the carbon content in their diet comes from microalgae (Lavaniegos, 1992), whereas adult stomach content includes microalgae (phytodetritus > 80%), and a wide variety of animal items (crustaceans > 9%, radiolarians 1%, and tintinnids > 1%) (Kanaeva & Pavlov, 1976). However, it is not known if the availability of food throughout the Gulf of California satisfies the physiological requirements for survival. Here, morphometric indices can help infer the physical condition of krill and whether the food is sufficient for survival.

The ratio between eye diameter and total length, as well as the hepatosomatic index (the ratio between hepatopancreas length and total length), and stomach fullness have been useful to infer health condition and recent feeding condition for polar, temperate and tropical euphausiids (Sun *et al*., 1995; Auerswald *et al*., 2009; Ambriz-Arreola *et al*., 2012, Riquelme-Bugeño *et al*., 2016a; 2016b). In euphausiids, eye diameter increases with age, but the total body length increases or shrinks from one molt to another depending on the amount and quality of diet (Sun *et al*., 1995). Accordingly, a large ratio between eye diameter and total length can be indicative of poor feeding conditions, whereas a large hepatosomatic index will reflect good feeding conditions. Finally, as food passes the stomach quickly (a few hours depending on species), a high percentage of stomach fullness will indicate a recent feeding event.

The objective of the present study was to evaluate the time scales at which these indices operate, and once validated, to assess the use of morphometric indices and fatty acid biomarkers to infer differences in food sources and diet quality for *N. simplex* in different hydrographic conditions in the Gulf of California during spring (March 2010). The underlying hypothesis was that hydrographic conditions leading towards upwelling conditions will be accompanied by more food resources with higher quality than those observed in other areas of the gulf.

3.3. Methods

3.3.1. Krill sampling and morphometrics

Zooplankton were collected at 9 of 36 CTD stations from the R/V 'El Puma' in an area of 76,460 km^2 in the northern and central regions of the Gulf of California during March, 2010 (Figure 3-1). Most of the epipelagic habitat of *Nyctiphanes simplex* occurs in the first 200-m depth (Tremblay *et al*., 2010; Ambriz-Arreola *et al.*, 2017), and so when possible, the maximum depth of CTD casts was 200-m depth. *In situ* water samples (Niskin bottles) for chlorophyll-*a* (mg m^{-3}) analysis (spectrophotometer) together with fluorescence data (WET Labs ECO-AFL/FL mg m^{-3}) were collected at different depths (5–100 m) and locations. A linear regression was used to estimate chlorophyll-*a* concentration across the study area. Opportunistic sampling of krill swarms occurred at night when a scientific echo sounder (Simrad EY-60, 120 kHz frequency) indicated a dense sound scattering layer at < 50 m depth. To avoid krill damage during collection, a 1 m diameter, 5 m long, and 300 μm mesh net equipped with an underwater lamp (Ikelite Pro-Video Lite II system, 50W) was used to attract zooplankton (instead of oblique tows). The net had a closed PVC cod end (0.215-m diameter and 0.70-m length, General Oceanics), and when deployed, the ship was allowed to drift for 30 minutes.

Animals with any indication of necropsy or physical damage were discarded and only intact animals were considered for analyses. Morphometric information and stomach condition was recorded on board for all live specimens: 1) hepatosomatic index (*HSI*), defined as the ratio between the long axis of the hepatopancreas (mm) and the total length (*TL*), measured from the forward rim of the carapace to the distal end of the telson (mm) (Ambriz-Arreola *et al*., 2012); 2) eye diameter index, defined as the ratio between eye diameter (mm) and *TL* (Sun *et al*., 1995); and 3) stomach fullness, estimated as the relative amount of colored material observed inside the stomach. This estimation was always performed by the same observer aided by a scale mounted in one of the eye pieces. After measurement, subsamples used for fatty acid analysis were rapidly rinsed with distilled water, briefly dried and placed in sealed cryogenic vials before being frozen in liquid nitrogen (-196°C) to avoid lipid hydrolysis and fatty acid oxidation.

3.3.2. Fasting experiments

Recently captured krill specimens (n = 83) from station 34 (Latitude: 29°38.05' N, Longitude 112°38.28' W; 101 m water depth; 51.5 km north of Isla Tiburón) were used for the onboard fasting experiment. Single individuals were placed in clean, transparent 1-L bottles filled with filtered seawater (0.7 µm-pore GF/F filter) to remove any potential food. Once in bottles, krill were incubated up to 140 h in an onboard dark cold-room at 16±0.5°C. Animals were observed every 12 h to detect molting or death. If moulted, the exuvia was removed to avoid ingestion. Batches of specimens were removed from bottles at different times to calculate morphometric indices (*HSI*, eye diameter index and stomach fullness) before being frozen in liquid nitrogen. A second set of animals was used to evaluate the effect of fasting time on lipid and fatty acid concentration (µg mg^{-1} of *DW*). Fasting time (0–118 h) was compared with values of morphometric indices using Pearson's correlations to identify responsiveness of morphometric

indices to fasting conditions. An ANOVA was used to assess the time at which significant differences first occurred between fasting and non-fasting krill. Effect of fasting time on lipids and fatty acids was studied for a longer period (0–137 h). Parameters for the effect of fasting time on morphometric indices, lipids and fatty acids were obtained from either logarithmic ($y = a + b \log_{10} x$) or linear regressions ($y = a + b\,x$) depending on best fit.

3.3.3. Weight, body condition and fatty acids

In the laboratory, krill specimens were freeze-dried (Virtis Lyophilizer 5L) for 24 h and weighed (*DW*) to the nearest 0.001 mg using a Cahn C-33 microbalance. To account for the possible variation in weight due to total length and sex, sexual size dimorphism was examined by ANCOVA fitting the equation between *DW* and *TL* to a power regression model ($DW = \gamma \cdot TL^{\beta}$) using $\log_{10}$-$\log_{10}$ transformations. Body condition (*BC*) was calculated as the ratio between observed dry weight (mg) and expected dry weight, which was computed from the measured *TL* (mm) using the power regression model.

Total lipids were extracted with chloroform:methanol:water 2:1:0.8 (Folch *et al*., 1957) using a modified protocol (Parrish, 1999), and a mixture of standards (dipalmitine, triestearine, cholesterol and lecithin) was run in parallel to account for losses during extraction. Lipid extracts were divided into two equal volumes and dried under a nitrogen stream. In one replicate, lipids were determined following a charring method (Marsh & Weinstein, 1966). The second replicate of the extract was methylated at 85°C for 2.5 h with hydrochloric acid and methanol 5:95 (Sato & Murata, 1988). Fatty acid methyl esters (*FAME*) were recovered in hexane before analysis in an Agilent GCD1800B gas chromatograph with a mass spectrometer detector (GC/MS), on a 30 m × 0.25 mm × 0.25 µm Omegawax 250 column (Supelco).

Fatty acids were identified by interpretation of mass spectra (Wsearch 32 software V.1.6 2005, Australia) and by comparison with retention times of commercial standards of 37 *FAME* commonly found in food products, including marine organisms (Supelco 47885-U). When fatty acid isomers were found, retention times of at least one of the isomers in the commercial standards allowed double bond positioning of the other isomer. Analyses of fatty acid picolinyl esters (Destaillats & Angers, 2002) were used to differentiate between isomers when required. Differences between fatty acid detector responses were calculated by plotting five different concentrations of commercial standards of 37 FAMEs ranging from 20 to 100 µg mL^{-1} (X axis) against their peak areas (Y axis). Hexane was used as blank. Linear regression analysis of each plot yielded the response factor for each fatty acid.

Fatty acids were initially analyzed using their relative contribution (%) to the overall fatty acid profile. To control for the potential effect of sex and gonad development on the fatty acid profile, we used two approaches: 1) testing the interactive effects of sex and gonad condition on fatty acid biomarkers via two-way ANOVA and 2) using permutational multivariate ANOVA based on distances (PERMANOVA) followed by principal coordinate analysis (PCO) of the fatty acid profile including both males and females. In both cases, the percent fatty acid values were transformed using the centered log ratio transformation (division by the geometric mean of the sample followed by $\log_{10}$ transformation) before testing (Aitchison, 1986; Loseto *et al*., 2009). For a depiction of the main sources of variation among fatty acids and among environmental conditions, correlations between orthogonal axes and the fatty acids or the environmental variables were calculated (PC-ORD Software V.6, Gleneden Beach, Oregon, USA). Environmental variables included longitude, latitude, sea floor depth (m), distance to an eastern

coast (km), temperature (ºC), salinity (PSU), density (kg m^{-3}), dissolved oxygen concentration (ml L^{-1}), oxygen saturation (%), and chlorophyll-*a* concentration (mg m^{-3}).

3.4. Results

3.4.1. Allometric parameters

The ANCOVA test confirmed that the allometric parameters (γ and β) of the equation ($DW = \gamma \cdot TL^{\beta}$) estimated via $\log_{10}$-$\log_{10}$ transformations were significantly different between males and females ($F_{1, 480} = 6.77$ $p = 0.010$). For males, the equation was: $DW = 0.00145\ TL^{2.96}$ and the 95% confidence intervals were [$2.95 \leq \beta \leq 2.97$] and [$0.00143 \leq \gamma \leq 0.00147$], whereas for females the equation was: $DW = 0.00189\ TL^{2.77}$, and the 95% confidence intervals were [$2.76 \leq \beta \leq 2.78$] and [$0.00186 \leq \gamma \leq 0.00191$]. The allometric exponents were slightly higher than those previously observed for the same species on samples collected in February 2002 (2.8 for males and 2.6 for females) (Chapter 2, this thesis).

3.4.2. Onboard fasting experiment and morphometric indices

After fasting *Nyctiphanes simplex* for 0–118 h (~5 days), the eye-diameter index did not show a significant increase with fasting time, *i.e.* no significant shrinkage occurred during short-term fasting. In contrast, stomach fullness, hepatosomatic index (*HSI*), and body condition (*BC*) all had significant negative correlations with fasting time (Table 3-1) and showed significant differences between fasting and non-fasting krill. This implies that after 30 to 40 h of fasting, krill substantially decreased their stomach-fullness > 36% ($F_{4, 74} = 13.64$, $p < 0.001$), due to food assimilation and evacuation. In the same period, *HSI* decreased > 7% (Figure 3-2a), *i.e.* the size of the digestive gland (hepatopancreas) had a significant decrease relative to the total length of the animals ($F_{4, 82} = 33.22$, $p < 0.001$), suggesting a decrease in storage lipids. Finally, a

significant decrease ($F_{3, 44} = 6.26$, $p = 0.001$) in *BC* (> 9%) was observed only after 86 h fasting (Figure 3-2b), *i.e*. the observed weight decreased compared with the expected weight for animals of a given total length.

3.4.3. Effect of fasting on lipid and fatty acid content

Lipid and fatty acid concentrations decreased with fasting time (0–137h). The concentration of most fatty acids decreased > 80% from their original values after fasting for 124 h, indicating fast physiological responsiveness of subtropical krill to short-fasting events (Figure 3-2c). However, not all fatty acids decreased at similar rates. Fasting conditions produced two main patterns: the first pattern was a steady decrease in fatty acid concentration (~40% after 57 h fasting), that fitted well with a linear regression: [*Fatty Acid*] = a + b·*t* where [*Fatty Acid*] = concentration of fatty acid (μg mg^{-1} *DW*), and *t* = fasting time (h). This pattern was observed in most fatty acids (%), the sums of saturated (Σ SFA), highly unsaturated fatty acids (Σ LC-PUFA), the sum of all polyunsaturated fatty acids (Σ PUFA = dienoic fatty acids plus LC-PUFA), the sum of ω3 fatty acids (Σ ω3) and the sum of ω6 fatty acids (Σ ω6). The second pattern was a rapid decrease in fatty acid concentration (≥ 60% after 57 h followed by a moderate decrease afterwards), fitting well ($R^2 > 0.75$) with a logarithmic regression: [*Fatty Acid*] = a + b·ln(*t*). This pattern was observed for the 18:0, *i*16:0, fatty acids, the sum of branched fatty acids (Σ Branched), 16:1ω9, 18:2ω6, the sum of dienoic fatty acids (Σ Dienoic), 16:3ω4, 18:3ω3, 18:4ω3, 22:5ω6, the sum of C_{18} polyunsaturated fatty acids (Σ C_{18} PUFA), and the total lipid content (as μg mg^{-1} DW) (Figure 3-2c). Parameters for the corresponding equations describing changes in fatty acid concentration as a function of fasting time were also calculated (Appendix 3-1).

The effect of fasting on fatty acid ratios used as trophic markers was also evaluated during the 137 h fasting period. As a consequence of similar decreasing rates between fatty acids, no significant changes were observed in 16:1ω7/16:0 (diatom marker) and 22:6ω3/20:5ω3 (dinoflagellate marker). On the other hand, other ratios such as ω6/ω3, C_{16}/C_{18} PUFA (marine *vs* terrestrial), 16:1ω7/18:4ω3 (diatom *vs* dinoflagellate) and 18:1ω9/18:1ω7 (zooplankton *vs* phytoplankton), increased as a consequence of different decreasing rates between fatty acids. These results highlight the importance of corroborating non-fasting conditions (control) before using fatty acids as trophic markers in wild krill.

3.4.4. CTD casts and environmental conditions

Using a T-S diagram (Appendix 3-2a), two water masses were detected within the 200-m CTD casts during March 2010: the Gulf of California Water mass (GCW), with salinities > 35 ‰ and temperatures > 12°C (Torres-Orozco, 1993), dominated the upper basin of the gulf and extended southward only in the upper layers of the lower basin. The other water mass, the Subtropical Subsurface Water (StSsW) with salinities ranging from 34.7 to 35.0‰ and temperatures < 16°C, was limited to the lower basin with a northern boundary south of 28.2°N, coincident with the Midriff Archipelago region. The upper boundary of the StSsW was deeper (180 m) along the west coast than along the east coast (70 m), as expected during east upwelling conditions (Appendix 3-2b). Similarly, the upper limit of the oxygen minimum zone, defined here as the depth with oxygen concentration < 1.5 mL L^{-1} (Tremblay *et al*., 2010; Ambriz-Arreola *et al*., 2017), was only detectable south of 28°N in CTD casts (≤ 200 m), being deeper in the west (~170 m) than in the central region (~145 m) or in the east (~70 m).

During March, the northern and northeastern dominant wind speeds ranged between 1.5 and 4.5 m s^{-1} (NOAA Pacific Marine Environmental Laboratory; http://ferret.wrc.noaa.gov/Ferret/).

Regional wind forcing produced a theoretical westward Ekman transport ranging between 200 and 700 kg m^{-1} s^{-1} (Figure 3-3a). The widely distributed 26 kg m^{-3} isopycnal was deeper (> 100 m) in the southwest portion of the gulf than in the east (15 to 30 m), particularly in the area south of Tiburón Island, near 27°N, where it was detected at the surface, indicating upwelling conditions along the eastern region (Figure 3-3b). Thermocline depth, defined as the region in which temperature showed rapid decreases compared with the layers above or below (≥ 1°C in depth intervals < 10 m), was deeper in the southern region (70 m) than in the north (< 20 m), or in the east (20 to 30 m), particularly south of Tiburón Island (Figure 3-3c). In general, the highest concentrations of surface chlorophyll-*a* (4.0 to 9.8 mg m^{-3}) occurred in eastern regions of the gulf, whereas the central and most of the southwestern region had lower (< 1.5 mg m^{-3}) surface concentrations (Figure 3-3d). The high positive correlation between fluorescence data and chlorophyll-*a* concentration ($R^2 = 0.743$, $F_{1,85} = 245.57$, $p < 0.001$ intercept=0.39 ± 0.14, slope=0.65 ± 0.08) was useful to infer higher chlorophyll-*a* concentrations (>1 mg m^{-3}) at subsurface (0–40 m) in the southwestern region.

3.4.5. Distribution of krill swarms

Krill swarms only occurred in a total of nine out of 34 sampling stations. The main hydrographic features of sampling stations are summarized in Table 3-2. Krill swarms at eastern stations were located close to mainland (< 36 km), where seafloor depths were 91–140 m, and bordering an area of high surface chlorophyll-*a* concentration (> 4 mg m^{-3}) (Figure 3-3d). In the northern basin (Stations S06 and S34), high surface chlorophyll-*a* concentrations (1.79–3.63 mg m^{-3}) decreased considerably (47–68%) at 40-m depth, and a shallow thermocline (> 10 m) suggests a shallow mixing layer (Figure 3-3c & d). In the southern basin (Station S47) local surface chlorophyll was lower (0.97 mg m^{-3}), but the mean chlorophyll-*a* concentration in the

upper 40 m was among the highest values for krill-sampled stations (1.28 mg m^{-3}). Here, upwelling conditions were manifested by low salinity (< 35.3‰), the lowest temperature (15.4°C), the highest density (> 26 kg m^{-3}), and low oxygen saturation (~59%) in the upper 40 m (Table 3-2).

Krill-sampled stations in the middle and along the west coast of the gulf had larger seafloor depths (372 to 491 m). Upwelling conditions were no longer evident in the central (Stations S36, S02) and west sections of the northern basin (Stations S12, S15) far from the east coast. Temperature, salinity and density were near overall means (Table 3-2). Chlorophyll-*a* concentrations were also near the overall mean for central stations (1.08–1.30 mg m^{-3}). The lowest chlorophyll-*a* concentration at surface (0.62 mg m^{-3}) occurred west to Angel de la Guarda Island (Station S12). Station 15 was the only station in the northern basin with a mean chlorophyll concentration > 0.9 mg m^{-3} for the upper 40 m.

In contrast, the southernmost locations along the west coast of the gulf (S57 and S80) were the only ones where the upper boundary of the StSsW was detected (at 90 and 130 m depth) (Appendix 3-2b). These stations were, on average, the warmest (> 17.7°C), with the lowest density (< 25.6 kg m^{-3}), and with the deepest thermocline (50 to 70 m) of all stations sampled (Figure 3-3c). Surface chlorophyll-*a* concentrations for both stations were near the overall mean (1.31 to 1.5 mg m^{-3}). However, the mean concentration of chlorophyll-*a* at station 80 (2.07 mg m^{-3}) and the oxygen saturation (≥ 80%; > 94% in the upper 10 m) were considerably higher for the upper 40 m (Table 3-2), suggesting elevated near-surface phytoplankton biomass.

3.4.6. Inferring feeding conditions in the field

All *N. simplex* specimens were adults (> 8 mm total length), but population structure (male–female ratio, and proportion of specimens at different gonadal stages) was variable among krill-

sampled stations. In general, stations with high proportions (> 48%) of females at resting gonadal stage (previtellogenic), had low average dry weights (≤ 3.5 mg ind^{-1}), and small total lengths (≤ 13.6 mm) (Table 3-2).

Four indices were selected to detect the occurrence of short-term fasting events in wild specimens of *N. simplex*: stomach fullness, *HSI*, *BC* and total lipid concentration. The 95% confidence limits for the mean values of those indices at different fasting times were used as a threshold to indicate unfavourable feeding conditions. From fasting experiments it was documented that krill fasted >40 h had stomach fullness <40%; krill fasted for 30–86 h had an *HSI* of 0.100–0.126, whereas *HSI* values < 0.100 were only observed for krill fasted 118 h. Similarly, lipid concentrations < 21.8 μg mg^{-1} were only observed in krill fasted > 57 h, and *BC* < 0.938 was only observed for krill fasting ≥ 86 h (Figure 3-2 and Section 3.4.2). Such differential responses allowed precision in fasting-time estimation at each station.

Significant differences in hepatosomatic index were observed among stations ($F_{7, 101} = 14.51$, $p < 0.001$). Average *HSI* of krill collected at S06 and S80 was higher than *HSI* of krill collected from other stations and > 0.126, indicating fasting times < 30 h (< 1.3 days). Average *HSI* for krill collected at 5 stations (Stations S02, S12, S15, S47, and S57) ranged between 0.100 and 0.126, indicating fasting times between 118 and 30 h respectively (Figure 3-4a). However, the average stomach fullness for specimens collected in most of those stations was consistently > 40% (Table 3-2), a high level of stomach fullness was only found in animals fasting < 40 h in fasting experiments. Therefore, the estimated range in fasting time for stations S02, S12, S15, S47, and S57 was 30–40 h (1.3–1.7 days). Krill collected at S36 had the lowest mean value for stomach fullness (< 40%), indicating fasting times > 40 h. However, all locations had average body conditions > 0.94 (Figure 3-4b), indicating krill collected here did not fast > 86

h (~3.5 days). Moreover, no location had lipid content below the threshold of 21.8 µg mg^{-1} *DW* (Figure 3-4c) decreasing the estimated range in fasting time for krill collected at station S36 to 40–57 h (1.7–2.4 days) before being captured.

3.4.7. Differences related to sex in fatty acid profile

Sex-location interactions were tested with a balanced design using krill samples with similar numbers of males and females (Stations S06, S47 & S57). A PERMANOVA main test (PRIMER+PERMANOVA V.6), with fatty acids > 0.5%, did not show significant interaction between sex and location (Pseudo-$F_{2,44}$ = 2.1356, $p_{(MC)}$ = 0.057). However, detailed pair-wise comparisons for the interaction term show significant differences in the fatty acid profile of males and females of station S47 (t_{20} = 2.8354, $p_{(MC)}$ = 0.0015). In contrast, no significant differences were observed for the fatty acid profile of males and females in station S06 (t_{15} = 1.5327, $p_{(MC)}$ = 0.0582), or station S57 (t_9 = 0.93965, $p_{(MC)}$ = 0.4355). Once sex differences were accounted for, same-sex pair-wise comparisons among krill collected at those three stations showed significant differences in their fatty acid profile (t > 3.0031, $p_{(MC)}$ < 0.0013 *d.f.*=14, 19 and 23 for pair-wise comparisons of females and *d.f.*=10, 10 and 12 for pair-wise comparisons of males collected at S06-S57, S47-S57, and S06-S57, respectively). These results highlight the importance of removing the effect of sex, when comparing fatty acid profiles, in order to detect differences among stations.

3.4.8. Geographical variability in *N. simplex* fatty acid profile

Three fatty acids comprised around 62% of all fatty acids detected for *N. simplex*: 22:6ω3 (23%), 16:0 (20%), and 20:5ω3 (19%). Other major fatty acids were 18:1ω9 (~7%), 14:0, 16:1ω7, and 18:1ω7 (~3% each). A SIMPER analysis (PRIMER V.6) showed high intra-station

similarity, ranging from 92.1% (Station S47) to 94.4% (Station S02). Moreover, average inter-station dissimilarity was considerably low (6.4 to 13.6%), suggesting a very stable fatty acid profile for *N. simplex* across this particular geographical range (Appendix 3-3 & Appendix 3-4).

During March 2010, sex differences in the fatty acid profile of *N. simplex* were larger than differences due to location. Data structure was tested with principal coordinates analysis (PCO) (Figure 3-5a). The first axis explained 47.9% of the total variation among samples. Here, two major groups (defined by 91.5% Bray-Curtis similarity via cluster analysis), were consistent with sex differences: most females were positioned at lower values of the main axis than their male counterparts from the same station. In contrast, the second coordinate explained 18.9% of the total variance, where subgroups (defined by 93.5% Bray-Curtis similarity via cluster analysis) were more related to oceanographic conditions: krill from warm stations (S57 & S80) with evidence of downwelling were positioned at lower values of the secondary axis, whereas those from colder areas (Station S47) with evidence of upwelling were located at higher values of the same axis (Figure 3-5a).

Pearson's correlations of major fatty acids (> 0.5%) with the first two principal coordinates of the PCO were useful to discriminate differences in fatty acid profiles due to sex or location. Animals with higher contents of 14:0, 16:2ω4, 16:3ω4, 16:4ω1, 18:4ω3 and phytanic acid were correlated (Pearson's correlations ≤ -0.7) with lower values of the main PCO axis, and therefore with most females (and males collected at S06). In contrast, animals with higher contents of 16:0, 17:0, 18:0, 18:1ω9, 18:2ω6, 20:1ω9, 20:4ω6, 22:5ω6, and 22:6ω3 were correlated (≥ 0.7) with the main PCO axis, indicating a higher content of those fatty acids in males. In terms of location, animals with higher proportions of 18:3ω3 and 18:4ω3, mostly from southwestern stations (S57 and S80), were negatively correlated (Pearson's correlations ≤ -0.7) with the

secondary PCO axis. Animals collected close to the upwelling plume south of Tiburón Island (Station S47), had a higher contribution of *i*16:0, 20:5ω3 and 21:5ω3 and a positive correlation (≥ 0.7) with PCO 2 (Figure 3-5b). The above results indicate that when samples are not separated by sex, differences in sex proportions can mask differences in the fatty acid profile of different locations.

3.5. Discussion

3.5.1. Onboard fasting experiment: indicators of unfavorable feeding conditions

Our results support the idea of sexual size dimorphism in *N. simplex* (where males tend to be heavier than same sized females). Accordingly, morphometric indices that are not affected by sex, like the hepatosomatic index, or indices that take into account sex differences, like body condition, become important in revealing fasting conditions. Fasting experiments were useful to set up thresholds for morphometric indices over unfavourable feeding conditions that might occur naturally in the Gulf of California. For instance, changes in the *HSI* have proven to be consistent with seasonal differences in food availability (upwelling *vs* non-upwelling conditions) for two tropical krill species (*Euphausia distinguenda* and *Euphausia lamelligera*) in the Mexican Tropical Eastern Pacific (Ambriz-Arreola *et al*., 2012). In the present study *HSI* not only had the highest Pearson's correlation with fasting time for the whole data set, but also was sensitive to short-term (< 5 days) changes in trophic condition, suggesting that, at least for subtropical krill, *HSI* is the most useful index to compare food availability at short time scales.

After fasting *N. simplex* for 118 h (5 days), there was no significant increase in the average eye-diameter index as evidence of body shrinkage after molting. Evidence indicates that *N. simplex* molted only once during the experiment, as estimated intermolt periods (*IMP*) for this species range between 2–9 days (Jerde & Lasker, 1966; Gómez-Gutiérrez *et al*., 2012), and the

estimated *IMP* for specimens collected at station 34, used in our fasting experiment, was 4.4 days. Shipboard incubation experiments indicate higher proportions of shrinking *N. simplex* in summer than in winter (Gómez-Gutiérrez *et al*., 2012). In the Antarctic krill, *E. superba*, a relatively high eye-diameter index is only observed after several moulting events under unfavourable winter conditions (Sun *et al*., 1995). Our result suggests that *N. simplex* also has to molt more than once in order to detect shrinkage via the eye-diameter index.

3.5.2. Onboard fasting experiment: Effect of fasting on lipid and fatty acid content

The fasting experiment indicates that subtropical *N. simplex* is not well adapted to withstand long-fasting periods, making it vulnerable to prolonged declines in primary productivity. After fasting for 57 h (2.4 days) at 16±0.5°C, the lipid content of *N. simplex* decreased from an average of ~67 μg mg^{-1} to ~21 μg mg^{-1}. This means that almost 70% of the lipid content was consumed within 3 days, producing a decrease of 4.6% *DW* via lipid consumption. In fact, when fasting > 10 days, *N. simplex* shows signs of body damage (decreased body transparency, contracted intestine, and arresting peristalsis) (Gómez-Gutiérrez, personal observations). In contrast, the Antarctic krill *E. superba*, a species well adapted to long periods of food limitation during winter, consumed < 30% of their lipids even after 18-days starvation (Auerswald *et al*., 2009), and fasting experiments have lasted between 18 and 44 days under cold sea-water conditions (Atkinson *et al*., 2002; Stübing *et al*., 2003; Auerswald *et al*., 2009; Pleuthner *et al*., 2016). The large changes in lipid content of *N. simplex* over a short period confirm that this subtropical species is adapted to a continuous food supply, and therefore is more sensitive to short-term temporal and geographical changes in food availability compared to temperate and polar species.

The fasting experiment also served to test the effect of fasting on relative proportions of fatty acids used as trophic markers. Most fatty acids exhibited significant decreases (40–60%) in concentrations after fasting for 57 h, and an even higher decrease (> 80%) after 124 h. Nonetheless, the relative ratios of some fatty acids commonly used as diatom (16:1ω7/16:0) and dinoflagellate markers (22:6ω3/20:5ω3) (Stevens *et al*., 2004), did not show significant differences during the 137-h fasting period, confirming that they are consistent trophic markers. Conversely, other ratios increased during fasting as consequence of differential fatty-acid decreasing rates (ω6/ω3, C_{16}/C_{18} PUFA, 16:1ω7/18:4ω3, and the zooplankton marker 18:1ω9/18:1ω7). These results highlight the need to evaluate overall feeding conditions before assessing and using fatty acid ratios as food biomarkers.

3.5.3. Inference of feeding conditions in the field

The observed geographical variability in fatty acid profiles of *N. simplex*, together with the inherent variation due to sex and gonad development, indicates that the environmental variability in the Gulf of California allows asynchronous gonad development (consistent with a species that reproduces throughout the year), and higher variation in body condition for this subtropical species compared with temperate and polar krill species. Fatty acid biomarkers suggest geographic differences in food quality and utilization even during a period with relatively high mean chlorophyll-*a* concentration.

Based on morphometric indices for wild krill collected at most stations, food-restricted conditions during spring were estimated at < 40 h (< 1.7 days). Only station S36 had longer estimated fasting times (1.7–2.4 days), which still can be considered short-term fasting. Additionally, fatty acid concentrations in wild males, including those from station 36, never reached values < 30 μg mg^{-1}. Both results suggest fasting times < 60 h (2.5 days) at all stations.

This means that the food consumed by this omnivorous krill species during spring was enough to fulfill its energy requirements between 1.7 to 2.5 days before collection.

Short fasting times for specimens collected in spring indicate favourable generalized feeding conditions. Still, evidence suggests differences in food quality: a parallel study with specimens collected the same month and year (Gómez-Gutiérrez *et al*., 2012) shows that specimens collected north of Tiburón Island had higher molting rates (35 to 50% after 48 h) and lower inter-molt periods (*IMP*) (< 6 days), a condition expected with better food quality. In the same study, krill at station 47 had a molting rate of 14% and an estimated *IMP* of 14.3 days, indicative of lower food quality. The lowest molting rate (< 4%) and the highest estimated *IMP* (> 50 days) was observed in warmer stations 57 and 80, suggesting that although food was available, quality was relatively poor for the requirements of *N. simplex*.

3.5.4. Differences related to sex on lipid and fatty acid profiles

The Antarctic krill, *Euphausia superba*, withstands long starvation periods during dark and cold winter months, which has been attributed to the comparatively large lipid reservoir. It has been suggested that in adult females the reservoir is large enough that it can buffer short-term diet-induced fatty acid variations (Stübing *et al*., 2003). We previously demonstrated that total fatty acid content in *N. simplex* postvitellogenic females is 1.2 to 2.2 times higher than in previtellogenic females of similar total length (Gómez-Gutiérrez *et al*., 2010), and in the present study, sex differences contributed largely to the variability in the fatty acid profile at specific sampling locations. Accordingly, a high proportion of postvitellogenic (gravid) females in any sampling station can obscure short-term diet differences. Additionally, *N. simplex* females are sac-spawners, carrying their eggs in ovisacs until they develop into metanauplii (Gómez-Gutiérrez & Robinson, 2005; Montuy-Gómez *et al*., 2012). Therefore, the embryo developmental

stage, brood size, and gonad development stage of females will likely account for most of the fatty acid variability in reproductive females.

3.5.5. Environmental conditions for wild krill

During March 2010 a shallow thermocline and a shallow 26 kg m^{-3} isopycnal, located south of the Midriff Archipelago and close to the east coast, revealed subsurface waters with low oxygen concentration rising up to the surface. Here, the estimated directions of the Ekman transport produced by wind stress were consistent with the observed upwelling and with the relatively high surface concentration of chlorophyll-*a* along the east coast. In the Gulf of California, cyclonic gyres can produce a shallow thermocline via upwelling, whereas anticyclonic gyres can produce a deeper thermocline via downwelling (Contreras-Catala *et al*., 2016). In fact, the continuous presence of cyclonic and anticyclonic gyres is particularly relevant to surface transport of plankton, nutrients and other drifting materials among different regions of the gulf (Lavín & Marinone, 2003; Marinone, 2012).

In contrast, the presence of a deep thermocline in the southwest region suggests an anticyclonic gyre, where introduction of nutrients to the area can trigger the growth of primary producers with higher tolerance of warm waters, promoting relatively high chlorophyll-*a* concentration at surface, and high dissolved oxygen percentage of saturation. A numerical hydrodynamic model in the Gulf of California indicates that in a typical March, the area south of the Midriff Archipelago continuously exports particles towards other areas in the gulf, whereas the Bahía Concepción region tends to import and retain particles from nearby areas (Marinone, 2012). The long and narrow shape of the Gulf of California, a semi-enclosed gulf, produces a coast-ocean-coast ecosystem that contributes to particle retention and to relatively fast transport of upwelling-generated nutrients from the east coast toward offshore waters.

Trophic habitats for *N. simplex* were defined geographically by the interaction among density, temperature, sea floor depth, percentage of oxygen saturation and distance to the closest coast on the windward east side. Morphometric and fatty acid analysis in *N. simplex* corroborate, at least partially, the proposed model: During March 2010, trophic habitats related on one hand to upwelling conditions bringing cold, low-oxygen water from the shallow-eastern portion of the gulf, and, on the other, to the subsequent transport of particles across the gulf into deeper and warmer waters, where active photosynthesis of eurythermal phytoplankton increases oxygen content.

3.5.6. Hydrographic conditions and food quality

As expected, krill distributed along the east coast (S06 and S47), close to the upwelling region, had higher proportions of diatom fatty acid biomarkers. Surface chlorophyll-*a* concentration in the northern section (Station S06) was very high and krill collected at this station had high concentration of C_{16} PUFA, an indicator of nutrient-replete diatoms as food sources (Parrish *et al*., 2009). Krill from this station also had high concentrations of 16:1ω7 and phytanic acid, suggesting the presence of phytodetritus as a food source. The occurrence of recently-produced phytoplankton together with phytodetritus, high *HSI*, and the largest fatty acid concentrations observed in this study, support the idea of continuous production in the area, and explain the relatively short *IMP* and high molting rates of *N. simplex*.

The dynamic conditions in the southern section of the upwelling region (Station S47) were different from the north, and restricted the retention of food for longer periods. Here, krill fed actively, as shown by stomach content, but the *HSI* and the total lipid concentration were lower than in the north. Additionally, the molting rates observed by Gómez-Gutiérrez *et al*. (2012) suggest below-optimal feeding conditions for this period and region. Upwelling areas are not

necessarily food-rich regions all the time; increases in phytoplankton biomass usually occur several days after nutrient inputs into the near-surface waters, and advection can export most of the phytoplankton bloom towards surrounding regions within days (Marinone, 2012).

During March 2010, food quality among stations within the gulf was large enough for subtropical *N. simplex* to survive and exhibit moderate to good feeding conditions even in moderately warm waters far from the upwelling plume. This feature seems specific to the Gulf of California and contrasts with the west coast of the Baja California peninsula where *N. simplex* is clearly associated with cold regional conditions, and population abundance decreases substantially from inshore to offshore and from spring to summer (Lavaniegos 1994, 1995; Gómez-Gutiérrez *et al*., 1996). In contrast, regional changes in abundance related to temperature are relatively less pronounced in the Gulf, and differences in secondary production seem strongly associated with circulation patterns and regional zooplankton connectivity (Gómez-Gutiérrez *et al*., 2012).

Stomach content evidence shows that *N. simplex* is an omnivorous species (Kanaeva & Pavlov, 1976), thus the ability to switch to different food sources can explain, at least partially, how this species copes with warm conditions in the gulf. Still, lipid content seemed dissociated from *HSI*, at least in the warm southwest region (S57 and S80), far from the upwelling plume. It is possible that *HSI* only reflects food availability whereas total lipid content is also associated with food quality. An alternative explanation is that total lipid content increases at lower rates than *HSI* after active feeding, then discrepancies between both signals might indicate previous fasting events, and only animals feeding continuously will exhibit both high *HSI* and high lipid content. Transitional conditions from non-feeding into active feeding have been used to explain similar discrepancies in *E. superba* at the onset of summer (Auerswald *et al*., 2009). In our study,

krill from the warm northern section (Station S57) had high lipid content, but lower *HSI* than those from the southern section (Station S80), indicating either prior favourable feeding conditions or better food quality. In contrast, krill from the warm southern section had large *HSI* indicating fasting times < 30 h, but with the lowest lipid content among all stations. Here, the presence of large krill with large dry weights suggests that most of the biomass consists of proteins, and a higher 18:1ω9/18:1ω7 ratio suggests higher zooplankton intake despite relatively high chlorophyll-*a* concentrations that show the presence of primary producers.

Compared with other regions, krill from the warm southwest region (S57 and S80) had significant higher proportions of 18:2ω6 and 18:3ω3, and low proportions of 20:5ω3. These fatty acids are ubiquitous in several microalgae groups, but marine green microalgae (Chlorophyceae), such as *Tetraselmis suecica*, exhibit relatively high proportions of 18:2ω6 (3–10%), and 18:3ω3 (13–15%), and lower proportions of 20:5ω3 (6–8%) than diatoms, and unlike dinoflagellates, Chlorophyceae have negligible proportions (0–0.53%) of 22:6ω3 (Rivero-Rodríguez *et al*., 2007; Fernández-Reiriz *et al*., 2011; Guedes *et al*., 2011). These two fatty acids are also among the three most abundant of several unicellular and filamentous blue-green algae. In fact, high proportions of 18:2ω6 (> 20%), and 18:3ω3 (25%) are common for several cyanobacteria, whereas 20:5ω3 (5.7%) and 22:6ω3 (0.3%) have been detected in only one out of 18 cyanobacteria species (Guedes *et al*., 2011).

Accordingly, either Chlorophyceae or cyanobacteria play an important trophic role in warmer regions, far from the influence of coastal upwelling events. Eurythermal adaptations have been reported for some cyanobacteria inside the Gulf of California (Díaz & Maske, 2000; Villalejo-Fuerte *et al*., 2005), although Chlorophyceae can also exist in warm waters. On an annual basis, secondary production of *N. simplex* in the Gulf of California has no correlation with chlorophyll-

a or fucoxantin concentrations (diatom-pigment proxy), whereas it has a strong positive correlation with alloxantin (a Cryptophyceae-pigment proxy), suggesting that this group can be a significant part of its diet (Gómez-Gutiérrez *et al*., 2012). Our biochemical analysis suggests that, depending on the hydrographic conditions, *N. simplex* can switch from diatoms into alternative food sources (detritus, copepods, and cyanobacteria or Chlorophyceae among others), either directly or indirectly, which can satisfy short-term requirements as shown by morphometric indices.

In the present study, I presented a complete suite of morphometric and fatty acid indices with high sensitivity to short-term fasting conditions (< 5 days) for *N. simplex*. Such indices proved useful to infer differences in food sources and diet quality for *N. simplex* in different hydrographic conditions during March 2010. Despite those differences, I concluded that spring hydrographic conditions allow *N. simplex* access to diverse food items in quantities sufficient to minimize fasting periods.

Future studies should evaluate subtropical and tropical habitats during summer conditions, when *N. simplex* population decreases, upwelling is considerably weaker than during winter, and circulation patterns differ (Tremblay *et al*., 2010; Gómez-Gutiérrez *et al*., 2012; Ambriz-Arreola *et al*., 2017). Such studies will provide better information on the timescale and ecological relevance of the associations between key zooplankton species like *N. simplex*, and their alternative food sources.

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Table 3-1 Pearson's correlations between fasting time (*t*) and the measured morphometric variables in *Nyctiphanes simplex*.

Morphometric variables	Pearson's correlation	p value	R^2 log (*t*)	Constant a	Slope b
Hepathosomatic index	-0.715	< 0.001	0.6006	0.1407	-0.0094
Body condition	-0.520	< 0.001	0.3028	1.0613	-0.0513
Stomach fullness	-0.493	< 0.001	0.3905	69.566	-11.826
Eye diameter index	0.072	0.518	-------	--------	--------

Log-regression parameters (*Morphometric Variable* = $a + b$ Log (t)) and R^2 were calculated only for significant correlations. Fasting time ranged from 0 to 118 h, $n = 83$.

Table 3-2 Average oceanographic conditions for the upper 40 m depth for stations where krill was collected for morphometric analysis, and relative distribution of gonad development stages, average (± 95% C.I.) size, and weight of krill collected per station*.

Variables/stations	S02	S06	S12	S15	S34**	S36	S47	S57	S80
Latitude	28°49.48'	29°17.21'	29°24.24'	28°39.89'	29°38.05'	29°25.31'	28°37.87'	27°44.69'	26°32.87'
Longitude	112°41.34'	112°33.18'	113°38.17'	113°05.01'	112°38.28'	113°13.16'	112°18.07'	112°32.78'	111°23.81'
Depth (m)	440	91	424	377	101	372	140	491	424
Distance to an eastern coast (km)	24.3	16.1	4.3	13.6	12.3	73.0	36.5	129.7	126.4
Temperature (°C)	17.30	17.48	16.35	16.77	17.26	16.77	15.41	17.72	17.96
Salinity	35.35	35.31	35.30	35.30	35.36	35.34	35.23	35.26	35.23
Oxygen (mL L^{-1})	4.73	4.53	4.07	4.35	4.33	4.42	3.33	4.14	4.53
Oxygen Saturation (%)	87.25	83.77	73.58	79.33	79.88	80.60	59.04	77.03	84.73
Chlorophyll-*a* at surface (mg m^{-3})§	1.30	1.79	0.62	1.30	3.63	1.08	0.97	1.31	1.50
Chlorophyll-*a* (mg m^{-3})ǂ	0.86	0.95	0.72	1.15	1.16	0.74	1.28	1.27	2.07
Density (kg m^{-3})	25.71	25.64	25.90	25.80	25.73	25.83	26.06	25.53	25.45
Males (%)	37.5	29.7	0.0	44.4	18.2	50.0	14.0	21.2	79.3
Females (%)	62.5	70.3	100.0	55.6	81.8	50.0	86.0	78.8	20.7
Previtellogenic Females (%)	0.0	16.2	0.0	0.0	81.8	0.0	14.0	9.1	0.0
Postvitellogenic Females (%)	12.5	8.1	66.7	22.2	0.0	14.3	10.5	6.1	0.0
Previtellogenic Ovigerous Females (%)	37.5	32.4	33.3	25.0	0.0	35.7	52.6	51.5	13.8
Postvitellogenic Ovigerous Females (%)	12.5	13.5	0.0	8.3	0.0	0.0	8.8	12.1	6.9
Abundance relative to other stations (%)	3.5	16.2	1.3	15.8	4.8	6.1	25.0	14.5	12.7
Total Length (mm)	13.1 ± 0.7	12 ± 0.6	13 ± 1.1	13.7 ± 0.4	12.8 ± 0.8	14.1 ± 0.6	11.7 ± 0.4	10.8 ± 0.3	13.1 ± 0.4
Dry Weight (mg)	3.8 ± 0.8	2.7 ± 0.4	4.1 ± 1.7	5 ± 0.8	2.8 ± 0.7	5.3 ± 1	2.5 ± 0.3	2.4 ± 0.4	4.8 ± 0.4

*Includes lipid samples and samples used in other projects.

**All euphausiids collected at station 34 were used in fasting experiments

§Surface values obtained from satellite data.

ǂ Obtained from CTD casts

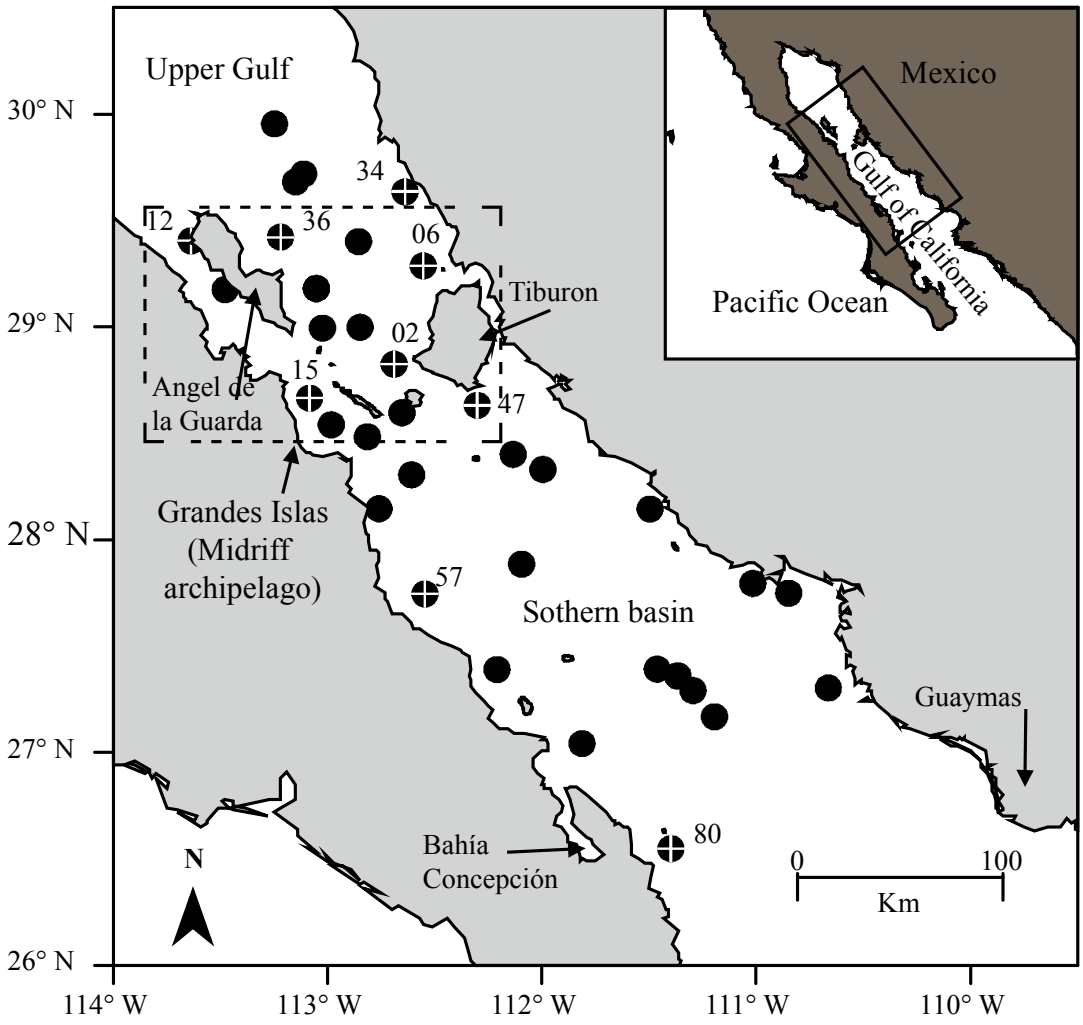


Figure 3-1 Study area of the oceanographic survey carried out during March 2010. Filled circles represent CTD sampling stations and crosses represent stations where krill was collected for lipid analysis and fasting experiments (numbers represent the sampling sequence among stations)

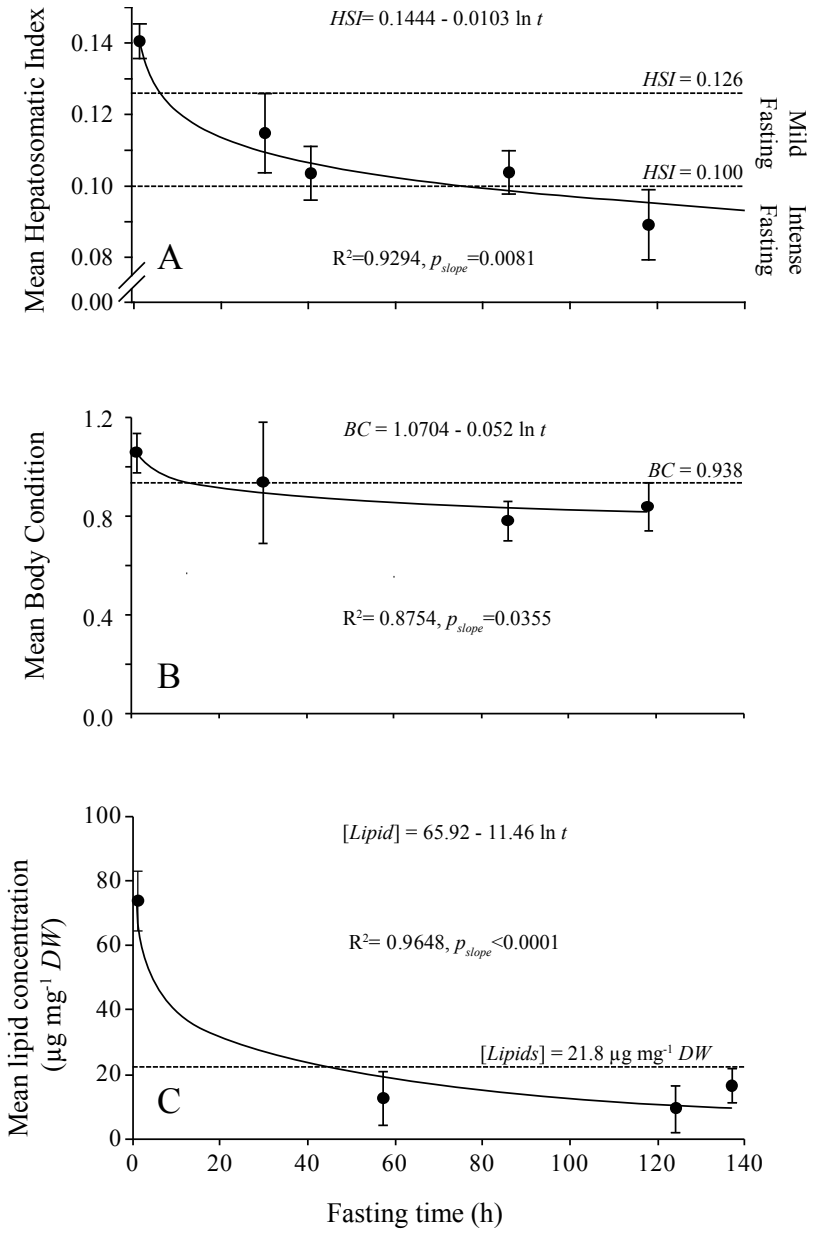


Figure 3-2 Changes in morphometric indices of *Nyctiphanes simplex* during fasting. a) Mean hepatosomatic index, b) Mean body condition, and c) Relative change in mean lipid concentration (µg mg^{-1} *DW*). Error bars represent the 95% confidence intervals of the mean. Dashed lines represent values where a significant reduction occurred due to fasting time

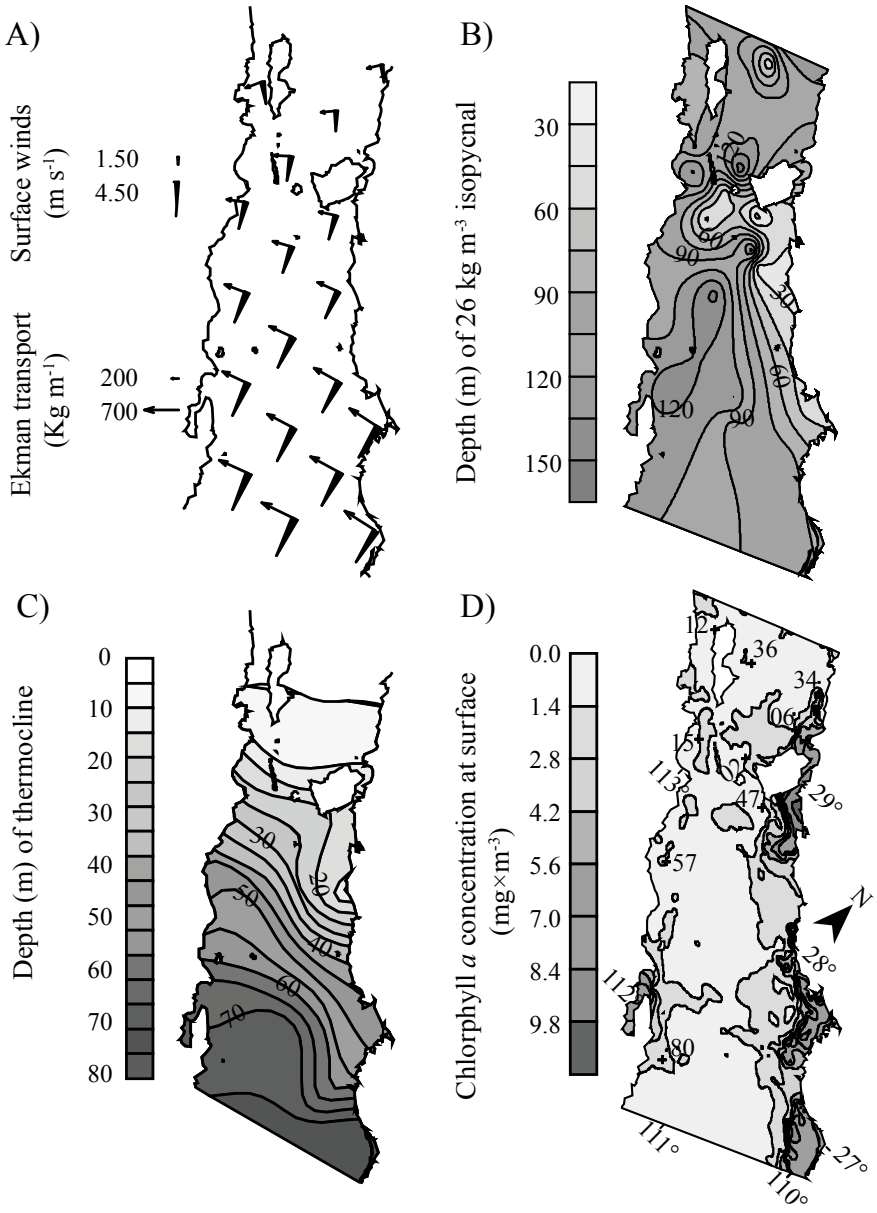


Figure 3-3 Environmental conditions recorded in the Gulf of California during March 2010. A) Surface winds (m s^{-1}) and the corresponding Ekman transport (kg m^{-1} s^{-1}), B) Depth of 26 kg m^{-3} isopycnal, C) Thermocline depth (m), as 1°C decrease in 10 m depth, and D) Satellite surface chlorophyll-*a* concentration (µg m^{-3})

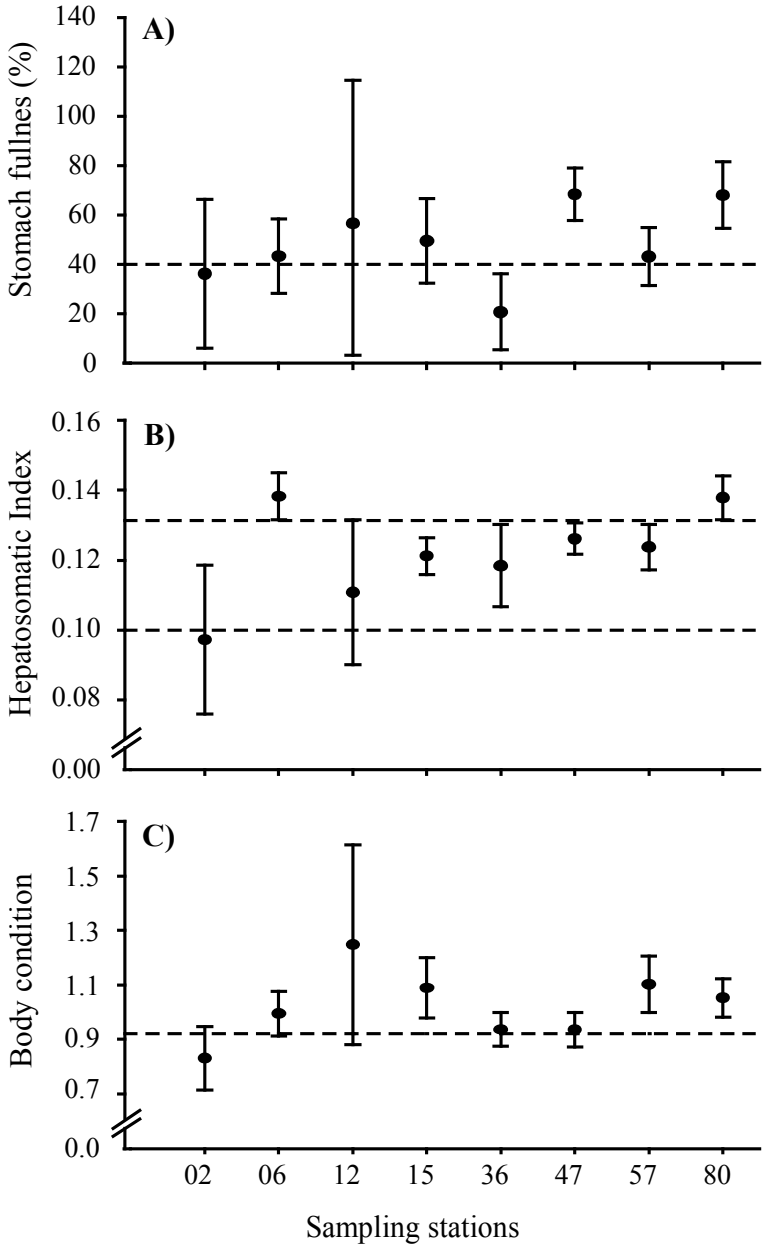


Figure 3-4 Mean ± 95% C.I. of *Nyctiphanes simplex* morphometric indices per sampling stations. A) Relative stomach fullness (%), B) Hepatosomatic index (HSI), and C) Body condition. Dashed lines represent range of values where fasting might be occurring

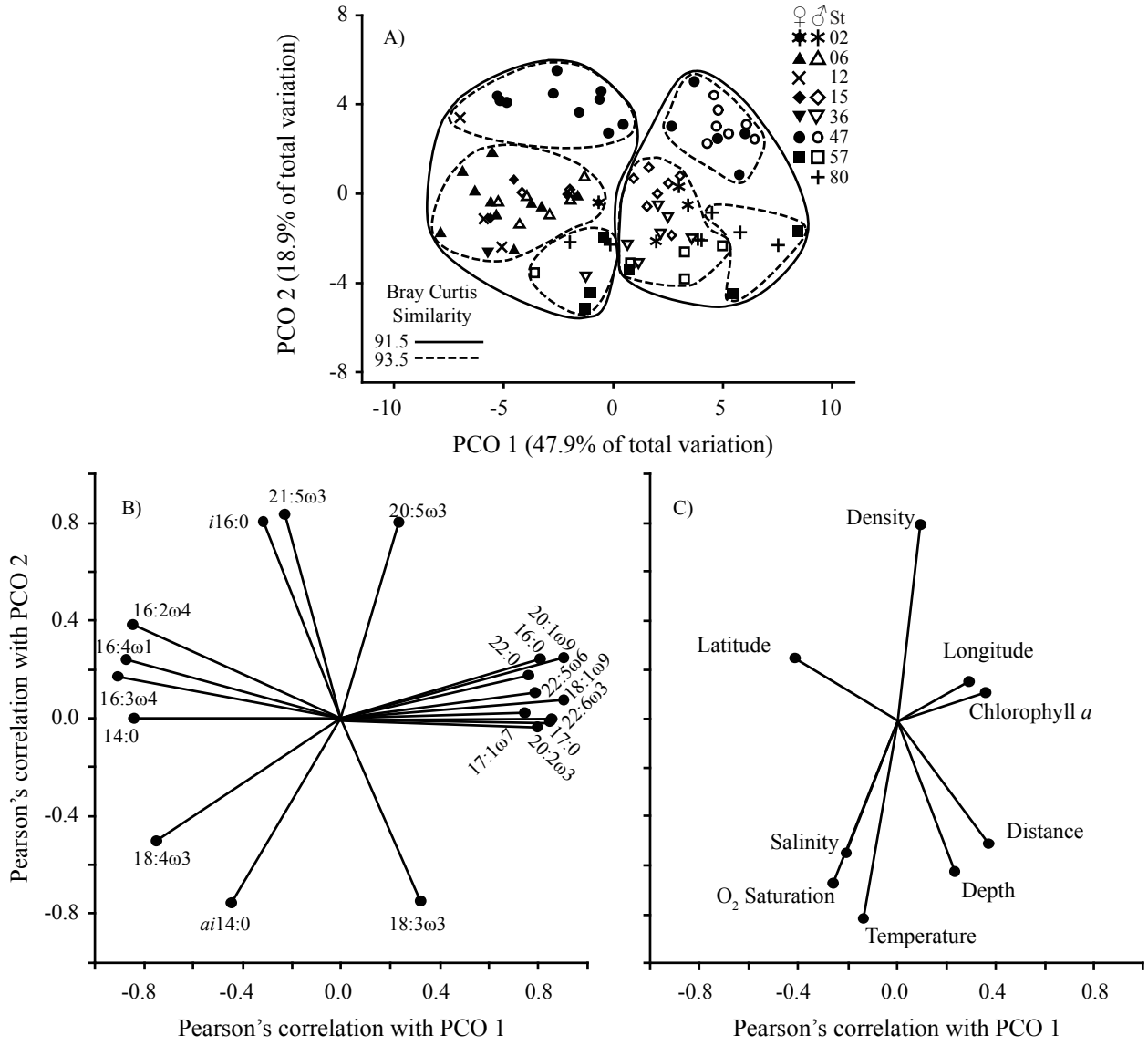
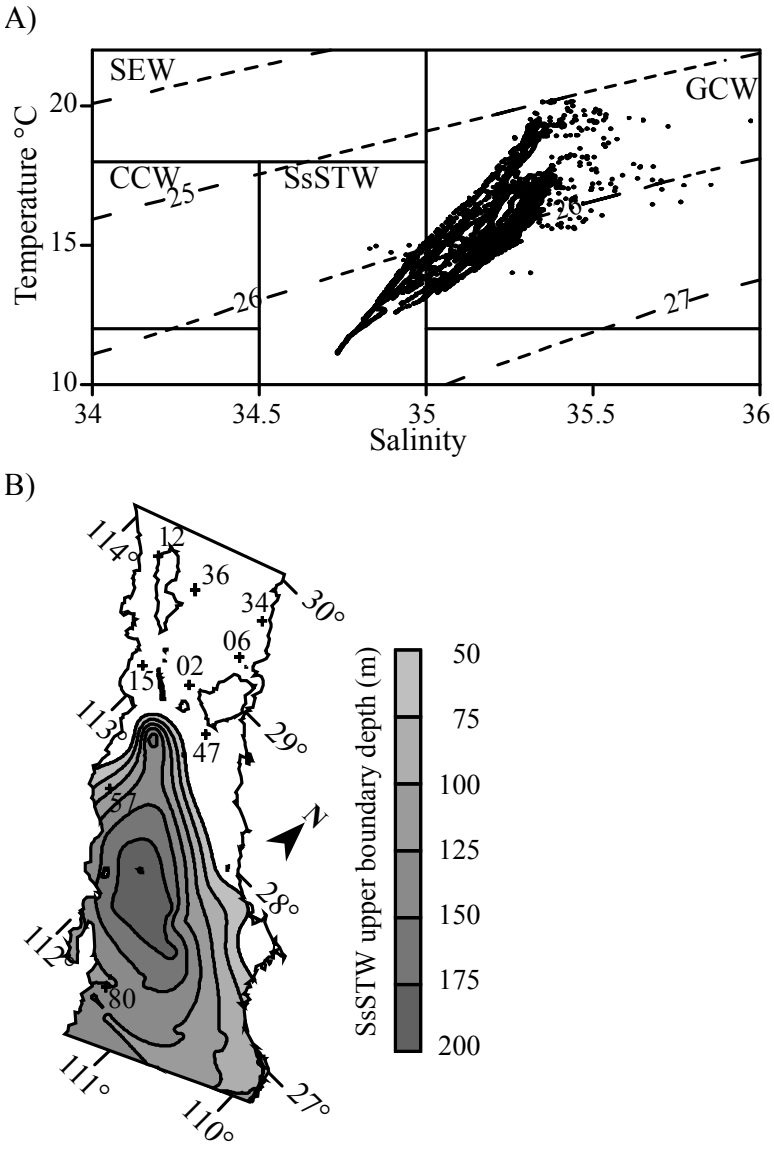


Figure 3-5 Principal coordinate analysis for fatty acids of male and female *Nyctiphanes simplex* collected in March 2010. A) Score plot of males and females collected at different stations, B) Pearson's correlations (> 0.7 and < -0.7) of major (> 0.5%) fatty acids with the first two principal coordinates, and C) Pearson's correlations of the environmental variables with the first two principal coordinates.

Appendix 3-1 Parameters and equations describing decreases in fatty acid concentration [*FA*] (µg mg^{-1} of *DW*) and fatty acid ratios of *Nyctiphanes simplex* relative to fasting time (t). Maximum fasting time =137 h fasting. *n* = 9, df = 1, 8

Fatty Acid	Trend	p-value for slope *b*	p-value for constant *a*	R^2	Slope *b*	Constant *a*
14:0	$[FAME]=a + b \times (t)$	< 0.001	< 0.001	0.9505	-0.0084	1.2211
15:0	$[FAME]=a + b \times (t)$	< 0.001	< 0.001	0.8857	-0.0012	0.199
16:0	$[FAME]=a + b \times (t)$	< 0.001	< 0.001	0.9551	**-0.0548**	8.3661
17:0	$[FAME]=a + b \times (t)$	< 0.001	< 0.001	0.8595	-0.0021	0.351
18:0	$[FAME]=a\, e^{(b \times t)}$	0.0030	< 0.001	0.7451	-0.0118	1.1893
Σ Odd chain[a]	$[FAME]=a + b \times (t)$	< 0.001	< 0.001	0.8450	-0.0039	0.6529
Σ Saturated[b]	$[FAME]=a + b \times (t)$	< 0.001	< 0.001	0.9373	-0.0814	12.5534
Phytanic	$[FAME]=a + b \times (t)$	< 0.001	< 0.001	0.9335	-0.0011	0.1554
Σ Branched[c]	$[FAME]=a + b \times (t)$	< 0.001	< 0.001	0.9015	-0.0043	0.6763
16:1ω7	$[FAME]=a + b \times (t)$	< 0.001	< 0.001	0.8697	-0.0069	1.0321
16:1ω9	$[FAME]=a\, e^{(b \times t)}$	< 0.001	< 0.001	0.9766	-0.0171	0.1230
18:1ω7	$[FAME]=a + b \times (t)$	< 0.001	< 0.001	0.9615	-0.0064	0.9671
18:1ω9	$[FAME]=a + b \times (t)$	< 0.001	< 0.001	0.8674	-0.017	2.8593
Σ Monoenoic[d]	$[FAME]=a + b \times (t)$	< 0.001	< 0.001	0.9152	-0.0344	5.5467
18:2ω6	$[FAME]=a + b \times (t)$	< 0.001	< 0.001	0.9215	-0.0055	0.8066
20:2ω3	$[FAME]=a + b \times (t)$	0.0010	< 0.001	0.8398	-0.0008	0.1164
Σ Dienoic[e]	$[FAME]=a + b \times (t)$	< 0.001	< 0.001	0.8976	-0.0065	1.0481
18:3ω3	$[FAME]=a\, e^{(b \times t)}$	0.0010	< 0.001	0.8384	**-0.0244**	0.8573
18:4ω3	$[FAME]=a\, e^{(b \times t)}$	< 0.001	< 0.001	0.9414	**-0.0392**	0.7461
20:3ω3	$[FAME]=a + b \times (t)$	< 0.001	< 0.001	0.8805	-0.0002	0.0327
20:4ω6	$[FAME]=a + b \times (t)$	0.004	< 0.001	0.7125	-0.0052	0.9217
20:5ω3	$[FAME]=a + b \times (t)$	< 0.001	< 0.001	0.9147	**-0.0556**	8.1092
22:6ω3	$[FAME]=a + b \times (t)$	< 0.001	< 0.001	0.8807	**-0.0993**	14.381
Σ LC-PUFA[f]	$[FAME]=a + b \times (t)$	< 0.001	< 0.001	0.9103	-0.1765	25.577
Σ PUFA[g]	$[FAME]=a + b \times (t)$	< 0.001	< 0.001	0.9107	-0.1830	26.625
Σ ω3	$[FAME]=a + b \times (t)$	< 0.001	< 0.001	0.9050	-0.1699	24.475
Σ ω6	$[FAME]=a + b \times (t)$	< 0.001	< 0.001	0.9232	-0.0126	1.9817
ω6/ω3	$[FAME]=a + b \times (t)$	0.001	< 0.001	0.8169	0.0004	0.0818
C_{16} PUFA	$[FAME]=a + b \times (t)$	0.005	< 0.001	0.7524	-0.0009	0.1459
C_{18} PUFA	$[FAME]=a\, e^{(b \times t)}$	0.0010	< 0.001	0.8177	-0.0236	2.4186
FA µg/mg	$[FAME]=a + b \times (t)$	< 0.001	< 0.001	0.9259	-0.2998	45.562
% DW	$[FAME]=a + b \times (t)$	< 0.001	< 0.001	0.9259	-0.0300	4.5562
Lipids µg/mg	$[FAME]=a\, e^{(b \times t)}$	0.0010	< 0.001	0.7879	-0.0121	60.3223

[a]Includes odd chain fatty acids < 0.5%: 13:0, 19:0, 21:0 and 23:0; [b]Incl. saturated < 0.5%: 12:0, 20:0, 22:0, and 24:0 plus the odd chain fatty acids; [c]Incl. branched < 0.5%: *ai*14:0, *ai*16:0, *i*15:0, and *i*17:0; [d]Incl. monoenoic fatty acids < 0.5%: 16:1ω5, 16:1ω9, 17:1ω7, 18:1ω5, 20:1ω7, 22:1ω9, and 22:1ω11; [e]Incl. dienoic < 0.5%: 20:2ω3; [f]Incl. polyenoic fatty acids < 0.5%: 16:4ω3, 18:5ω3 and 20:3ω3; [g]Includes dienoic fatty acids and LC-PUFA. Bolded values indicate highest negative slopes (*b*) for individual fatty acids.



Appendix 3-2 Water masses detected in the first 200-m depth in the Gulf of California during March 2010. A) Temperature and salinity (T-S) profiles at sampling stations compared with a T-S Diagram of water masses in the Gulf of California and its vicinity (Torres-Orozco, 1993). GCW = Gulf of California water; StSsW = Subtropical subsurface water; CCW = California Current water; SEW = Subequatorial water. Dotted lines represent isopycnals (lines of equal density) in kg m^{-3}, and B) Upper boundary of the subtropical subsurface water mass

Appendix 3-3 Relative proportions (%) ± 95% C.I. of the mayor fatty acids of mature females of *Nyctiphanes simplex* collected at different sampling stations during March 2010.

Fatty acids/stations	Station 02* $n = 1$	Station 06 $n = 10$	Station 12 $n = 3$	Station 15 $n = 3$	Station 36* $n = 1$	Station 47 $n = 15$	Station 57 $n = 6$	*p*-value	C.V.
14:0	4.5	5.0± 0.3	**5.4**± 0.4	5.1± 0.8	5.6	3.7± 0.5	3.6± 0.7	< 0.001	24
15:0	0.76	**0.83**± 0.06	0.77± 0.17	0.75± 0.12	0.84	0.50± 0.03	0.69± 0.11	< 0.001	25
16:0	23	21± 1	19± 1	19± 1	18	21± 1	**23**± 1	0.005	11
17:0	0.75	0.79± 0.05	0.76± 0.17	0.75± 0.07	0.72	0.72± 0.04	0.85± 0.12	0.165	13
18:0	2.6	2.5± 0.3	2.2± 0.4	2.2± 0.1	2.2	2.7± 0.2	2.8± 0.3	0.125	16
Σ Odd chain[a]	1.7	**1.9**± 0.1	1.8± 0.3	1.8± 0.1	1.8	1.4± 0.1	1.9± 0.2	< 0.001	15
Σ Saturated[b]	32.4	30.9± 1.8	29.0± 1.3	28.4± 0.4	28.4	29.2± 0.8	**32.5**± 1.5	0.010	8
Phytanic	0.4	**1.0**± 0.2	0.8± 0.1	0.7± 0.1	0.7	0.8± 0.1	0.5± 0.2	0.017	39
Σ Branched[c]	1.51	**2.42**± 0.13	2.05± 0.05	1.99± 0.32	2.14	1.86± 0.17	1.81± 0.28	0.001	18
16:1ω7	4.4	4.3± 0.3	4.2± 1.0	**4.4**± 0.9	4.4	3.8± 0.4	2.5± 0.5	0.001	23
18:1ω7	3.4	3.4± 0.1	3.1± 0.2	3.2± 0.4	2.8	3.4± 0.2	3.1± 0.1	0.138	10
18:1ω9	8.0	5.8± 0.2	5.5± 0.1	6.1± 0.1	6.6	7.3± 0.4	**9.0**± 0.8	< 0.001	19
Σ Monoenoic[d]	18.0	16.0± 0.3	15.3± 1.5	16.1± 0.9	16.3	16.7± 0.4	**16.7**± 0.4	0.015	5
16:2ω4	0.3	0.5± 0.1	**0.7**± 0.3	0.5± 0.1	0.5	0.5± 0.1	0.2± 0.1	0.005	48
18:2ω6	1.5	1.6± 0.1	1.5± 0.2	1.7± 0.1	2.0	1.5± 0.1	**2.5**± 0.3	< 0.001	25
Σ Dienoic[e]	2.25	2.23± 0.10	2.35± 0.08	2.42± 0.05	2.70	2.15± 0.09	**3.03**± 0.32	< 0.001	16
16:4ω1	0.2	0.5± 0.1	**0.9**± 0.4	0.5± 0.1	0.6	0.4± 0.2	0.2± 0.1	0.004	62
18:3ω3	1.5	1.7± 0.2	2.1± 0.7	2.0± 0.1	1.9	1.3± 0.1	**2.5**± 0.4	< 0.001	30
18:4ω3	1.1	2.1± 0.4	**2.9**± 0.3	1.9± 0.3	3.0	1.1± 0.2	1.7± 0.6	< 0.001	46
20:4ω6	2.2	1.8± 0.1	2.0± 0.4	**2.7**± 0.5	1.7	2.7± 0.2	2.0± 0.4	< 0.001	25
20:5ω3	17	19± 1	21± 2	20± 1	19	**21**± 1	15± 1	< 0.001	14
22:6ω3	21	21± 1	21± 3	22± 1	22	21± 1	23± 2	0.215	9
Σ LC-PUFA[f]	46	49± 2	**51**± 2	51± 1	50	50± 1	46± 2	0.001	5
Σ PUFA[g]	48	51± 2	**54**± 2	54± 1	53	52± 1	49± 2	0.007	5
Σ n3	43	46± 2	**49**± 2	48± 1	48	47± 1	44± 2	0.006	5
Σ n6	4.3	3.8± 0.2	3.8± 0.2	4.7± 0.5	4.0	4.7± 0.3	**5.1**± 0.4	< 0.001	14
Σ n3/Σ n6	10	12± 1	**13**± 1	10± 1	12	10± 1	9± 1	< 0.001	16
Σ C16 PUFA[h]	1.0	1.6± 0.3	**2.1**± 0.8	1.6± 0.3	1.8	1.2± 0.3	0.6± 0.2	0.003	50
Σ C18 PUFA[i]	4.2	5.6± 0.6	6.7± 1.3	5.7± 0.6	7.2	3.9± 0.2	**6.8**± 1.2	< 0.001	28
C16/C18 PUFA	0.23	0.28± 0.04	**0.34**± 0.20	0.27± 0.07	0.25	0.30± 0.08	0.09± 0.04	0.011	52
16:1 (ω7)/16:0	0.19	0.21± 0.02	0.22± 0.06	0.24± 0.06	0.24	0.19± 0.03	0.11± 0.03	0.001	30
16:1(ω7)/18:4(ω3)	4.1	2.1± 0.4	1.5± 0.5	2.3± 0.7	1.5	**3.8**± 0.6	1.7± 0.5	< 0.001	48
18:1(ω9)/18:1(ω7)	2.4	1.7± 0.1	1.8± 0.1	1.9± 0.3	2.3	2.2± 0.2	**2.9**± 0.4	< 0.001	23
18:5(ω3)/18:3(ω3)	0.08	**0.10**± 0.02	0.07± 0.02	0.06± 0.01	0.14	0.05± 0.01	0.05± 0.02	< 0.001	41
22:6(ω3)/20:5(ω3)	1.3	1.1± 0.1	1.0± 0.2	1.1± 0.1	1.2	1.0± 0.1	**1.6**± 0.2	< 0.001	22
LC-PUFA/Saturated	1.4	1.6± 0.1	**1.8**± 0.1	1.8± 0.01	1.8	1.7± 0.1	1.4± 0.1	0.004	12
Total % DW	5	12± 2	6± 1	10± 3	11	**12**± 1	10± 2	0.035	31

[a]Includes the odd chain fatty acids < 0.9%: 13:0, 19:0, 21:0 and 23:0; [b]Includes the saturated < 0.9%: 12:0, 20:0, 22:0, and 24:0 plus the odd chain fatty acids; [c]Includes the branched < 0.9%: *ai*14:0, *ai*16:0, *i*15:0, *i*16:0 and *i*17:0; [d]Includes the monoenoic fatty acids < 0.9%: 16:1ω5, 16:1ω9, 17:1ω7, 18:1ω5, 20:1ω7, 20:1ω9, 22:1ω9, 22:1ω11, and 24:1ω9; [e]Includes the dienoic < 0.9%: 20:2ω3; [f]Includes the polyenoic fatty acids < 0.9%: 16:3ω4, 16:4ω3, 18:5ω3, 20:3ω3, 20:4ω3, 21:5ω3, 22:5ω3, 22:5ω6; [g]Includes dienoic and LC-PUFA; [h]Includes 16:3ω4 and 16:4ω3. [i]Includes 18:5ω3. Maximum values are in bold when differences are significant ($p < 0.05$). Rounding of significant figures was based on the magnitude of the error. *Not included in ANOVA

Appendix 3-4 Relative proportions (%) ± 95% C.I. of the major fatty acids of mature males of *Nyctiphanes simplex* collected at different stations during March 2010.

Station	E02	E06	E15	E36	E47	E57	E80	*p*-value	C.V.
	n = 3	*n* = 7	*n* = 9	*n* = 7	*n* = 7	*n* = 5	*n* = 6		
14:0	3.4± 0.5	**3.9**± 0.2	3.5± 0.4	3.2± 0.3	2.2± 0.2	3.1± 0.9	3.5± 0.8	0.001	24
15:0	0.60± 0.12	**0.78**± 0.05	0.67± 0.05	0.61± 0.03	0.43± 0.04	0.61± 0.07	0.54± 0.06	< 0.001	20
16:0	20.7± 1.4	20.8± 1.8	19.4± 0.9	19.2± 0.5	**21.5**± 1.3	20.9± 0.7	19.2± 1.1	0.037	8
17:0	0.83± 0.02	0.81± 0.04	0.79± 0.04	0.82± 0.03	0.76± 0.02	0.68± 0.04	**0.87**± 0.11	0.002	10
18:0	2.5± 0.1	2.5± 0.3	2.0± 0.1	2.1± 0.1	2.3± 0.1	2.1± 0.1	**2.6**± 0.2	< 0.001	14
Σ Odd chain[a]	1.6± 0.1	**1.8**± 0.1	1.7± 0.1	1.6± 0.1	1.4± 0.1	1.6± 0.1	1.7± 0.1	< 0.001	11
Σ Saturated[b]	29± 2	30± 2	27± 1	27± 1	28± 2	28± 2	28± 1	0.145	8
Phytanic	0.3± 0.1	**0.8**± 0.1	0.4± 0.1	0.3± 0.1	0.5± 0.1	0.6± 0.4	0.4± 0.1	0.001	48
Σ Branched[c]	1.4± 0.1	**2.1**± 0.1	1.5± 0.1	1.5± 0.1	1.5± 0.1	1.7± 0.4	1.6± 0.1	< 0.001	19
16:1ω7	3.4± 0.1	**3.9**± 0.2	3.1± 0.3	2.9± 0.2	2.4± 0.2	2.5± 0.7	2.7± 0.5	< 0.001	21
18:1ω7	2.6± 0.2	**3.2**± 0.1	3.1± 0.2	2.7± 0.2	3.1± 0.1	2.9± 0.1	2.3± 0.2	< 0.001	13
18:1ω9	8.1± 0.7	5.6± 0.3	6.7± 0.4	7.2± 0.5	7.8± 0.4	8.1± 0.6	**8.2**± 0.7	< 0.001	15
Σ Monoenoic[d]	16.3± 0.9	15.0± 0.5	14.9± 0.5	15.0± 0.4	15.3± 0.5	15.5± 0.2	15.1± 0.5	0.049	5
16:2ω4	0.205± 0.003	**0.372**± 0.066	0.266± 0.063	0.227± 0.037	0.118± 0.030	0.181± 0.090	0.209± 0.075	< 0.001	46
18:2ω6	1.8± 0.3	1.5± 0.1	1.7± 0.1	2.0± 0.1	1.5± 0.1	**2.8**± 0.1	2.4± 0.2	< 0.001	24
Σ Dienoic[e]	2.2± 0.3	2.1± 0.1	2.2± 0.1	2.5± 0.1	1.9± 0.1	**3.2**± 0.1	2.9± 0.2	< 0.001	19
16:4ω1	0.17± 0.02	**0.35**± 0.10	0.26± 0.08	0.22± 0.05	0.09± 0.02	0.18± 0.10	0.22± 0.11	0.004	58
18:3ω3	1.8± 0.6	1.8± 0.1	1.9± 0.2	2.2± 0.2	1.3± 0.2	**2.6**± 0.1	2.0± 0.1	< 0.001	22
18:4ω3	0.9± 0.2	**1.9**± 0.3	1.2± 0.2	1.6± 0.6	0.5± 0.1	1.7± 0.4	1.4± 0.4	< 0.001	47
20:4ω6	2.4± 0.5	1.9± 0.1	**3.0**± 0.3	2.1± 0.2	3.0± 0.5	2.4± 0.2	2.9± 0.4	< 0.001	23
20:5ω3	17± 1	20± 1	19± 1	18± 1	**20**± 1	17± 1	17± 1	< 0.001	9
22:6ω3	26± 1	23± 1	26± 1	**27**± 1	26± 2	25± 2	27± 2	0.010	9
Σ LC-PUFA[f]	51± 1	51± 3	54± 2	54± 1	53± 2	51± 2	53± 2	0.082	5
Σ PUFA[g]	53± 1	53± 3	56± 2	57± 1	55± 2	54± 2	56± 2	0.086	5
Σ n3	48± 1	49± 2	51± 2	52± 1	50± 2	48± 2	49± 1	0.110	5
Σ n6	4.9± 0.2	3.9± 0.1	5.1± 0.2	4.8± 0.2	5.2± 0.5	5.6± 0.3	**6.0**± 0.5	< 0.001	15
Σ n3/Σ n6	9.9± 0.1	**12.6**± 0.3	9.9± 0.3	10.9± 0.5	9.7± 0.9	8.6± 0.4	8.3± 0.6	< 0.001	15
Σ C16 PUFA[h]	0.6± 0.1	**1.2**± 0.3	0.8± 0.2	0.8± 0.1	0.3± 0.1	0.6± 0.3	0.6± 0.3	< 0.001	50
Σ C18 PUFA[i]	4.5± 1.2	5.3± 0.5	4.9± 0.4	5.9± 0.9	3.4± 0.3	**7.1**± 0.3	5.9± 0.6	< 0.001	24
C16/C18 PUFA	0.15± 0.04	**0.22**± 0.04	0.16± 0.04	0.13± 0.01	0.10± 0.02	0.08± 0.04	0.10± 0.03	< 0.001	42
16:1 (ω7)/16:0	0.16± 0.02	**0.19**± 0.01	0.16± 0.01	0.15± 0.01	0.11± 0.01	0.12± 0.03	0.14± 0.03	< 0.001	21
16:1(ω7)/18:4(ω3)	3.8± 1.0	2.2± 0.5	2.7± 0.4	2.1± 0.6	**5.0**± 0.8	1.5± 0.3	2.1± 0.5	< 0.001	48
18:1(ω9)/18:1(ω7)	3.12± 0.24	1.74± 0.04	2.21± 0.24	2.70± 0.27	2.55± 0.22	2.81± 0.27	**3.62**± 0.49	< 0.001	25
18:5(ω3)/18:3(ω3)	0.04± 0.02	**0.08**± 0.02	0.04± 0.01	0.05± 0.02	0.05± 0.01	0.05± 0.02	0.08± 0.03	0.002	49
22:6(ω3)/20:5(ω3)	1.55± 0.02	1.17± 0.05	1.35± 0.10	1.49± 0.06	1.30± 0.15	1.49± 0.16	**1.64**± 0.24	< 0.001	16
LC-PUFA/Saturated	1.8± 0.2	1.7± 0.2	2.0± 0.1	2.0± 0.1	1.9± 0.2	1.8± 0.2	1.9± 0.2	0.138	12
Total % DW	7± 2	**10**± 1	7± 2	7± 1	6± 2	9± 2	6± 1	0.048	39

[a]Includes the odd chain fatty acids < 0.9%: 13:0, 19:0, 21:0 and 23:0; [b]Includes the saturated < 0.9%: 12:0, 20:0, 22:0, and 24:0 plus the odd chain fatty acids; [c]Includes the branched < 0.9%: *ai*14:0, *ai*16:0, *i*15:0, *i*16:0 and *i*17:0; [d]Includes the monoenoic fatty acids < 0.9%: 16:1ω5, 16:1ω9, 17:1ω7, 18:1ω5, 20:1ω7, 20:1ω9, 22:1ω9, 22:1ω11, and 24:1ω9; [e]Includes the dienoic < 0.9%: 20:2ω3; [f]Includes the polyenoic fatty acids < 0.9%: 16:3ω4, 16:4ω3, 18:5ω3, 20:3ω3, 20:4ω3, 21:5ω3, 22:5ω3, 22:5ω6; [g]Includes dienoic and LC-PUFA; [h]Includes 16:3ω4 and 16:4ω3. [i]Includes 18:5ω3. Maximum values are in bold when differences are significant ($p < 0.05$). Rounding of significant figures was based on the magnitude of the error.

Chapter 4. **Seasonal changes in biochemical composition, body condition and energy content of *Nyctiphanes simplex* males off the southwest coast of the Gulf of California, Mexico**

4.1. Abstract

Small changes in energy content of prey can produce important impacts in large predators. Seasonal variation in the energy content of the subtropical krill, *N. simplex* (Crustacea), has not been studied despite their significant role in the trophodynamics of the Gulf of California. In this region, seasonal changes in oceanographic conditions are caused by wind stress, cyclonic gyres, and local upwelling, with strong interactions among climatic regimes, winds, vertical stratification of the water column, and phytoplankton abundance. Consequently, seasonal changes in energy content and body condition of *N. simplex* are expected. Such changes were investigated through analyses of the biochemical composition and morphometric features of males collected in the southern region of the Gulf of California during May, July, and October 2000, and February 2001. Body condition was calculated as the ratio of the observed and predicted dry weight estimated from a total length–dry weight regression model. Dry weight content of proteins, carbohydrates, and structural and storage lipids were calculated. Fatty acid analyses were conducted for neutral and polar lipid fractions. Male krill collected in winter (February) and the spring-summer transition (May) exhibited larger lipid storage likely related to the main reproductive period and to the winter-spring phytoplankton bloom (mostly diatoms). In summer (July), at the end of the reproductive peak, the lipid content, and dry weight reached the lowest values (an unlikely condition for reproduction). Fatty acid analysis suggested a diatom-based diet from winter to summer. Small-sized adult males collected in autumn (October)

exhibited still remained with low lipid content, but protein content increased noticeably together with dry weight producing the best body condition. High protein content and high energy content appeared to be related to alternative food sources to diatoms. Whereas high lipid content seems to drive the reproductive process in males of this subtropical krill, high protein content can improve essential amino acid and energy content of krill as prey during autumn.

Keywords: Biochemical composition, fatty acids, food chain, krill, trophic relationships, seasonal change

4.2. Introduction

The euphausiid *Nyctiphanes simplex* proliferates in regions close to coastal upwelling areas of the north and south temperate margins of the Eastern Tropical Pacific Ocean (ETP). In the Northern Hemisphere, it is particularly abundant in the shoreward region of the California Current along the coast of Southern California, the west coast of the Baja California Peninsula and the Gulf of California, though it may extend variable distances to the north and south or be carried to the west in tongues of distribution (Boden *et al*., 1955*;* Brinton, 1960; 1979; Brinton and Townsend, 1980).

The southernmost part of the Baja California Peninsula is an oceanographic transition zone between tropical and temperate waters, where *N. simplex* inhabits neritic areas, and in the Gulf of California it is the most abundant and widespread euphausiid species year-round (Brinton & Townsend, 1980; Brinton *et al*., 1986*;* Gendron, 1992). Several upwelling regions along the coasts of the Baja California Peninsula support considerable production of this euphausiid (Gómez-Gutiérrez *et al*., 1995, 1996, 2012, De Silva-Dávila & Palomares-García, 1998).

Small changes in energy content of preferred prey can cause important impacts in large predators. For instance, estimations of the prey biomass required may change considerably

(Mårtensson *et al.,* 1996), and, if biomass requirements are not met, result in decline in the condition of predators. However, energy content of *Nyctiphanes simplex* has not been studied in terms of seasonal variations despite the significant role of *N. simplex* in the trophodynamics of this subtropical region as prey for other zooplanktonic organisms, fish, seabirds, and marine mammals. In the southern Gulf of California seasonal changes in oceanographic conditions caused by wind stress, cyclonic gyres, and local upwelling (Jiménez-Illescas *et al.,* 1997*;* Sánchez-Velasco *et al.,* 2006), produce strong interactions among climatic regimes, winds, vertical stratification of the water column, and phytoplankton abundance (Martínez-López *et al.,* 2001). As a consequence, I expect seasonal and geographical differences in the energy content of *N. simplex*.

The underlying hypothesis for the present work was that this euphausiid will have higher energy content during winter–spring months, when overall phytoplankton abundance and biomass increase, and when krill reproductive stages increase in abundance. Nevertheless, consumption of alternative food sources (omnivory and carnivory) during summer, and ecological adaptations to a subtropical environment might compensate the decrease in primary productivity. Biochemical composition of *N. simplex* changes throughout its life stages (Chapter 2), and during gonad development, especially in females (Gómez-Gutierrez *et al*., 2010), and the use of different life stages or variable degrees of gonadal maturity might obscure seasonal variations in energy content. Accordingly, seasonal variations in biochemical composition, body condition, and energy content were investigated only in adult males, where investment in reproduction is considerably smaller than in females (Chapter 2). Additionally, fatty acids were investigated, as several authors have demonstrated that in zooplankton, fatty acids can be used as trophic markers for specific taxonomic groups such as diatoms, dinoflagellates,

prymnesiophytes, ciliates, detritus and metazoans (Dalsgaard *et al.,* 2003*;* Pepin *et al.,* 2011), and in other euphausiid species around the world, fatty acids have been valuable in discerning food habits (Mayzaud *et al.,* 1999*;* Cripps & Atkinson, 2000; Virtue *et al.,* 2000*;* Stübing *et al.,* 2003).

4.3. Materials and Methods

4.3.1. Chlorophyll-*a* and temperature

Monthly data of sea surface temperature from 1982 to 2000 were provided by ICOADS at the NOAA-CIRES Climate Diagnostics Center, Boulder, Colorado, USA (http://www.cdc.noaa.gov), and *in situ* oceanographic CTD data were used to describe warm and cold seasons. Data obtained from the SeaWiFS Project, NASA/Goddard Space Flight Center, and ORBIMAGE (http://oceancolor.gsfc.nasa.gov/cgi/level3.pl) for the same sampling period were used to estimate mean surface chlorophyll *a* concentrations (mg m^{-3}). Monthly phytoplankton abundance (diatoms, dinoflagellates, silicoflagellates and nanoplankton) was obtained from a 1998–1999 study with plankton nets in the same area (Villalejo-Fuerte *et al.,* 2005).

4.3.2. Sampling

During May, July, and October 2001, and February 2002, a 51-station zooplankton field survey was performed between the coastal towns of Loreto and La Paz in the state of Baja California Sur (Figure 4-1). Zooplankton hauls were carried out at night with a bongo net (0.6 m diameter, 505-µm mesh), using oblique tows from 300 m depth to the surface. Vessel speed was 2 knots (~3.7 km h^{-1}) and the net was towed for 30 minutes. Several life stages of *Nyctiphanes simplex* of both sexes were collected in the field; however, only mature males were sufficiently

represented in the four seasons. Males were identified by the presence of petasma —a modified endopodite of the first pair of abdominal appendages (pleopods)—, or a flat-shaped lappet in the rostrum distinctively longer than the forward-canted lappet of females. However, only those bearing an internal or protruding spermatophore visible through the cuticle were selected for the present study (Appendix 4-1).

Selected mature males were removed gently, placed in sealed containers, frozen, transported in liquid nitrogen (–196°C), and stored at –80°C until analysis. Single specimens were rinsed with distilled water, measured to the nearest mm from the end of the rostrum to the tip of the telson (*TL*), briefly blotted with Whatman No.1 filter paper, and weighed wet (*WW*) to the nearest 0.1 mg. Samples were freeze-dried for 24 h and weighed dry (*DW*) to the nearest 0.001 mg with a Cahn C-33 microbalance.

4.3.3. Biochemical composition and energy content

Using D-glucose as a standard, Dreywood's anthrone reagent was used to assess carbohydrate content (Morris, 1948). For determining protein concentration (Bradford, 1976), freeze-dried samples were digested with 5 mL of 0.1 N NaOH for 30 min at 80°C (Mayzaud & Martín, 1975) and aliquots of 50 to 200 µL of this solution were adjusted to 800 µL with distilled water. No neutralization was required after use of NaOH, since the pH of the solution after addition of 200 µL of dye-binding reagent (Bio-Rad, Hercules, CA) was equal to the pH obtained in solutions without NaOH.

For lipid determinations, single freeze-dried specimens (< 3 mg) were homogenized, re-hydrated with 0.15 mL of distilled water, and homogenized in 3 mL of a $CHCl_3$:CH_3OH (2:1) solvent system (exceeding the required 20-fold volume to mass ratio). A two-phase system was obtained by adding 0.6 mL of distilled water to achieve a mixture of $CHCl_3$:CH_3OH:H_2O (8:4:3)

(Folch *et al.,* 1957). After extraction and evaporation to concentrate the volume to 0.5 mL, samples were separated into polar and neutral fractions on a silica-gel (60–200 μm) microcolumn (Palacios *et al.,* 2002*;* Christie, 2003) and collected in tubes containing an internal standard (23:0) and buthylated hydroxytoluene (BHT, 0.01 %) as an antioxidant. A mixture of standards (dipalmitine, triestearine, cholesterol and lecithin) was run in parallel to account for losses during separation and elution. Each lipid fraction was divided into two equal volumes and dried under a nitrogen stream. Lipid content of one replicate in each fraction was determined using a charring method (Marsh & Weinstein, 1966).

Individual energy content (J ind^{-1}), and energy content per dry weight (J mg^{-1}) was calculated as the amount of energy provided by partial oxidation of carbohydrates (17.2 J mg^{-1}), proteins (21.4 J mg^{-1}) and lipids (35.6 J mg^{-1}) (Postel *et al.,* 2000).

4.3.4. Fatty acids

The fatty acids from the second replicate of each fraction were transesterified (Sato & Murata, 1988) and methyl esters were analyzed by gas chromatography on a 30 m × 0.25 mm fused silica capillary column (Supelco Omegawax, Bellefonte, PA), with polyethylenglycol as the stationary phase with a thickness of 0.25 μm, and helium as the carrier gas. The column was mounted in a gas chromatograph coupled to a mass-spectrometric detector (GCD 1800B Hewlett-Packard, Palo Alto, CA). Chromatographic conditions were set as follows: helium flow 0.9 mL min^{-1}, and injector temperature 250°C. After injection, the temperature of the column was subjected to the following sequence: hold at 110°C for 3 min, increase to 165°C at a rate of 30°C min^{-1}, hold at 165°C for 2 min, increase to 220°C at a rate of 2°C min^{-1}, and hold at 220°C for 16 min. The detector was set at 260°C and the ion source was set at 70 eV.

Fatty acid identification was based on interpretation of their mass spectra and comparison with those generated from commercial standards of 37 fatty acid methyl esters (FAME) commonly found in food products, including marine organisms (Supelco 47885-U, Appendix 4-3). When fatty acid isomers were detected, retention times of at least one of the isomers in commercial standards allowed double-bond positioning of the other isomers, because the isomers with double bonds closer to the ester group elute earlier than isomers with more remote double bonds. Differences between fatty acid detector responses were calculated by plotting five different concentrations of FAME ranging from 20 to 100 $\mu g\ mL^{-1}$ (X axis) against their peak areas (Y axis). Regression analysis of each plot yielded the response factor for each fatty acid.

The relative contribution of each fatty acid to the overall fatty acid composition was computed. Individual fatty acids, and their sums or ratios, were examined as possible biomarkers or indices of nutritional status. For instance, the ratio between docosahexaenoic and eicosapentaenoic fatty acids (22:6ω3/20:5ω3 or DHA/EPA) is considered a reliable nutritional index for halibut larvae (Shields *et al.,* 1999) as is the Σω3/Σω6 ratio for sea scallops (Milke *et al.,* 2004). The 18:1ω7/18:1ω9 ratio was used as a trophic marker of carnivory (Dalsgaard *et al.,* 2003); the 22:6ω3/20:5ω3 ratio as a trophic marker of dinoflagellate intake (Budge & Parrish, 1998); the sum of 18:1ω9 + 18:4ω3 to indicate intake of prymnesiophytes (Dalsgaard *et al.,* 2003) and the sum of 18:2ω6 + 18:3ω3 to show terrestrial input (Budge & Parrish, 1998). Similarly the sum of 16:1ω7 + 16:4ω1 + 20:5ω3 and the ratios 16:1ω7/16:0 and $\Sigma C_{16}/\Sigma C_{18}$ were used as trophic markers of diatoms (Budge & Parrish, 1998; Dalsgaard *et al.,* 2003), whereas the C_{16} PUFA index was an indicator of nutrient sufficiency in diatoms (Parrish *et al.,* 2005).

This last index was originally called 'Polyunsaturation Index of C_{16} fatty acids' (Shin *et al.,* 2000) and was proposed as a measure of the biochemical status of diatoms in the diet of

zooplankters. It consisted of the ratio between C_{16} polyunsaturated fatty acids (16:2ω7 + 16:3ω4 + 16:4ω1) to all C_{16} fatty acids (16:0 + 16:1ω9 + 16:1ω7 + 16:1ω5 + 16:2ω7 + 16:3ω4 + 16:4ω1). This index was further modified (Parrish *et al.,* 2005), adding the polyunsaturated 16:2ω4 and 16:4ω3, which comprise a significant portion (>2%) of the total fatty acids in some diatoms and was called the C_{16} PUFA ratio. Its calculation comprised the ratio of C_{16} PUFA (16:2ω4 + 16:3ω4 + 16:4ω3 + 16:4ω1) to all C_{16} FA (16:0 + 16:1ω7 + 16:1ω5 + 16:2ω4 + 16:3ω4+ 16:4ω3 + 16:4ω1). This ratio provides an index of nutrient sufficiency in diatoms and has been shown to decline rapidly as nitrate concentrations in the water column fall following the spring bloom (Parrish *et al.,* 2005). In the present study, this index was calculated as the ratio between all C_{16} polyunsaturated fatty acids to all C_{16} fatty acids (saturated, monounsaturated and polyunsaturated) found in *N. simplex*.

4.3.5. Statistics

Differences among sampling seasons were assessed with univariate ANOVA tests, using the Fisher statistic (*F*). Assumptions of normality, homogeneity and independence of residuals were tested with residual analyses. When those assumptions were not met and the *p* values were close to the α value (0.05), a *p*-randomization was conducted 1,000 times to test the data empirically. A similar approach was used to calculate non-symmetrical 95% confidence intervals for variables with residuals not meeting ANOVA assumptions. PRIMER statistical software was used for SIMPER analysis of the main (>1%) fatty acids among seasons, and for permutational multivariate ANOVA based on distances (PERMANOVA) followed by principal coordinate analysis (PCO). Pearson correlations were calculated for orthogonal axes of the fatty acids.

4.4. Results

4.4.1. Sea surface temperature and chlorophyll *a*

The average temperature for the complete time series (24.2ºC) was used to separate warm and cold seasons. Cooler sea surface temperatures (< 24.2ºC) were recorded from December to June, and the warm period (>24.2ºC) extended from July to November (Figure 4-2a). Mean surface chlorophyll *a* concentrations revealed a high value of 1.9 ± 0.1 mg m^{-3} during May 2001, with a decrease during the warm months to 1.4 ± 0.1 mg m^{-3} in July, and a minimum of 0.6 ± 0.1 mg m^{-3} in October. Finally, at the beginning of the cold period in February 2002, the mean chlorophyll-*a* concentration increased to 2.0 ± 0.2 mg m^{-3} (Figure 4-2b). Diatom abundance data from 1998–1999 (Villalejo-Fuerte *et al.,* 2005) also showed higher abundances in colder months and lower abundances during warmer months, *i.e.*, a similar pattern to the observed changes in chlorophyll-*a* during the 2001–2002 period. (Figure 4-2b).

4.4.2. Length, weight, and body condition

Seasonal differences in morphometric data and biochemical content of males of *Nyctiphanes simplex* in the area surrounding Bahía de La Paz are summarized in Table 4-1. Males collected during colder months (February and May) were larger on average (>10.6 mm) than those collected during warmer months (July and October, <10.2 mm). However, lower dry weight was recorded in specimens collected in May and July (~1.2 mg ind^{-1}), and higher dry weight in samples collected in October and February (~1.8 mg ind^{-1}), suggesting differences in biochemical composition within warm and cold months.

Although larvae (nauplii, pseudometanauplii, metanauplii, calyptopis, and furcilia) and subadult males were not analyzed in this study, morphometrical data of those forms were

included together with data of adult males to calculate the relationship between dry weight (*DW*) and total length (*TL*). Given the properties of the equation $DW = \gamma\ TL^{\beta}$, lack of information at early stages has larger impacts on allometric relationships than underrepresented mature stages, especially with small sample sizes. Regression was significant ($R^2 = 0.94$; $F_{1, 220} = 3663$, $p < 0.05$), with an allometric exponent of 2.96 ± 0.1 at 95% confidence interval.

Log *DW* = 2.9586 (Log *TL*) –2.7849

or

$DW = 0.00164\ TL^{2.9586}$

Body condition (*BC*) was defined as the ratio between the observed and the estimated *DW* for a given *TL*:

$BC = DW_o / DW$

where DW_o is the observed dry weight and *DW* is the average estimated from the *DW vs TL* regression model. Accordingly, body condition displayed significant differences among all sampled months ($F_{3, 162} = 79.94$), with the lowest value occurring during the winter-spring transition (May) and the highest at the end of the summer (October).

4.4.3. Biochemical composition and energy content

Biochemical composition of mature males changed during the four sampling periods (Table 4-2), with the exception of structural lipids that did not show significant changes throughout the year, neither in terms of absolute concentrations ($F_{3, 101} = 2.06$, $p = 0.11$ for μg ind^{-1}), nor in relative contribution to dry weight ($F_{3, 101} = 0.20$, $p = 0.89$ for % *DW*). The dominance of neutral lipids over polar lipids seems consistent with previous reports of lower phospholipid content in crustaceans living in warmer conditions, whereas a marked increase in the concentration of phosphatidylethanolamine is observed when crustaceans are acclimated to lower temperatures

(Chapelle *et al.*, 1979 and references therein). Carbohydrates provided the lowest contribution to total energy content with a small but significant difference between February and May-July. Storage lipid content exhibited a similar pattern to that observed for total length, increasing in February and May and decreasing in July and October (Table 4-2). The lowest protein content occurred in May and the highest in October, with moderate values in the intermediate months of July and February. Throughout the year, proteins were the largest proportion of dry weight, so that calculated energy content was almost always a direct function of protein and body condition variability.

4.4.4. Fatty acids

According to PERMANOVA, there were statistically significant differences in the fatty acid profiles of neutral lipids of adult males sampled among seasons (Pseudo-$F_{3,15}$=5.3496, P_{MC}=0.0003), whereas within sampling periods, samples had moderate to high similarities (86–90%) in neutral lipids. SIMPER analysis and pair-wise tests (Appendix 4-2) show that during the transition from colder to warmer conditions (May–July) the dissimilarities in fatty acid profiles were moderate (~17%) but significant (t=2.5923, P_{MC}=0.0063), mostly caused by strong decreases in 20:5ω3 (20 to 12%) and 16:1ω7 (5 to 2%), together with moderate increases in 22:6ω3 (20 to 21%), 16:0 (23 to 27%), and 18:0 (5 to 9%). During the warm period (July–October) and during the transition from warmer to colder conditions (October–February) the dissimilarities were slightly smaller (<16%) and not significant (t<1.6, P_{MC}>0.12). Surprisingly, the largest dissimilarity (~23%) occurred between sample sets collected during cold conditions: May 2001 and February 2002 (t=3.3026, P_{MC}=0.0038), and were characterized by a strong decrease in 20:5ω3 (20–11%), 22:6ω3 (20–14%), and 16:1ω7 (5–2%); together with a strong increase in 16:0 (23–31%) and 18:0 (5–8%). This suggest that, on an annual cycle, contribution

of diatom as prey for *N. simplex* peaks during May and decreases gradually throughout the warmer months and part of the colder months that follow, before peaking again in May (Table 4-3, Figure 4-3).

According to univariate analyses, during May, at the end of the colder period, the proportions of the phytoplankton markers 16:1ω7 and 18:1ω7, and the diatom markers 14:0 and 20:5ω3 and the EPA/DHA ratio (the inverse of the 22:6ω3/20:5ω3 ratio) were higher than in the other three seasons, whereas the copepod marker 20:1ω9 remained at low proportions. During spring and summer (May–July), the dinoflagellate marker 22:6ω3 comprised a significantly higher proportion of the fatty acid profile than in winter (February). In summer two minor fatty acids (16:1ω9 and 18:3ω3) also reached higher proportions, and the 22:6ω3/20:5ω3 ratio reached its highest value (~1.8). The summer–fall transition and the winter were depicted by an increase in the proportion of some saturated fatty acids (17:0, 18:0 and 20:0 in October, and 12:0, 15:0, 16:0 in February). By winter, there was also an increase in some polyunsaturated fatty acids (16:2ω4, 16:3ω3, 18:2ω6, 18:4ω3) and a decrease in others such as the arachidonic acid (20:4ω6 or ARA). The fatty acid profile of neutral lipids is summarized in Table 4-3.

According to PERMANOVA, there were no significant differences in the fatty acid profile of polar lipids among seasons (Pseudo-$F_{3,14}$=1.8888, P_{MC}=0.1148). The univariate analysis shows that, throughout the year, there were no significant differences in the total contribution of fatty acids from polar lipids to *DW* (μg mg^{-1} or %*DW*). Significant changes occurred mostly in minor (1–3%) fatty acids that were mostly saturated, but also monounsaturated and a few polyunsaturated fatty acids (mainly of the ω6 family). Few major fatty acids had significant variation among seasons and therefore fatty acid ratios in polar lipids were not very useful. For instance, the saturated 16:0 and 18:0 comprised respectively >22% and >6% of the fatty acid

profile of polar lipids in May and July, whereas in October and February they comprised <22% and <6% respectively. However, the variability for 16:0 fatty acid was very high (CV=27%) in May (Table 4-4). In contrast the relative contribution of the monounsaturated 18:1ω9 to the fatty acid profile of polar lipids increased from <8% in May to > 9% in October. In general, there were no significant differences in the individual relative contribution of essential fatty acids (ARA, EPA & DHA) to the fatty acid profile of polar lipids in adult males of *N. simplex* (Table 4-4). However, the EPA/DHA ratio exhibited a small, but significant decrease from May to October.

4.5. Discussion

4.5.1. Physical and biological conditions: Possible effects on reproductive strategy

The study of males throughout the year avoided the effect of different life stages or variable degrees of gonadal maturity on biochemical composition. This is particularly obvious in females, where oogenesis is better known than spermatogenesis (Gómez-Gutiérrez *et al*., 2010; Chapter 2, this thesis). In fact, it is typically assumed that energy expense for male reproduction is negligible, but this perspective is likely the result of little research on the subject. In this study, storage lipid content of *Nyctiphanes simplex* related well to the male reproductive peak of the species, suggesting that lipid assimilation in males is enhanced by the increase in food abundance and might enhance the synchronicity of the reproductive process for the population in the Gulf of California.

According to Pardo *et al*. (2013) surface chlorophyll-a concentration in the southwestern gulf follows a bimodal pattern with a maximum in January, a decrease in March, a secondary peak at the end of May, and the main minimum in September. However, this pattern can shift one or two months from year to year. Using satellite surface chlorophyll-a concentrations provided by the

SeaWiFS Project, NASA/Goddard Space Flight Center and ORBIMAGE, I estimated that during the cold months, chlorophyll-a values were higher in February 2002 (2.3±0.6 mg m^{-3}), and May 2001 (1.94±0.2 mg m^{-3}). In contrast, chlorophyll-a values for July 2001 (1.4±0.1 mg m^{-3}) and October 2001 (0.7±0.1 mg m^{-3}) were lower in Bahía de La Paz. Monthly phytoplankton abundances per taxonomic group (diatoms, dinoflagellates, silicoflagellates, and nanoplankton), collected in Bahía de La Paz between 1998 and 1999 (Villalejo-Fuerte *et al*., 2005) (Figure 4-2b, c) were assumed to represent typical changes in phytoplankton assemblage throughout the year. In general, diatom abundance showed a similar pattern to the observed changes in surface chlorophyll-a concentration during 2001–2002. However, for the 1998–1999 period, diatom concentration remained at similar levels between July and October (32–37 × 10^3 cells L^{-1}) whereas surface chlorophyll-a concentration in 2001 was particularly low in October compared to July (Figure 4-2b). This disparity between diatoms and surface chlorophyll-*a* might either be a consequence of interannual differences in the area, or a consequence of interaction between higher irradiance and vertical distribution of diatoms (migration to deeper layers) or reduction in chlorophyll-a production.

According to several reports, diatoms dominate the phytoplankton assemblage year-round (Signoret & Santoyo, 1980; Lavaniegos-Espejo & López-Cortes, 1997; Martínez-López et al., 2001; Villalejo-Fuerte *et al*., 2005), although density increases particularly from February to June (Figure 4-2b). In contrast, dinoflagellate, silicoflagellate and nanoplankton abundances increased from July to January (Figure 4-2c).

Maximum and minimum values of diatom abundance and surface chlorophyll-*a* concentration observed respectively during colder and warmer months can be explained by changes in local oceanographic processes in Bahía de La Paz. Intensification of the northwest

winds during colder months produces a coastal cyclonic current and a cyclonic gyre, causing divergence at the surface and upwelling (Jiménez-Illescas *et al.,* 1997). Additionally, the combination of northern winds with atmospheric cold fronts in winter contribute to mixing process in the water column, increasing nutrient availability, as well as phytoplankton abundance, particularly diatoms (Martínez-López *et al.,* 2001). During warmer months southeast winds reverse the current patterns at sea, causing occasional short-term anticyclonic gyres and convergence at the surface at the bay (Jiménez-Illescas *et al.,* 1997), whereas high irradiance promotes a strong stratification of the water column that diminishes the vertical transport of dissolved and particulate material from deep layers (Martínez-López *et al.,* 2001). Additionally, during summer the southern region of the Gulf of California is dominated by less productive subsurface subtropical waters from the Pacific Ocean (Obeso-Nieblas *et al.,* 2004; Sánchez-Velasco et al., 2006).

4.5.2. Colder months

Larger average total lengths of *N. simplex* (Table 4-1) and accumulation of storage lipids during colder months (Table 4-2) are consistent with favourable feeding conditions, and with the intense reproductive period reported from February to April (Brinton & Townsend, 1980; De Silva-Dávila & Palomares-García, 1998; Gómez-Gutiérrez *et al*., 2012). Additionally, high chlorophyll-*a* concentrations during the cold season (Figure 4-2b), are consistent with increased diatom abundances (Lavaniegos-Espejo & López-Cortes, 1997; Villalejo-Fuerte *et al.,* 2005*;* Pardo *et al.,* 2013), and indicate the presence of the winter-spring diatom bloom. In other studies (Chapter 3, Appendix 3-4), I found that proportions of 14:0 >4% and 20:5ω3 >20% are likely indicative of diatom consumption for *N. simplex*. Similar proportions of diatom biomarkers in storage lipids were only observed at the end of the colder months (May; Table 4-3), suggesting

that, at the end of the winter, *N. simplex* feed mostly on diatoms (either directly or indirectly through diatom feeders). In February, those diatom biomarkers were not as high as in May (Table 4-3), despite the high levels of chlorophyll-*a* in the area and high lipid content in krill collected (Figure 4-2b; Table 4-2). Still, the high levels of the C_{16}PUFA index (>3%) observed in February (Table 4-3) might indicate a different physiological condition of diatoms in winter. The enzymes responsible for C_{16} PUFA production reside in the inner chloroplast, and evidence suggests that the most abundant C_{16} PUFA differs among diatoms, green algae and higher plants (Khozin-Goldberg, 2016). Under nutrient-rich conditions their chloroplast production increases (and the concomitant chlorophyll-*a* in chloroplasts), and enzymes that synthesize those C_{16}PUFA also increase. Once nutrients are consumed (particularly nitrogen), the stationary growth-phase occurs and enzyme production rate becomes slower, stalling production of C_{16}PUFA. This is consistent with the use of high levels of C_{16}PUFA as indicative of early growth stages (exponential phase) in diatoms (Parrish *et al.,* 2009).

Total length and dry weight, two relatively simple morphometric features, were useful for determination of body condition of male *N. simplex*, mirroring major seasonal variations in protein and energy content, but not sensitive enough to follow smaller variations in lipid content. Hence, body condition can be used, but with caution, as a general index of health condition of *N. simplex*. For instance, although reproduction can be an energy-demanding process in both sexes (Ross & Quetin, 2000; Chapter 2, section 2.5.4), the intense reproductive activity at the end of the diatom bloom in the region does not explain by itself the low *DW* and the consequent low *BC* of large-sized males during the winter-spring transition (May). This decrease was mostly an effect of low protein content (Table 4-2), which is consistent with a predominantly non-carnivorous diet, and suggests a direct intake of diatoms. Based on the low C_{16}PUFA index (<

1.5%) observed in May (Table 4-3) it is probable that diatoms were at the stationary growth-phase.

4.5.3. Warmer months

During summer (July), high temperatures, strong stratification, low phytoplankton densities, and low chlorophyll-*a* values prevailed (Figure 4-2), suggesting overall poor feeding conditions. Still, average length of males was smaller but dry weight was similar to the previous season. Smaller animals with higher protein content resulted in slightly better body condition than that observed in May (Table 4-1; Table 4-2; Figure 4-2D).

Some observations support the idea that decrease in body size is a physiological response that allows survival in summer conditions for the overall population of *N. simplex* in the southern Gulf of California. For instance, daily instantaneous growth rates, estimated from shipboard incubations (Gómez-Gutiérrez *et al.,* 2012), indicated that the number of individuals of *N. simplex* that did not grow or shrunk in summer months was relatively higher than in winter months (January and November). Other euphausiid species, such as *Nyctiphanes australis* shrunk in total length under laboratory conditions after feeding a deficient diet (Hosie & Ritz, 1989), or after starvation (Iguchi & Ikeda, 1995), and similar responses to the lack of food in *E. superba* have been interpreted as a decrease in metabolic demand (Ikeda & Dixon, 1982; Thomas & Ikeda, 1987; Nicol *et al.,* 1992).

However, lack of adequate food is probably not the only reason for body shrinkage in this subtropical species. During summer, *N. simplex* can also experience unfavourable physiological condition (oxidative stress) in response to high temperature or relatively low oxygen concentration (Tremblay *et al.,* 2010), and some temperate species such as *E. pacifica* can shrink if exposed to high temperatures, even when food supply is abundant (Buchholz, 1985; Marinovic

& Mangel, 1999). This process can explain the presence of small animals with high protein content during summer months.

In October, during the warm-cold transition, chlorophyll-*a* values decreased and total phytoplankton abundance declined in Bahía de La Paz (Figure 4-2), males exhibited small sizes (Table 4-1) and low neutral lipid content in both absolute and relative terms (Table 4-2). Here, energy content was higher and body condition improved as a consequence of the increase in dry weight and protein content (Table 4-2), suggesting the use of alternative food sources during this transitional period. Such behaviour would be consistent with the euryphagous habits reported for *N. simplex* in Peruvian waters (Kanaeva & Pavlov, 1976). Apparently, other plankton sources might play an important role in the feeding strategy of *N. simplex* after the end of the diatom bloom (Fig. 4.2c). However, alternative food sources can be different from year to year or among regions: For instance, in 1998–1999, dinoflagellates, silicoflagellates, and nanoplankton increased from July to January (Fig. 4-2c). In contrast, biomass production of *N. simplex* in the Gulf of California during 2005 and 2007 was better associated with alloxanthin than with chlorophyll *a* or *b* concentrations (Gómez-Gutiérrez *et al.,* 2012). The same authors also report a weak but significant association with zeaxanthin. Both alloxanthin and zeaxanthin are accessory carotenoid pigments, the former commonly found in microalgae of the division Cryptophyta, whereas the latter is found in autotrophic prokaryotes (Vidussi *et al.,* 1996). Such results are not necessarily conflicting; autotrophic prokaryotes such as cyanobacteria are commonly found in the nanoplankton fraction, however, not all nanoplankton can be assumed to be cyanobacteria.

4.5.4. Fatty acids from neutral *vs* polar lipids

In the present study, fatty acids from neutral and polar lipids of *N. simplex* were analyzed separately, giving additional information compared with total fatty acid extraction. For instance,

fatty acid profiles in polar lipids, in particular essential fatty acids (EFA), were not significantly different among seasons (Table 4-4), suggesting that the constancy of the observed values is fundamental to krill phospholipid structures. ARA averaged 2.7%, EPA 10.5% , DHA 27.5%, and the Σω3/Σω6 7.5. Still, some indices like the 22:6ω3/20:5ω3 ratio exhibited significant changes among seasons (Table 4-4). Those changes should be considered moderate (~20%) as they arise from slight and non-significant changes in both DHA and EPA. In contrast, the fatty acid profiles in neutral lipids showed significant differences among seasons for almost all fatty acids, including EFA (Table 4-3), despite their tendency to be preserved in biological systems. Here, the 22:6ω3/20:5ω3 ratio exhibited significant and pronounced (>45%) changes among seasons suggesting strong changes in diet. Accordingly, the separate analysis of fatty acids from neutral and polar lipids provides valuable physiological information on required EFA levels and ratios in membranes as well as on their ecological use as trophic markers.

4.5.5. Predominant food sources during the warm period revealed by fatty acid biomarkers

Dinoflagellates

In subtropical environments, several dinoflagellate species have the ability to switch from photosynthetic to heterotrophic trophic strategy, a strategy called mixotrophy (Faust, 1998). Mixotrophy can provide considerable amounts of protein to dinoflagellates (John & Flynn, 1998), which together with carnivory might explain higher protein content during summer for *N. simplex*. Dinoflagellates typically have high percentages of 18:4ω3, 18:5ω3, and especially 22:6ω3, but are deficient in 16:1ω7 (Falk-Petersen *et al.,* 2000*;* Virtue *et al.,* 2000). In storage lipids of *N. simplex*, 18:4ω3 and 22:6ω3 had different seasonal patterns (Table 4-3): the highest proportions of 18:4ω3 occurred in February, when the lowest proportion of 16:1ω7 and 22:6ω3 were observed. In contrast, males of *N. simplex* exhibited higher proportions of 22:6ω3 in July,

and the dinoflagellate marker 22:6ω3/20:5ω3 (Budge & Parrish, 1998; Pepin *et al*., 2011) increased significantly (almost twice) from May to July, decreasing afterwards.

Accordingly, both the increase in protein content and the increase in 22:6ω3/20:5ω3 markers indicate that dinoflagellates are frequent prey contributing to the survival of *N. simplex* in summer. Experimental work is necessary to determine if *N. simplex* prefers dinoflagellates over diatoms. However, a study on preferences must consider if subtropical krill species have limited capacity to detect and avoid unpalatable food as *Nyctiphanes australis* does, detecting and avoiding unpalatable prey like the chlorophytes *Dunaliella* and *Nannochloris*, whereas being susceptible to the toxic dinoflagellate *Alexandrium minutum* (Haywood & Burns, 2003). In this sense, a study on prey preferences of *N. simplex* which also investigates if dinoflagellates promote higher increases than diatoms in secondary productivity (growth, molt, and reproduction) might be more conclusive.

Zooplankton

Seasonal changes in species richness and assemblage structure of the main zooplankters in the area suggest major changes at the base of the trophic web after the spring bloom. According to Signoret & Santoyo (1980), phytophagous copepods related to the post bloom period contribute largely to the highest densities of zooplankton observed in May. In August, the abundance of carnivorous forms of zooplankton increases, despite the decline in overall zooplankton density. By December, community's structure becomes more homogeneous, but ovigerous copepod females dominate the community almost entirely.

Carnivorous copepods often produce considerable amounts of SFA, especially 16:0. In these species, fatty acid biosynthesis is quite simple, and it typically ends with carbon chain elongation to 18:0, which is almost completely desaturated to 18:lω9. This last fatty acid is used as a general

marker of carnivory taking into account that it is a major FA in most marine animals. In addition, the 18:1ω7/18:1ω9 ratio has been used in marine pelagic predators to distinguish herbivores from carnivores (Dalsgaard *et al*., 2003).

Although the abundance of *N. simplex* in Bahía de La Paz has been associated with high zooplanktonic biomass throughout the year (De Silva-Dávila & Palomares-García, 2002), the low protein content during May (Table 4-2) suggest that during spring, *N. simplex* does not rely largely on animal intake. In contrast, the high protein content observed in males of *N. simplex* collected in October and February (Table 4-2) might indicate animal intake at the summer-winter transition and during early winter. However, the fatty acid profile does not provide strong evidence towards the intake of carnivorous forms of zooplankton in late summer and early winter. In the present study, there were no significant changes throughout the year in the contribution of 18:1ω9 to the overall profile of neutral lipids nor in the 18:1ω7/18:1ω9 ratio, aside from a slight, non-significant decrease in the 18:1ω7/18:1ω9 ratio during October (Table 4-3).

On the other hand, predominantly herbivorous copepods (like *Calanus* and *Calanoides*), are able to elongate 18:1ω9 into 20:1ω9 and 20:1ω11 into 22:1ω11. Accordingly, calanoid copepods contain considerable amounts of C_{20} and C_{22} monounsaturated fatty acids and fatty alcohols biosynthesized *de novo*, and these moieties can be recognized (as FA) in copepod predators (Sargent & Falk-Petersen, 1988). The presence of 20:1ω9 has also been observed in feeding experiments of the tropical copepod *Centropages furcatus* from Bahía de La Paz, irrespective of the diet provided (Band-Schmidt *et al*., 2009). In the present work, the neutral lipid fraction of males of *N. simplex* increased 20:1ω9 more than twice (0.4–1.0%) from May throughout the year

into the next winter (February), suggesting that at least during early winter, herbivorous copepods might be part of the diet of *N. simplex*.

Terrestrial input in the marine trophic web

It has been suggested that the sum of 18:2ω6 + 18:3ω3 is either a direct or indirect indicator of terrestrial input in the diet of some invertebrates (Budge & Parrish, 1998; Pepin *et al*., 2011). This index increased in July and was significantly higher than the ratio observed in October. According to the daily weather data of the CLICOM-SMN (National Weather Service in Mexico), obtained via the web platform at CICESE (http://clicom-mex.cicese.mx), during 2001, mean precipitation around Bahía de La Paz was 0.9 mm in May, 47.3 mm in July, and 12.3 in October. In February 2002, mean precipitation was 15.0 mm. Consequently, the precipitation data seem to confirm that the increase in the 18:2ω6 + 18:3ω3 is, in fact, evidence of an increase in terrestrial input into the area.

Other sources

Knowledge of fatty acid composition in less-known marine heterotrophs has increased in recent years. This enhances the possibility to infer their use as prey for other taxonomic groups. For instance, some species of estuarine oomycetes (fungus) do not produce any 22:6ω3, but the percentage of 20:4ω6 (7–25%), and of 20:5ω3 (0.01–18.42%) can be quite high depending on the strain (Lee Chang *et al*., 2012*;* Pang *et al*., 2015). Similarly, thraustochytrids (heterotrophic protists ubiquitous in the marine environment), tend to have high contents of 22:6ω3 (~19–61%), and depending on the strain, low or high contents of 20:4ω6 (0.4–12%) and 20:5ω3 (~2–11%) (Lee Chang *et al*., 2012). Thraustochytrids also had unusually high proportions (~9%) of ω6DPA (22:5ω6) which acts as an essential fatty acid, at least for early life stages of certain marine fauna (Parrish *et al*., 2007). To the date, the study of such taxonomic groups is absent in Bahía de La

Paz or in the Gulf of California. However, environmental conditions required for their presence (temperate to tropical, salt-rich estuarine environments) are likely fulfilled in the study area. Further research is required (coupled with analysis of soil or dust) to evaluate the role of such groups in trophic food webs and the scope of their area of influence, which might explain larger quantities of 20:4ω6 in October, at the end of the hurricane season.

4.6. Conclusions

Males of *N. simplex* exhibited high content of neutral (storage) lipids and longer total lengths (~12%) during colder months (February and May), suggesting better feeding conditions consistent with the documented diatom blooms and the bimodal peaks in chlorophyll-*a* reported for the area during the same period. Apparently, availability of lipid-rich food promotes faster growth (mostly as increase in size with shorter intermolt periods). Previously published data have shown that the proportion of *N. simplex* individuals with positive growth was higher in January (50%) than in July (27%) (Gómez-Gutiérrez *et al*., 2012). The estimated lifespan for *N. simplex* is ~8 months (Lavaniegos-Espejo, 1992), and therefore, size differences for animals collected in warmer months are likely a consequence of sampling different cohorts growing under less favourable feeding conditions.

Specimens collected during the winter-spring transition and early summer (May and July) had a dry weight ~30% lower than that of males collected during the summer-fall transition and early winter (October and February). The lowest *BC* was observed during the winter-spring transition (large animals with low *DW*) and the highest *BC* at the end of the summer (small animals with high *DW*). This difference in body condition seems related to protein content, as the lowest protein content also occurred in May and the highest in October, with moderate values in the intermediate months (small animals with low *DW* in July and large animals with high *DW* in

February). Proteins constituted the largest proportion of dry weight throughout the year, so that calculated energy content for males was almost a direct function of protein content and body condition.

Fatty acids in storage lipids clearly varied with season, and while feeding conditions were less favourable during warmer months, several biomarkers indicate alternative food sources after the diatom bloom playing an important role in survival of *N. simplex* in this area.

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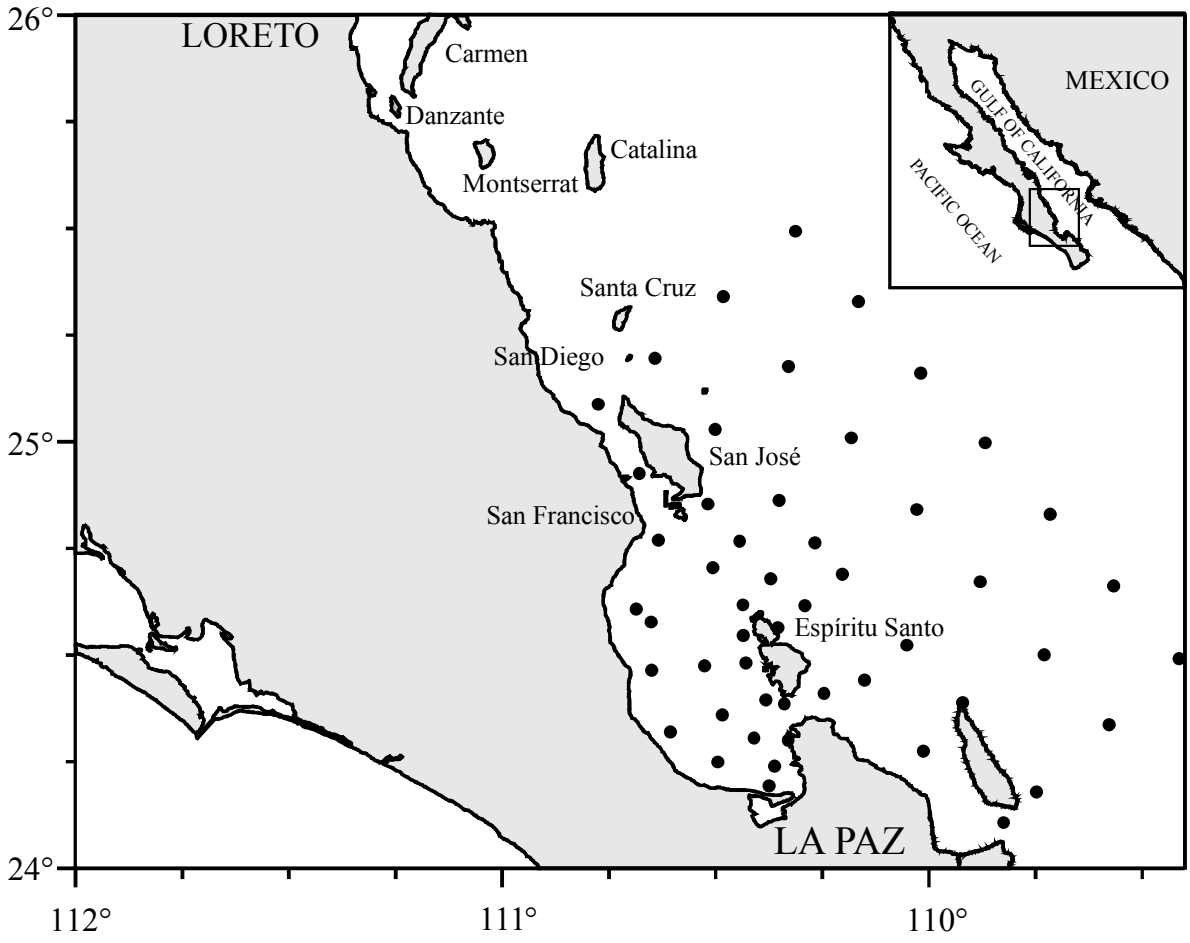


Figure 4-1 Map of the study area showing the closest towns (La Paz and Loreto), and main islands. Dots represent the stations recurrently sampled throughout the four surveys.

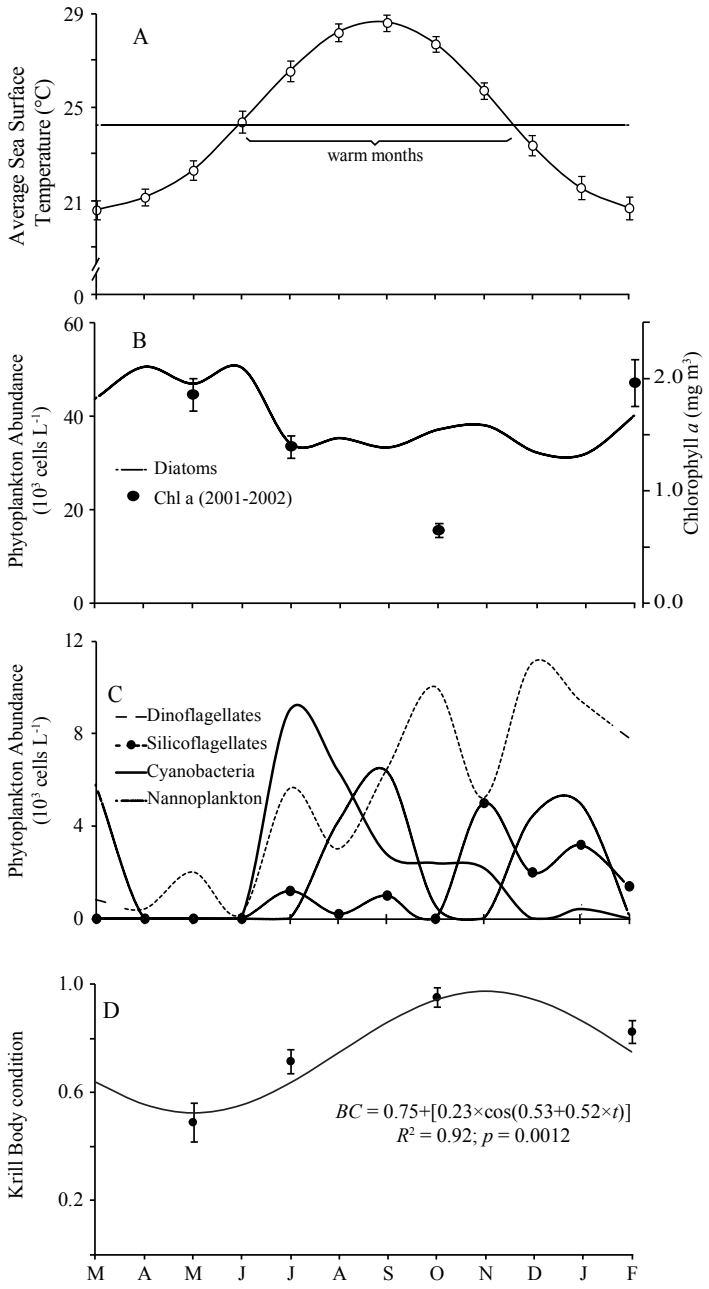


Figure 4-2 Relationships among: A) Sea surface temperature (1982–2000), B) Monthly diatom abundance (1998–1999) and chlorophyll *a* concentration (2001–2002); C) Monthly abundance of other groups of phytoplankton (1998–1999), and D) Body condition of males of *Nyctiphanes simplex* (2001–2001) and least–squares adjustment to a cosine function. Horizontal line in a) represents the average temperature (24.2ºC) for the complete time series. Vertical lines in A), B) and D) represent the 95% confidence interval for the mean. Phytoplankton data on B) and C) from Villalejo-Fuerte *et al*. (2005), with permission.

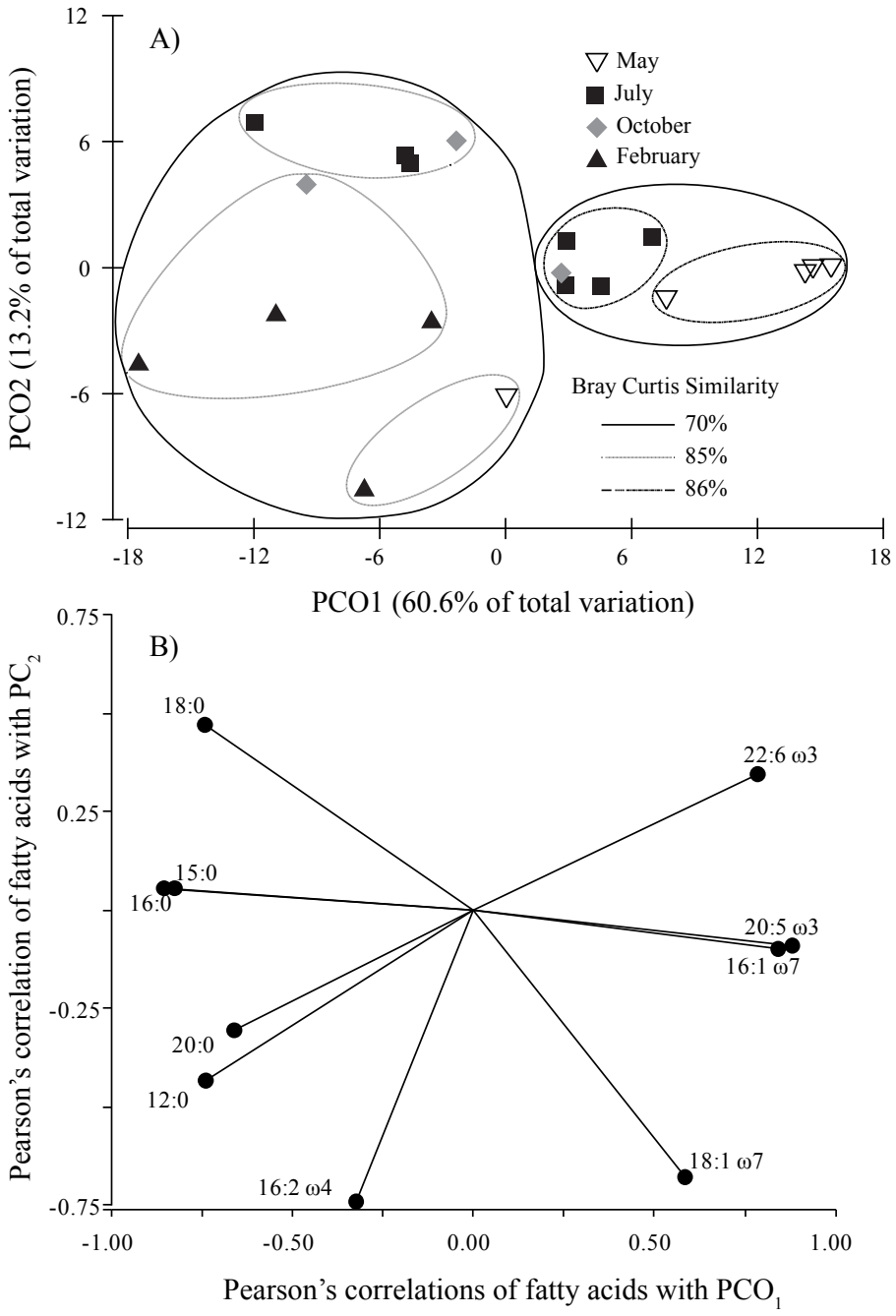


Figure 4-3 Principal coordinates analysis (PCO) for fatty acids in neutral lipids of mature males of *Nyctiphanes simplex*. A) Score plot of males collected in May, July and October 2001, and February 2002, B) Significant (≥ 0.7) Pearson correlations of major fatty acids (> 1%) with the first two principal coordinate axes.

Table 4-1 Total length, dry weight and body condition of mature males of *Nyctiphanes simplex* per sampling season. Mean ± 95% C.I.

	Sample size (n)	Total Length (mm)	Dry Weight (mg)	Body Condition
May 2001	16	11.0± 0.5 [b]	1.3± 0.3 [a, b]	0.55± 0.06 [a]
July 2001	41	9.4± 0.3 [a]	1.2± 0.2 [a]	0.79± 0.04 [b]
October 2001	64	9.6± 0.3 [a]	1.7± 0.1 [b, c]	1.06± 0.03 [d]
February 2002	45	10.4± 0.3 [b]	1.9± 0.2 [c]	0.92± 0.04 [c]

Univariate ANOVA test were performed to analyze differences among groups. Significant differences ($p<0.05$) between means are indicated with a different superscript in the same column.

2400 **Table 4-2 Biochemical composition of mature males of *Nyctiphanes simplex* by sampling season. Mean ± 95% C.I.**

		Protein		Neutral Lipids		Polar Lipids		Carbohydrates	Energy
	(n)	(µg ind^{-1})	(n)	(µg ind^{-1})	(n)	(µg ind^{-1})	(n)	(µg ind^{-1})	(J ind^{-1})
May 2001	16	593 ± 94 ^{a}	15	170 ± 19 ^{b}	18	97 ± 27	20	27 ± 3 ^{c}	23 ± 4 ^{a}
July 2001	40	797 ± 60 ^{b}	60	126 ± 10 ^{a}	57	78 ± 15	44	21 ± 2 $^{b, c}$	25 ± 2 $^{a, b}$
October 2001	12	1077 ± 109 ^{c}	12	126 ± 21 ^{a}	12	88 ± 34	8	18 ± 5 $^{a, b, c}$	31 ± 4 ^{b}
February 2002	60	806 ± 49 ^{b}	12	172 ± 21 ^{b}	18	111 ± 27	60	15 ± 2 ^{a}	28 ± 3 $^{a, b}$
		(% of DW)		(% of DW)		(% of DW)		(% of DW)	(J mg^{-1})
May 2001		45 ± 5 ^{a}		12 ± 2 $^{a, b}$		7 ± 1		1.8 ± 0.3 $^{b, c}$	16 ± 2 ^{a}
July 2001		65 ± 3 ^{b}		11 ± 1 $^{a, b}$		7 ± 1		1.9 ± 0.2 ^{c}	21 ± 1 ^{b}
October 2001		66 ± 6 ^{b}		9 ± 2 ^{a}		6 ± 1		1.5 ± 0.4 $^{a, b, c}$	20 ± 2 $^{a, b}$
February 2002		41 ± 3 ^{a}		14 ± 2 ^{b}		7 ± 1		1.0 ± 0.1 ^{a}	16 ± 2 $^{a, b}$

Univariate ANOVA test were performed to analyze differences among groups. Significant differences (p<0.05) between means are indicated with a different superscript in the same column. Sample size (n) precedes each variable.

Table 4-3 Relative contribution (%) of fatty acids from storage lipids of *Nyctiphanes simplex* males by sampling season. Mean ± 95% C.I.

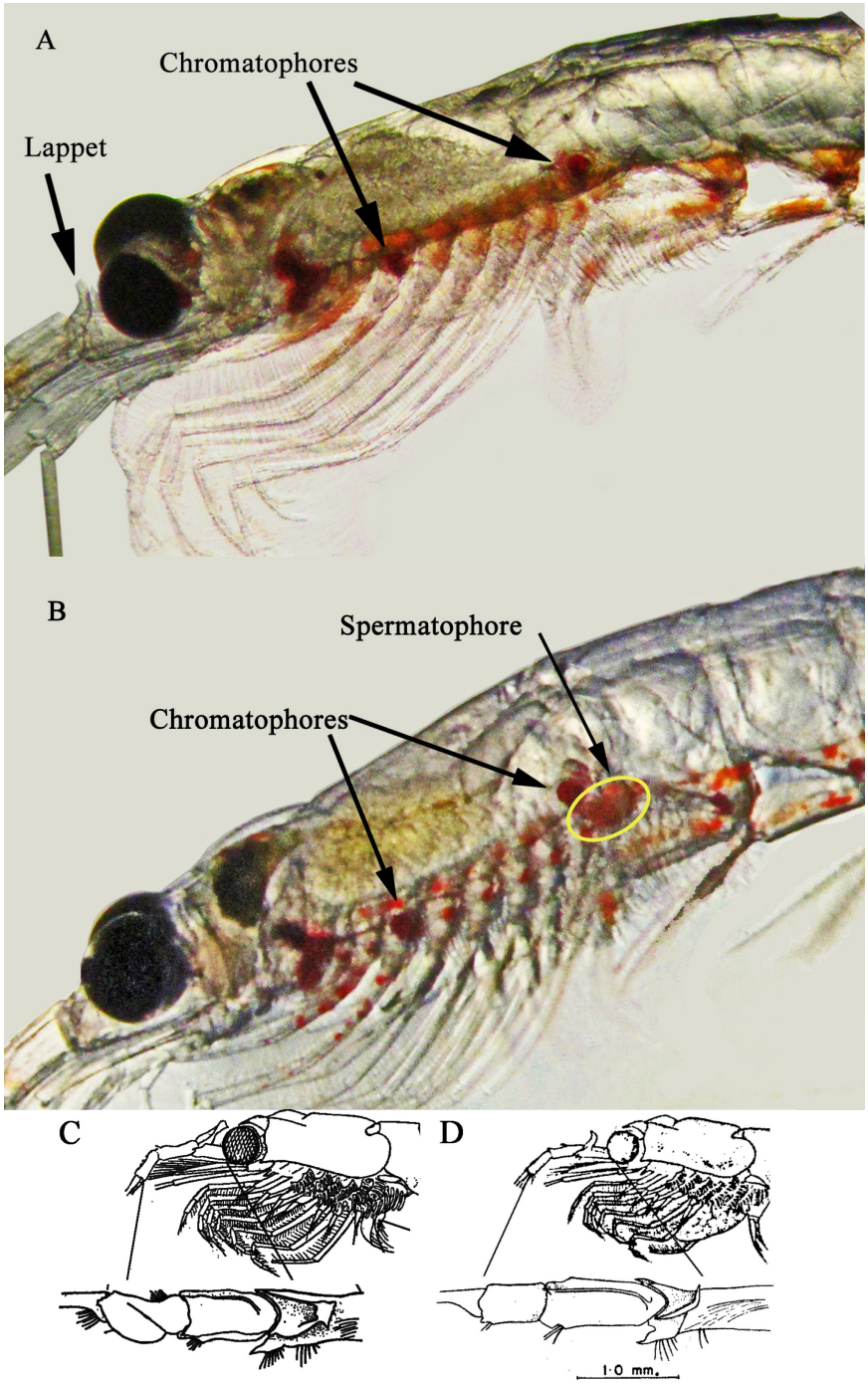
Fatty Acid	May n=5	July n=7	October n=4	February n=4	
12:0	0.1± 0.1	0.4± 0.1	0.3± 0.1	**1.4**± 0.5	sig
14:0	**4.1**± 0.4	4.1± 0.8	3.4± 0.2	3.5± 0.6	sig
15:0	0.7± 0.0	0.9± 0.1	1.0± 0.2	**1.3**± 0.3	sig
16:0	23± 1	27± 1	28± 3	**31**± 4	sig
17:0	0.9± 0.1	1.1± 0.0	**1.2**± 0.1	1.1± 0.2	sig
18:0	5± 2	9± 3	**14**± 6	8± 1	sig
20:0	0.6± 0.4	0.4± 0.1	**1.2**± 0.8	0.9± 0.3	sig
Saturated[a]	36± 4	46± 5	**52**± 7	50± 7	sig
16:1ω7	**5.1**± 0.6	2.4± 0.3	1.6± 0.4	1.5± 0.2	sig
16:1ω9	0.4± 0.1	**1.5**± 0.4	1.4± 0.4	1.0± 0.3	sig
18:1ω7	**2.6**± 0.2	2.0± 0.3	1.9± 0.4	2.2± 0.2	sig
18:1ω9	7± 2	6± 1	8± 3	7± 2	n.s.
20:1ω9	0.4± 0.1	0.7± 0.1	0.7± 0.3	**1.0**± 0.3	sig
Monounsaturated[b]	16± 2	13± 1	15± 3	13± 2	n.s.
16:2ω4	0.7± 0.3	0.5± 0.2	0.4± 0.3	**3.5**± 2.2	sig
18:2ω6	1.9± 0.1	1.9± 0.2	1.4± 0.3	**2.1**± 0.8	sig
16:3ω3	0.3± 0.0	0.2± 0.1	0.2± 0.1	**0.8**± 0.2	sig
18:3ω3	1.2± 0.1	**1.8**± 0.3	1.1± 0.3	1.1± 0.3	sig
18:4ω3	0.6± 0.1	0.8± 0.3	1.0± 0.7	**1.6**± 0.4	sig
20:4ω6	3.2± 0.4	2.3± 0.4	**3.3**± 0.8	2.1± 0.4	sig
20:5ω3	**19**± 2	12± 1	9± 4	11± 2	sig
22:6ω3	20± 3	**21**± 3	15± 6	14± 3	sig
Polyunsaturated[c]	**48**± 5	41± 4	32± 10	37± 5	sig
Σω3	**42**± 5	36± 4	27± 8	29± 5	sig
Σω6	5.6± 0.5	4.7± 0.6	5.4± 0.9	5.2± 1.0	n.s.
ΣC16 PUFA	1.0± 0.4	0.7± 0.3	0.6± 0.3	**4.3**± 2.0	sig
ΣC18 PUFA	3.8± 0.2	4.6± 0.6	3.5± 0.7	**5.0**± 0.9	sig
ΣSFA/ΣPUFA	0.8± 0.2	1.2± 0.3	**2.0**± 1.0	1.5± 0.4	sig
16:1ω7 + 20:5ω3	**24**± 3	14± 1	11± 4	13± 2	sig
16:1ω7/16:0	**0.23**± 0.03	0.09± 0.02	0.06± 0.02	0.05± 0.01	sig
ΣC16/ΣC18	1.7± 0.3	1.5± 0.1	1.3± 0.3	1.8± 0.3	n.s.
C16 PUFA index[d]	0.03± 0.01	0.02± 0.01	0.02± 0.01	**0.12**± 0.06	sig
18:1ω9 + 18:4ω3	8± 2	9± 3	7± 1	8± 2	n.s.
18:1ω7/18:1ω9	0.42± 0.09	0.32± 0.03	0.27± 0.09	0.37± 0.07	n.s.
18:2ω6 + 18:3ω3	3.2± 0.2	**3.8**± 0.4	2.5± 0.4	3.3± 1.0	sig
Σω3/Σω6	7.5± 0.6	**7.7**± 0.5	4.8± 0.9	6.0± 1.3	sig
22:6ω3/20:5ω3	1.0± 0.1	**1.8**± 0.2	1.5± 0.2	1.2± 0.1	sig
EPA/DHA	**1.0**± 0.1	0.6± 0.1	0.7± 0.1	0.8± 0.1	sig
Total μg mg^{-1}	**104**± 15	35± 7	23± 6	45± 7	sig
% DW	**10**± 1	3± 1	2± 1	4± 1	sig

[a] Includes SFA < 1%: 13:0, 19:0, 21:0, 22:0, 24:0, *ai*14:0, *i*17:0, *i*15:0, *i*16:0, and phytanic acid; [b] includes MUFA < 1%: 16:1ω5 and 20:1ω7; [c] includes PUFA < 1%: 20:2ω6 and 20:3ω6. Bold numbers indicate the highest value if differences between means are significant ($p<0.05$). [d] Calculated as the ratio between all C_{16} PUFA to all C_{16} (saturated, monounsaturated and polyunsaturated) found in *N. simplex*. Rounding of significant figures was based on the magnitude of the error.

Table 4-4 Relative contribution (%) of fatty acids from structural lipids of *Nyctiphanes simplex* males by sampling season. Mean ± 95% C.I.

Fatty Acid	May n=5	July n=7	October n=4	February n=3	
12:0	0.13 ± 0.03	0.35 ± 0.28	**1.01** ± 0.91	0.27 ± 0.02	sig
13:0	0.06 ± 0.03	0.04 ± 0.01	0.04 ± 0.01	**0.90** ± 0.11	sig
14:0	**2.7** ± 0.7	2.0 ± 0.2	1.5 ± 0.4	1.5 ± 0.1	sig
15:0	**0.85** ± 0.18	0.67 ± 0.11	0.75 ± 0.44	0.52 ± 0.02	sig
16:0	**31** ± 8	23 ± 3	18 ± 3	18 ± 2	sig
17:0	**1.5** ± 0.3	1.1 ± 0.1	1.0 ± 0.2	0.8 ± 0.1	sig
18:0	**7** ± 2	6 ± 2	5 ± 1	4 ± 1	sig
19:0	0.32 ± 0.09	0.33 ± 0.05	0.62 ± 0.36	0.23 ± 0.06	n.s.
20:0	0.5 ± 0.1	0.5 ± 0.3	0.6 ± 0.4	0.5 ± 0.1	n.s.
22:0	**1.4** ± 0.6	0.5 ± 0.1	0.6 ± 0.3	0.8 ± 0.2	sig
i15:0	**0.84** ± 0.44	0.24 ± 0.06	0.24 ± 0.11	0.19 ± 0.05	sig
Phytanic	**1.3** ± 0.5	0.4 ± 0.1	0.2 ± 0.1	0.5 ± 0.1	sig
Saturated[a]	**48** ± 13	36 ± 6	34 ± 4	29 ± 3	sig
16:1ω7	1.5 ± 0.5	1.8 ± 0.2	1.5 ± 0.2	1.9 ± 0.2	n.s.
16:1ω9	1.2 ± 0.5	1.0 ± 0.6	2.3 ± 2.0	0.5 ± 0.2	n.s.
18:1ω7	0.8 ± 0.3	**1.8** ± 0.3	1.6 ± 0.2	1.6 ± 0.2	sig
18:1ω9	6 ± 2	10 ± 2	**10** ± 1	10 ± 2	sig
Monounsaturated[b]	10 ± 3	15 ± 3	**19** ± 3	15 ± 3	sig
16:2ω4	0.5 ± 0.2	1.5 ± 1.1	1.6 ± 1.2	2.3 ± 2.0	n.s.
18:2ω6	1.6 ± 0.4	2.0 ± 0.2	1.6 ± 0.5	1.7 ± 0.3	n.s.
18:3ω3	0.9 ± 0.2	**1.4** ± 0.2	0.8 ± 0.2	1.2 ± 0.2	sig
18:4ω3	0.6 ± 0.3	0.5 ± 0.2	0.6 ± 0.3	**1.0** ± 0.2	sig
20:3ω6	0.19 ± 0.04	0.14 ± 0.05	0.19 ± 0.07	**1.50** ± 1.12	sig
20:4ω6	2.6 ± 0.4	2.7 ± 0.5	3.1 ± 1.1	2.4 ± 0.1	n.s.
20:5ω3	10 ± 3	12 ± 2	8 ± 3	12 ± 3	n.s.
22:6ω3	24 ± 7	29 ± 6	24 ± 8	33 ± 5	n.s.
Polyunsaturated[c]	41 ± 11	49 ± 8	46 ± 7	57 ± 5	n.s.
Σω3	36 ± 10	42 ± 8	38 ± 8	48 ± 8	n.s.
Σω6	5 ± 1	5 ± 1	6 ± 1	6 ± 1	n.s.
ΣC16PUFA	0.5 ± 0.2	1.6 ± 1.1	2.7 ± 2.0	2.8 ± 2.1	n.s.
ΣC18 PUFA	3.0 ± 0.6	4.0 ± 0.5	3.3 ± 0.4	4.0 ± 0.7	n.s.
ΣSFA/ΣPUFA	**1.5** ± 0.8	0.8 ± 0.3	0.8 ± 0.2	0.5 ± 0.1	sig
16:1ω7 + 20:5ω3	12 ± 3	13 ± 2	11 ± 2	14 ± 3	n.s.
16:1ω7/16:0	0.060 ± 0.029	0.081 ± 0.012	0.087 ± 0.012	**0.105** ± 0.002	sig
ΣC16/ΣC18	**2.1** ± 0.6	1.3 ± 0.2	1.2 ± 0.2	1.3 ± 0.2	sig
C16 PUFA index[d]	0.02 ± 0.01	0.05 ± 0.04	0.09 ± 0.07	0.11 ± 0.08	n.s.
18:1ω9 + 18:4ω3	7 ± 2	10 ± 2	**14** ± 3	11 ± 2	sig
18:1ω7/18:1ω9	0.13 ± 0.02	**0.19** ± 0.03	0.15 ± 0.02	0.18 ± 0.03	sig
18:2ω6 + 18:3ω3	2.5 ± 0.6	3.5 ± 0.3	2.7 ± 0.4	3.0 ± 0.5	n.s.
Σω3/Σω6	7 ± 1	8 ± 1	6 ± 1	9 ± 3	n.s.
22:6ω3/20:5ω3	2.3 ± 0.2	2.5 ± 0.2	**2.9** ± 0.3	2.7 ± 0.3	sig
EPA/DHA	**0.44** ± 0.04	0.40 ± 0.03	0.35 ± 0.04	0.38 ± 0.04	sig
Total µg/mg	20 ± 8	36 ± 12	28 ± 10	28 ± 1	n.s.
% DW	2.0 ± 0.7	3.6 ± 1.3	2.8 ± 1.0	2.8 ± 0.1	n.s.

[a] Includes SFA < 1%: 21:0, 24:0, *ai*14:0, *i*17:0, and *i*16:0; [b] includes MUFA < 1%: 16:1ω5, 20:1ω7, and 20:1ω9; [c] includes PUFA < 1%: 16:3ω3 and 20:2 ω6. Bold numbers indicate the highest value if differences between means are significant ($p<0.05$). [d] Calculated as the ratio between all C_{16} PUFA to all C_{16} (saturated, monounsaturated and polyunsaturated) found in *N. simplex.* Rounding of significant figures was based on the magnitude of the error..



Appendix 4-1 *Nyctiphanes simplex* males. A) Adult sized individual without a visible spermatophore, B) Adult sized individual with protruded spermatophore. Lateral views and dorsal details of lappet's shape in the rostrum of a C) male individual and D) female individual (Modified from Boden *et al*., 1955).

Appendix 4-2 *Nyctiphanes simplex* males. SIMPER analysis and pair-wise tests on neutral lipid fatty acids in samples collected in different seasons.

Bray Curtis Dissimilarities (%)	May	July	October
July	17.1		
October	18.1	11.6	
February	23.5	17.0	15.5
t values			
July	2.59		
October	2.45	0.97	
February	3.30	2.12	1.56
PMC values			
July	0.006		
October	0.020	0.375	
February	0.004	0.018	0.117

Appendix 4-3 Names and formulae of the 37 fatty acid methyl esters (FAME) used in the present study as references for identification and quantification purposes.

Common Name	Short Name	%	Molecular Weight (MW)	Formula
Butyric Acid Methyl Ester	4:0	4	102	$C_5H_{10}O_2$
Caproic Acid Methyl Ester	6:0	4	130	$C_7H_{14}O_2$
Caprylic Acid Methyl Ester	8:0	4	158	$C_9H_{18}O_2$
Capric Acid Methyl Ester	10:0	4	186	$C_{11}H_{22}O_2$
Undecanoic Acid Methyl Ester	11:0	2	200	$C_{12}H_{24}O_2$
Lauric Acid Methyl Ester	12:0	4	214	$C_{13}H_{26}O_2$
Tridecanoic Acid Methyl Ester	13:0	2	228	$C_{14}H_{28}O_2$
Myristic Acid Methyl Ester	14:0	4	242	$C_{15}H_{30}O_2$
Myristoleic Acid Methyl Ester	14:1ω5	2	240	$C_{15}H_{28}O_2$
Pentadecanoic Acid Methyl Ester	15:0	2	256	$C_{16}H_{32}O_2$
cis-10-Pentadecenoic Acid Methyl Ester	15:1ω5	2	254	$C_{16}H_{30}O_2$
Palmitic Acid Methyl Ester	16:0	6	270	$C_{17}H_{34}O_2$
Palmitoleic Acid Methyl Ester	16:1ω7	2	268	$C_{17}H_{32}O_2$
Heptadecanoic Acid Methyl Ester	17:0	2	284	$C_{18}H_{36}O_2$
cis-10-Heptadecenoic Acid Methyl Ester	17:1ω7	2	282	$C_{18}H_{34}O_2$
Stearic Acid Methyl Ester	18:0	4	298	$C_{19}H_{38}O_2$
Elaidic Acid Methyl Ester	18:1ω9 t	2	296	$C_{19}H_{36}O_2$
Oleic Acid Methyl Ester	18:1ω9 c	4	296	$C_{19}H_{36}O_2$
Linolelaidic Acid Methyl Ester	18:2ω6 t	2	294	$C_{19}H_{34}O_2$
Linoleic Acid Methyl Ester	18:2ω6 c	2	294	$C_{19}H_{34}O_2$
γ-Linolenic Acid Methyl Ester	18:3ω6	2	292	$C_{19}H_{32}O_2$
α-Linolenic Acid Methyl Ester	18:3ω3	2	292	$C_{19}H_{32}O_2$
Arachidic Acid Methyl Ester	20:0	4	326	$C_{21}H_{42}O_2$
cis-11-Eicosenoic Acid Methyl Ester	20:1ω9	2	324	$C_{21}H_{40}O_2$
cis-11,14-Eicosadienoic Acid Methyl Ester	20:2ω6	2	322	$C_{21}H_{38}O_2$
cis-8,11,14-Eicosatrienoic Acid Methyl Ester	20:3ω6	2	320	$C_{21}H_{36}O_2$
Heneicosanoic Acid Methyl Ester	21:0	2	340	$C_{22}H_{44}O_2$
Arachidonic Acid Methyl Ester	20:4ω6	2	318	$C_{21}H_{34}O_2$
cis-11,14,17-Eicosatrienoic Acid Methyl Ester	20:3ω3	2	320	$C_{21}H_{36}O_2$
cis-5,8,11,14,17-Eicosapentaenoic Acid Methyl Ester	20:5ω3	2	316	$C_{21}H_{32}O_2$
Behenic Acid Methyl Ester	22:0	4	354	$C_{23}H_{46}O_2$
Erucic Acid Methyl Ester	22:1ω9	2	352	$C_{23}H_{44}O_2$
cis-13,16-Docasadienoic Acid Methyl Ester	22:2ω6	2	350	$C_{23}H_{42}O_2$
Tricosanoic Acid Methyl Ester	23:0	2	368	$C_{24}H_{48}O_2$
Lignoceric Acid Methyl Ester	24:0	4	382	$C_{25}H_{50}O_2$
cis-4,7,10,13,16,19-Docasahexaenoic Acid Methyl Ester	22:6ω3	2	342	$C_{23}H_{34}O_2$
Nervonic Acid Methyl Ester	24:1ω9	2	380	$C_{25}H_{48}O_2$

The Supelco 37 Component FAME Mix (47885-U) has been upgraded to a certified reference material (CRM47885)

Chapter 5. **Estimates of prey biomass demand and minimum swarm density for blue and fin whales (*Balaenoptera musculus* and *B. physalus*) in the Gulf of California, Mexico**

5.1. Abstract

The Gulf of California is a key area in the survivorship and recovery of Balaenopterid whale populations with unique biological features in the North Pacific. It is a well-known nursing and feeding area of the eastern North Pacific blue whale population (*Balaenoptera musculus*) during winter migrations to lower latitudes. This population appears to be recovering strongly from exploitation. The diet of blue whales in the Northeast Pacific consist mostly of euphausiids, and during winter they have access to swarms along both coasts of the Baja California peninsula. In addition, there is evidence of a resident population of fin whales (*B. physalus*) inhabiting the Gulf of California throughout the year, which appears to use multiple food sources. In order to evaluate if maximum observed densities of krill are sufficient to fulfill the maximum energy requirements of whales in the area, the maximum biomass demand of preferred prey in the area (*Nyctiphanes simplex*) and the maximum swarm density required were estimated. Biomass demands were modeled from estimations of the basal and active metabolic rates of both whale species; the metabolic efficiency and the expected seasonal differences in consumption. To estimate the prey density required to sustain these whale species, I used the energy value of the prey. The parameter with greatest uncertainly was the ratio between active and basal metabolic rates. However, even with a large and improbable value, an average density of 40–51 g m^{-3} was estimated to satisfy the daily energy requirements of a female blue whale. The estimated swarm densities were within krill densities observed in the Gulf of California (32–88 g m^{-3}). From

February to May, and as consequence of lower energy content of *N. simplex*, fin whales increase the maximum density of required prey form 47–62 g m^{-3} to 59–75 g m^{-3}. Compared with biomass demand of whales in temperate and polar areas, neither *B. musculus* nor *B. physalus* appear to consume a significant biomass in the Gulf of California and adjacent waters. Still, those estimates are required to evaluate the effect of decadal-scale changes in food supply on population demographics (particularly birth rates and survival to reproductive maturity), and to evaluate the impact of whale populations on *N. simplex*.

5.2. Introduction

In the past, feeding studies of fin whales (*Balaenoptera physalus*), blue whales (*B. musculus*), and other Balaenopterid whales were restricted to temperate and polar regions, where summer abundance of food appear to fulfill the requirements of large-whale species (Mackintosh, 1966; Jonsgård, 1966; Lockyer & Brown, 1981). It is well known that during winter migration of those whales to lower latitudes, food consumption rates decrease and reproduction (mating, calving, lactating and breeding) takes a central role. Evidence collected from whaling vessels shows that winter migration towards subtropical and tropical regions is accompanied with lower frequency of feeding events; small amounts of food in their stomachs, and lower yields of fat and oil (Mackintosh, 1966; Lockyer & Brown, 1981).

During the four months of intensive feeding (summer), overall body weight of blue and fin whales increases ~50%, mostly as fat. In contrast, pregnant blue and fin whales increase their body weights at least 60–65%, and their blubber is ~25% thicker than that of resting (anoestrous) females (Lockyer, 1981a). In order to accumulate this extra fat, pregnant whales tend to remain longer on the feeding grounds. As the initial energy costs of pregnancy are minimal and only become important in the last half or third of gestation, Lockyer (1978) suggests that most of this

extra energy is channelled catabolically into milk production in winter and spring (once they leave the summer feeding grounds).

However, if summer fattening is not as efficient as predicted, low body weight and low lipid reserves will be critical for reproductive performance. Failure to replete energy stores is predicted to be correlated with an increase in the reproductive interval, and with a decrease in the apparent pregnancy rate in the population (Lockyer, 1987). Furthermore, calf survival can be compromised if mothers fail to recover fat body condition, as lactation can be highly demanding in terms of energy. In fin whales, lactation costs are estimated to be at least 1.4 times higher than those of total gestation and foetal development (Lockyer, 1987) and are in excess of gestation costs for blue, sei and minke whales (Lockyer, 1978; 1981a; 1981b).

Nevertheless, if sporadic and opportunistic feeding occurs during winter, whales might fatten up earlier in the year and regain body fat condition quickly, compensating the energy deficit (Lockyer, 1987). Overcoming food shortage at winter feeding grounds seems particularly important for lactating females. If sufficient amounts of food and time to consume it are available, a full recovery of body condition before the next reproductive cycle is more likely.

Winter feeding grounds can contribute significantly to the survival of migratory Balaenopterid whales. Lockyer (1981a) assumed daily body weight losses in southern-hemisphere whales similar to those in other long fasting animals (0.2–0.3% day^{-1}) and estimated that, if whales were only feeding heavily for ~4 months and on nothing for the remaining 8 months of the year, then their total weight loss during their migration span will be about 45%. This figure is higher than the 22–30% of body weight observed for blue and fin whales by Lockyer (1981a), and therefore suggests sporadic and opportunistic feeding at lower latitudes

during winter. This difference between estimated and observed weight loss implies that at least for some migratory populations, summer prey demands are overestimated.

One of these winter-feeding grounds is the Gulf of California. Here, the distribution of blue whales (*Balaenoptera musculus*) in the southwest part of the Gulf has been associated with the presence of surface swarms of the euphausiid *Nyctiphanes simplex* (Gendron, 1990, 1992; 2002), suggesting that blue whales use the Gulf of California as feeding area. Those whales are part of the Eastern North Pacific population, one of the various other populations of blue whales that appears to be recovering from exploitation (Calambokidis *et al*., 1990). Blue whales migrate from California to the south at the end of the summer, arriving in Mexican Pacific waters during winter and early spring, and inhabiting the Gulf of California from December–May. Their occupancy in the gulf can be variable among years (Gendron 1990, 1992, 2002). Those whales have been reported particularly in the areas between Carmen Island and Bahía de La Paz (Figure 5–1a) as late as March and April (Leatherwood *et al*., 1988; Calambokidis *et al*., 1990*;* Gendron, 1990, 2002).

In contrast, fin whale sightings in the Gulf of California are reported throughout the year (Rojas-Bracho, 1984) although they are more frequent around the Midriff Archipelago from April to August (Tershy *et al*., 1993a; Ladrón de Guevara *et al*., 2008). This suggests the presence of a resident population (Gilmore, 1957; Gambell, 1985; Leatherwood *et al*., 1988), an idea supported by the negligible genetic exchange observed between the fin whales inhabiting the Gulf of California and those inhabiting other regions of the Pacific Ocean (Bérubé *et al*., 1998; 2002). Such a resident population will require year-round regional food sources, or a high amount of seasonally and spatially-concentrated food sources to satisfy annual energy requirements. Although fin whales distribute widely in the Gulf of California (Tershy *et al*.,

1991*;* Gendron, 1993), in winter and in spring they often aggregate near the midriff archipelago (Isla Tiburón and Isla Angel de la Guarda) and in the southern portion of the Gulf (Figure 4–1b), where they feed mainly on the euphausiid *Nyctiphanes simplex* (Gendron, 1990; Tershy *et al*., 1993b*;* Croll *et al*., 1998; Ladrón de Guevara *et al*., 2008; 2015).

During winter and spring, fin whales and blue whales coexist in the Gulf of California where they both feed actively on zooplankton and occasionally (and likely incidentally) on nekton (Del Angel-Rodríguez, 1997; Ladrón de Guevara *et al*., 2008; Jiménez-Pinedo, 2010). The analysis of blue and fin whale feces from the region of Loreto and Bahía de La Paz during winter and spring, showed a strong overlap in diet, feeding mainly on adult and juvenile forms of the euphausiid *N. simplex* (Del Angel-Rodríguez, 1997; Jiménez-Pinedo, 2010). This krill species forms dense swarms, sometimes at surface during daytime, and is the numerically dominant species among the 12 euphausiid species in the Gulf of California (Brinton & Townsend, 1980; Brinton *et al*., 1986; Gendron, 1992; Ambriz-Arreola *et al*., 2017). In the region comprised between Loreto and Bahía de La Paz, blue whales are found mostly in deep areas, whereas fin whales inhabit mainly shallower regions near the coast. Once the blue whales leave this southwest area of the gulf, fin whale numbers increase and they continue feeding on euphausiid swarms (Del Angel-Rodríguez, 1997; Flores-Ramírez *et al*., 1996; Pardo *et al*., 2013).

Consumption rate estimates (properly: prey biomass demands) for these species have been published for high latitude feeding regions (Sigurjónsson & Víkingsson, 1997; Trites & Pauly, 1998). However, to my knowledge, there has not been any formal attempt to estimate either prey biomass demand or minimum swarm density of both species for subtropical latitudes. Such estimations are necessary to evaluate the contribution of wintering grounds to whale's energetics, to evaluate the impact of whale's populations on *N. simplex* in the Gulf of California, and to

evaluate the effect of decadal-scale changes in food supply on population demographics of whales (particularly birth rates and survival to reproductive maturity). Additionally, biomass demands at lower latitudes can help to correct overestimated summer prey demands in higher latitudes,

In the present work, the underlaying hypothesis was that maximum observed densities of krill are sufficient to fulfill the energy requirements of whales in the area, at least during winter time. To that end, I considered observational physiological studies of small cetaceans in captivity, studies with direct observations of stomach contents, and filling times from whaling operations. I also considered theoretical calculations on energy requirements and, when no experimental or observational data existed for the specific population, I assumed physiological parameters such as metabolic rates and assimilation efficiency using references from studies in other mammals or other populations.

The decision on many parameters was based on the most plausible value, therefore this chapter should be considered merely as an exploration of the possible consequences of seasonal and ontogenic changes in the energy content of krill over estimates of prey biomass demand for *B. musculus* and *B. physalus* in the Gulf of California. One important step into estimating minimum swarm density and prey biomass demand was the consideration of an overall variation in energy content of *N. simplex* up to 1.7 fold due to swarm structure (Chapter 2 of this thesis) and up to 1.3 fold due to seasonal conditions (Chapter 4 of this thesis). A conservative estimate still allows comparisons to find differences between species, or among seasons and regions, which ultimately can help to characterize feeding strategies for both species of baleen whales.

5.3. Material and methods

An allometric model was used to estimate daily individual energy requirements of whales:

$$WDR_{ij} = e \times f \times g \times c\, M_{ij}^{d} \quad (1)$$

where WDR_{ij} = winter daily energy (kcal whale^{-1} day^{-1}) required by an individual of species *i* and sex *j*; *e* = Ratio between active metabolic rate (*AMR*) or field metabolic rate (*FMR*) and basal metabolic rate (*BMR*) (kcal active kcal^{-1} at rest); *f* = Ratio between ingested and assimilated energy (kcal ingested kcal^{-1} assimilated); *g* = Winter-spring daily rate (*WDR*) (kcal day^{-1} winter rate kcal^{-1} day average rate); *c* = Intercept for the equation of the Basal Metabolic Rate (*BMR or Q*) (kcal day^{-1} kg^{-d}); *d* = exponential slope for the equation of *BMR* at rest, and *M* = average individual body mass (kg) of species *i* and sex *j* estimated according to Trites & Pauly (1998) as:

$$\ln M_{mean} = a + b \ln L_{max} \quad (2)$$

where M_{mean} is the average mass (kg) and L_{max} corresponds to the maximum recorded length (cm) for each species. Alternatively, winter daily rate was also estimated as:

$$WDR_{ij} = f \times g \times c_1\, M_{ij}^{d}{}_1 \quad (3)$$

where c_1 = Intercept for the equation of field metabolic rate (*FMR*) in kcal day^{-1} kg$^{-d}{}_1$; d_1 = exponential slope for the equation of *FMR*.

Monte Carlo simulations were used to incorporate parameter uncertainty into energy requirement and biomass-demand estimates (Winship *et al*., 2002). In each run of the model, parameter values were randomly selected from assigned sampling distributions that best described their uncertainty. Thus, each run of the model produced 1 estimate of energy requirement or biomass demand, and multiple runs produced a distribution of requirement estimates. Details on decisions and exceptions on the values used for each parameter are explained below.

5.3.1. Average mass and maximum recorded length

In Balaenopterids, females are usually larger than males both in size and mass. According to Trites & Pauly (1998), the values of the parameters of equation (1) for females of *B. musculus* and *B. physalus* are a = -7.503 and b=2.347, whereas for the males of both species it will be: a = -7.347 and b = 2.329. The average mass for female blue whales in the gulf were calculated using the maximum lengths reported by True (1904) in the northern hemisphere (26.8 m). Male length was assumed to follow similar sex differences in size of the Antarctic populations, *i.e.* 5% smaller than females (25.46 m). These lengths yielded mass values of 61,280 kg for females and 55,142 kg for males of *B. musculus*. For fin whales, Jefferson *et al*. (1993) reports that most Northern Hemisphere adult fin whales are less than 24 m long, and it is reasonable to assume that larger individuals are females. Using Equation (2), the estimated weight was 47,298 kg. This value is similar to the average mass of 49,000 kg reported by Merrick (1997) in the North Pacific. Assuming 5% sex differences, the maximum length for a male fin whale will be 23.15 m with an average mass of 44,168 kg. Table 5-1 shows the reported and estimated weight for blue and fin whales in different areas of the world.

5.3.2. Basal metabolic rate.

A common procedure to calculate the prey biomass required for mammal predators starts with the estimation of the daily individual amount of energy required to satisfy the minimum metabolic demands. This amount of energy, known as basal metabolic rate (*BMR* or Q) is a power law function of body mass ($Q = c \times M^d$), where Q is expressed in kcal day^{-1}, and M is the body mass in kg. Units for c are kcal day^{-1} kg^{-d}, and the d exponent (the scaling coefficient or allometric slope) has no units.

Brody (1968) and Kleiber (1975) proposed values close to 3/4 for the scaling coefficient of mammals ($d \sim 0.75$), and both early and recent estimations of prey biomass demand for large whales have used classical values for the parameters (Overholtz & Nicolas, 1979; Hinga, 1979; Kenney *et al*., 1986; Trites & Pauly, 1998). However, other specific values of the parameters have been proposed in studies with larger sample sizes and higher taxa diversity (Hayssen & Lacy, 1985). Deviations from the original pattern have also been associated with food and habitat (McNab, 1986), and more recently with distribution within zoogeographical zones (Lovergrove, 2000), and mobility (Lovergrove, 2000). Additionally, Williams *et al*. (2001) found that when measured under equivalent experimental conditions *BMR*s of adults of a large number of marine-mammals were 1.4–2.8 times higher than those predicted for terrestrial carnivores of similar body mass.

On the other hand, Lavinge *et al*. (1986) observed that the perception that marine mammals have higher metabolic rates than terrestrial mammals of similar size arises from determinations that did not satisfy Kleiber's criteria. This conclusion was also reached by Hunter *et al*. (2000) selecting rates appeasing the conditions defined by Kleiber (1975), i. e., non-growing animals, thermoneutral, post-absorptive, non-reproductive, and quiescent. These authors concluded that marine mammals have similar *BMR* as terrestrial mammals of similar body size.

I evaluated three different models for *BMR*.

1) An early model proposed by Kleiber (1961):

$$Q = 67.6 \times M^{0.756} \quad (4)$$

2) An alternative model described by Brody (1968) with a slightly lower exponent:

$$Q = 70.5 \times M^{0.7325} \quad (5)$$

3) The widely used model described by Kleiber (1975), included for comparative purposes with other publications:

$$Q = 70 \times M^{0.75} \quad (6)$$

A controversial figure for cetaceans suggested by Williams *et al*. (2001) proposes that

$$Q = 2.3 \times (70 \times M^{0.75}) \quad (7)$$

This last equation results in very high energy requirements and therefore was not used. Research on cetaceans suggest alternative methods to estimate prey biomass demand. Accordingly, those methods were also explored in the following sections.

5.3.3. Active metabolic rate

Active metabolic rate (*AMR*) is the energy consumption due to normal body activity. Estimates of the ratio between basal metabolic rate and active metabolic rate (*e*) of cetaceans in captivity range from *e* = 2 to 5 times (Overholtz & Nicolas, 1979; Hinga, 1979; Kenney *et al*., 1986).

A review of data on metabolic rates derived from experimental work done with small captive marine mammals (Lockyer, 1981b) suggests that total energy expenditure of cetaceans (while swimming slowly from place to place or feeding) can be calculated directly with the formula:

$$AMR = 110 \times M^{0.783} \quad (8)$$

Where *AMR* is expressed in kcal day^{-1} and *M* in kg. Lockyer (1981b) used this equation to estimate the *AMR* of *Balaenoptera acutorostrata*, a Balaenopterid with a similar shape but smaller size than *B. musculus* and *B. physalus*. According to this model, for a 23–27 m length whale (the estimated range of blue and fin whales in the Gulf of California), *AMR* would be roughly 2.8 times larger than Kleiber's *BMR*. Such a value falls within the 2–5 estimated range

for cetaceans and is close to the one used for marine birds (Kooyman *et al.,* 1982; Schneider *et al*., 1986).

5.3.4. Field metabolic rate

One limitation of *AMR* is that it involves the *BMR* plus the energy required for the main activities using both direct and indirect estimations. However, strict conditions required to perform direct or indirect calorimetric measurements of the *BMR* on large cetaceans in the wild are currently impossible to meet. Alternatives rely on estimates derived from terrestrial mammals. A solution to this dilemma is the measurement of the field metabolic rate (*FMR*), as it calculates directly the whole energy used for animals without any physical restriction using the doubly labeled water method. Nagy (2005) proposed that *FMR* in mammals adjusts well to the allometric equation:

$$FMR = 4.82\ M^{0.734} \quad (9)$$

where *FMR* is the field metabolic rate in kJ day^{-1}, and *M* is the body mass in g.

For large whales, the most exhaustive analysis was made by Leaper & Lavigne (2007). These authors analyzed different parameters of general regressions for *FMR* and daily consumption (*R*) and compared them with estimates of Kleiber's *BMR* and with data from other sources, including rates of filter feeding, oxygen consumption and seasonal changes in energy stores. These authors suggest a parameter space for average energy intake for large whales bounded at the high end by the equation proposed by Innes et al. (1986) to estimate feeding rates,

$$R = 0.42M^{0.67} \quad (10)$$

Where *R* is the daily consumption in kg d^{-1}, and *M* is body mass in kg. Here, the average prey calorific value is estimated at 5,450 kJ kg^{-1} and the assimilation efficiency at 80%.

The proposed lower end is given by Boyd (2002), who estimated *FMR* (kJ d^{-1}) as:

$$FMR = 2529.2\ M^{0.524} \quad (11)$$

An example of calculations of the upper limit of energy demand as *AMR* from *R* and the lower limit from the *FMR* adjusted for assimilation efficiency is shown in Appendix 5-1. Here it can be shown that the ratio *e* (*AMR*/*BMR*) when *AMR* is estimated from equation (8) is 2.7–2.8 kcal active kcal^{-1} at rest. Similarly, the ratio *e* (*FMR*/*BMR*) when *FMR* is estimated with equation (9) is 2.7–2.8 kcal active kcal^{-1} at rest, i. e., falls within the limits proposed by Leaper & Lavigne (2007).

In contrast, it can be demonstrated that the allometric regression developed by Williams *et al*., (2004) for active otariids and small odontocetes

$$FMR = 19.65\ M^{0.756} \quad (12)$$

where *FMR* is expressed in watts (J s^{-1}), and *M* is the body mass in kg, results in a ratio of ~6 times Klieber's *BMR*, far from the proposed limits. Here, the result in watts (J s^{-1}) was transformed into kcal day^{-1} using a factor of (3600 s h^{-1} × 24 h day^{-1} / 4184 J kcal^{-1}) = 20.65 kcal s day^{-1} J$^{-1.}$

Based on all these considerations, the interval for the parameter *e* was 0.8–4.1 for blue whales and 0.9–4.3 for fin whales

5.3.5. Apparent digestible efficiency and average daily rate (*ADR*)

Lockyer (1981b) considered a 0.80 absorption efficiency or efficiency of food assimilation to calculate the actual daily energy (or average daily rate) required for large whales (*ADR*). This value is similar to the 0.81–0.83 apparent digestible efficiency observed in krill (Mårtensson, 1994a; 1994b). For the present study, a range for assimilation efficiency of 0.80–0.83 was considered appropriate. The inverse of these values resulted in the range of ratios between ingested and assimilated energy (*f*) between 1.205 and 1.250 kcal ingested kcal^{-1} assimilated.

5.3.6. Seasonal variation in energy requirements

Assuming no seasonal variation in energy demands, a crude estimate of annual metabolic energy expenditure of large whales could be calculated from *ADR* kcal day^{-1} × 365 days $year^{-1}$. However, according to data on subcutaneous fat thickness and average mass of whales in different seasons, the amount of energy required by migratory whales in summer is about 83% of their annual energy requirements (Lockyer, 1981b). A similar value (80%) was considered by Sigurjónsson & Víkingsson (1997). They considered an average residence time of four months (120 days) in the summer regions and an eight-month migration period (245 days). These authors estimated the summer daily rate as 0.80 × 365 ÷ 120 = 2.433 times the *ADR*. Similarly, the upper limit for the winter-spring daily rate (*WDR*) or *g* in equation (1) can be estimated as 0.20 × 365 ÷ 245 = 0.298 times the *ADR*. The lower limit of *g* can be estimated as 0.17 × 365 ÷ 245 = 0.253 times the *ADR*. In other words, *g* ranges from 0.253 up to 0.298 kcal day^{-1} winter rate $kcal^{-1}$ day average rate.

5.3.7. Winter biomass demand (*WBD*)

Winter biomass demand (kg day^{-1}) was estimated from *WDR* (*ADR* for winter; kcal day^{-1}) and the average energy content (kcal kg^{-1}) of typical *N. simplex* swarms during February (for blue whales) and May (for fin whales) (Table 2-3; Table 4-2.). To calculate the average energy content of typical swarms of *N. simplex* I first estimated the relative proportions for each age and sex class (as described in Table 2-1) in a matrix that included four different types of swarms. To that end, I considered that the proportion of females and males in swarms of *N. simplex* in the Gulf of California varies from 1:1 to 2:1 during the winter-spring aggregations (Gendron, 1992). Likewise, the proportion of adults of both sexes ranges from swarms with 8% mature organisms of both sexes, to swarms in which 43% of females were mature and 72% of males were mature

(Gendron, 1992). Apparently, this proportion of adults within swarms is not rare. A more recent study on swarm structure reports that in 50% of swarms < 17% of the population were adults, and in 78% of swarms < 46% were adult krill. Only 22% of swarms exhibit > 50% adult krill (Ladrón de Guevara *et al*., 2008). From these data, I calculated the relative proportions of the different classes in which energy values are known (Table 5-2**.**).

5.3.8. Local food density

In most cases, prey biomass demand is expressed in units of mass which is not sufficient to establish a criterion of sufficiency or scarcity of food in a region. For a particular area, available prey biomass is reported in terms of density (organisms m^{-2}, organisms m^{-3}, kg m^{-2}, or kg m^{-3}), and it becomes necessary to estimate the density of prey required to satisfy a given rate of consumption. Variations in local food density are important as survival depends on feeding at high prey densities in swarms, not at average prey densities. For this purpose, I estimated the filtered volume on each whale species following the empirical equation that relates mouth size with swallowing-filtered volume (Lockyer, 1981b):

$$V_L = 0.82\ [L_j\ W_j\ (S\text{-}s)] \qquad (13)$$

V_L is the volume (m^3) engulfed on a single lunging event; L_j is the maximum jaw length (m); W_j is the maximum jaw width (m); s is the maximum depth of the jaw sac whilst swimming (contracted lower jaw sac) (m); S is the maximum depth of the jaw sac whilst feeding (expanded lower jaw sac) (m); and S-s is the difference in maximum depth of the sac at the point of greatest jaw width (m).

Based upon data provided by Lockyer (1981b), jaw length and jaw width relate linearly to body length in both blue whales (n=13) and fin whales (n=13) and can be calculated using linear equations:

$$L_j = a_1 + b_1 L \quad (14)$$

$$W_j = a_2 + b_2 L \quad (15)$$

where L_j is the jaw length (m); L is total length (m), a_1 = -1.4555 and b_1 = 0.2577 for blue whales and a_1 = -0.9529 and b_1 = 0.2470 for fin whales. Similarly, W_j is jaw width (m); L is total length (m), a_2 = -0.2436 and b_2 = 0.1224 for blue whales and a_2 = -0.2008 and b_2 = 0.1138 for fin whales.

Similarly, the difference in depths of the sac (S-s) can be related to total length using linear equations. Here less data exist for both blue whales (n=5) and for fin whales (n=3):

$$(S\text{-}s) = a_3 + b_3 L \quad (16)$$

where (S-s) is the difference in maximum depth of the sac at the point of greatest jaw width (m); L is total length (m), a_3 = -0.8078 and b_3 = 0.1076 for blue whales and a_3 = -0.3466 and b_3 = 0.0953 for fin whales.

Computations of prey density required knowledge of swimming speeds, filtered volume, and daily time dedicated to feeding (Kenney *et al*., 1985; 1986; 1997). To this end, the diving behaviour of blue and fin whales was considered (Croll, 2001; Acevedo-Gutiérrez *et al*., 2002). Briefly, whales swim toward krill patches where they perform a session of several foraging dives. Each dive normally involves more than 1 lunging event before returning back to the surface where they recover for a period of time proportional to the number of lunging cycles performed. The filtering rate can then be estimated with the formula:

$$F = V_s/t_s = [V_L \times LC \times D] / [((t_D + t_R) \times D) + t_T] \quad (17)$$

where F is the filtering rate ($m^3\ h^{-1}$), V_s is the total volume filtered within a session (m^3) and t_s is the total time elapsed within a session. V_L is the filtered volume on a single lunging event; LC is the average number of lunging cycles; D is the average number of foraging dives within a

session; t_D is the average time of a dive; t_R is the average resting time after a dive; and t_T is the transit time required to move from one krill patch into another.

The required winter densities can then be estimated as:

$$\delta_w = (WBD \times 1000)/(24 \times F) \quad (18)$$

where δ_w is the estimated winter's swarm density (g m^{-3}), *WBD* is the winter biomass demand (kg day^{-1}) and *F* is the is the filtering rate (m^3 h^{-1}).

While each of the above studies emphasize some aspects to estimate Balaenopterid prey biomass demand, no single study has considered them all together, and no direct measurements have been attempted so far. In the case of estimates that include the blue whale or fin whale, this chapter represent the first estimation of prey biomass demand of these two species in their winter regions, based on empirical data of the energy content of krill prey in the Gulf of California.

5.4. Results

5.4.1. Winter daily rates

There was a strong variation in size and biomass of whales in the literature. For instance, the difference in biomass between large blue whales and small ones was 80–86% for females and males. Similarly, the difference between large fin whales and smaller individuals was 85–90% (Table 5-1). As expected, average energy requirements estimated (kJ ind^{-1} day^{-1}) for female blue whales were larger (1,073,258) than for male blue whales (1,031,032). Similarly, average requirements for female fin whales (694,064) were larger than for male fin whales (661,486). However, the uncertainty in other parameters (particularly the large range between *AMR* and *BMR*, or *FMR* and *BMR*) resulted in large confidence intervals for the average winter daily rates (kJ ind^{-1} day^{-1}) and no significant differences were observed among the size range of both species, neither between species, nor among sexes (Table 5-3). Still the differences in the

maximum winter daily rates (upper limits) between large and small individuals of *B. musculus* (47–48%) and *B. physalus* (45–48%) were important and deserved further examination as they are the most conservative way to estimate maximum winter biomass demand.

5.4.2. Winter-spring biomass demand

In the southeastern Gulf of California, blue whales (*B. musculus*) are observed more frequently during the first part of the year whereas fin whales (*B. physalus*) exhibit higher abundances in the same area after May (Del Angel-Rodríguez, 1997; Pardo *et al*., 2013). If seasonal changes in the energy content of the main prey species are incorporated (Table 5-2), then whales foraging in May are expected to acquire ~18% less energy per mass unit (wet basis) than whales feeding in winter (February). For a fin whale of a given mass, in the present study, the lower energy content of prey observed in spring (May) resulted on an average biomass demand 20–23% higher than that required for prey in winter (February) with higher energy content (Table 5-4). One interesting consequence is that, the maximum winter biomass demand of prey can be 15% higher for a fin whale foraging in May compared to a blue whale of similar size feeding in February in the same area (Table 5-4).

5.4.3. Food density and daily required feeding time

Following equation (13) a 26.8 m female *B. musculus* will be able to engulf 28.24 m^3, whereas a 25.46 m male of the same species will engulf 23.28 m^3. Similarly, a 24 m female *B. physalus* will engulf 21.16 m^3 and a 23.15 m male *B. physalus* 17.72 m^3. From telemetry data, Mate *et al*. (1999) and Lagerquist *et al.* (2005) estimate an average speed of 4.5 km h^{-1} for *B. musculus* during winter migration. This value integrates both migratory displacement and feeding events during migration. In the case of *B. physalus*, Bose & Lien (1989) observed

displacement speeds of 9 km h^{-1}, but data from Ray *et al*. (1978) suggest slower speeds of 6.7 km h^{-1}. Both for *B. musculus* and *B. physalus*, Croll *et al.* (2001) and Acevedo-Gutiérrez *et al.* (2002) provide their respective average dive times (7.8 and 6.3 min), the average resting times after a dive (2.5 and 1.5 min), the average number of feeding events by dive (2.4 and 1.7), the average number of foraging dives per session (9.1 and 10.9) and average distances between diving sessions (0.5254 and 0.8957 km).Accordingly, the estimated filtered capacity of a female blue whale was estimated as:

$$F = V_s/t_s \quad (19)$$

$F = [28.24 \times 2.4 \times 9.1] / [((7.8 + 2.5)/60) \times 9.1) + (0.5254/4.5)] = 616.8/1.68 = 367.\ 3\ m^3\ h^{-1}$. Similarly, the maximum filtering capacity for male *B. musculus* will be 302.8 $m^3\ h^{-1}$, for a female *B. physalus* 252.8 $m^3\ h^{-1}$, and for a male *B. physalus* it will be 211.8 $m^3\ h^{-1}$.

To satisfy their daily energy requirements, a female blue whale with a winter daily biomass demand of 245 kg of *N. simplex* day^{-1} and a filtering capacity of 367.3 $m^3\ h^{-1}$ will require a minimum density of:

$\delta w = (245 \times 1000)/(24 \times 367.53) = 27.8\ g\ m^{-3}$

Table 5-5 shows the maximum winter-spring biomass demand of prey (*WBD*) as kg $ind^{-1}\ day^{-1}$, the maximum required densities of *N. simplex* swarms (g m^{-3}) and the estimated time invested on feeding (h day^{-1}). For a given sex and whale species, the ratio between maximum and minimum estimated density was ~6.6. As larger animals can filter larger volumes and have larger filtering rates, in general larger animas will require smaller densities to satisfy their winter biomass demand of prey (Table 5-5).

All estimated densities were above the 17.5 g m^{-3} estimated by Brodie *et al.* (1978). Relatedly, Gendron (1992) estimated *N. simplex* swarm densities as high as 32.6 g m^{-3} in the

southern part of the Gulf. In the present work, considering the high energy value of prey during winter, average densities estimated for female and male blue and fin whales were below that value. However, during spring, as the energy value of prey decreases, only male fin whales shown average required densities below 32.6 g m^{-3} (Table 5-5). High prey density requirements for whales imply that if real densities are above but closer to estimated densities, then feeding periods will be longer (including the time devoted to engulfing krill, the time devoted to dive, rest and traveling among krill swarms). For instance, a density of 88 g m^{-3} as the observed in swarms of *Nyctiphanes simplex* in the upper gulf (Ladrón de Guevara *et al*., 2008) would require a feeding period of 11–14 h day^{-1} for blue whales to satisfy their maximum winter biomass demand. For fin whales the same density will require an investment on feeding of 13–17 h day^{-1}. Such periods seem reasonable as none of the estimated densities for the winter-spring period was above 88 g m^{-3} (Table 5-5). On the other hand, if real densities are below the required densities, then the animals will not fulfill their daily biomass demand at that particular location. Furthermore, densities close to 150 g m^{-3}, similar to those of krill swarms off the coast of California (Croll *et al*., 2005) appear adequate to sustain maximum biomass demand for large whales in the Gulf of California. Here, feeding periods will be 7–8 h day^{-1} for blue whales and 8–10 h day^{-1} for fin whales during winter, and 9–12 h day^{-1} for fin whales during spring.

5.4.4. Overall biomass demand in the Gulf of California

According to Urbán-Ramírez (2000), the *B. physalus* population in the Gulf of California is around 300 individuals. Another report (Gerrodette & Palacios, 1996), suggests a population of 820 (594–3229) for the Eastern Tropical Pacific, but the sex ratio in the area is not known. In the case of *B. musculus*, Calambokidis & Barlow (2004) estimated 2,994 blue whales for the Northeast Pacific population based on line-transect surveys. Gendron (2002), using distance

sampling methodology, estimated an average of 576 whales reaching the Pacific side of the Baja California peninsula during winter, of which 370 are females, resulting in a male: female ratio of 1:1.8. The same author estimated an average of 283 whales entering the Gulf of California every year. A similar result (209 whales) is reported by Ugalde de la Cruz (2008) using photo-recapture methods for the same area which also confirms a higher use of the area by blue whale females. Based on the maximum prey biomass demand, during winter (February), 283 blue whales will be consuming a maximum of 104.24 metric tons a day (t day^{-1}), whereas 300 fin whales will consume 89.78 t day^{-1}. On the other hand, the same number of fin whales will require 110.84 t day^{-1} during spring time, i. e., an increase of ~20 t day^{-1} as consequence of lower energy content of *N. simplex* during spring time.

In other words, when seasonal differences in prey composition are considered, the estimated biomass required for all *B. physalus* in the Gulf of California during spring is ~23% higher than the requirements for the same species in winter, and ~6% higher than the requirements of all *B. musculus* in the same area. Apparently, seasonal differences in prey composition will produce a higher biomass demand of *N. simplex* by fin whales in order to fulfill their daily requirements in the area. However, this estimation does not include the occupancy estimates (residence time) for each species and sex, or the fact that high densities of each whale species do not occur throughout the full season, but rather during short weekly periods over winter and spring. For instance, Gendron (2002) observed that 20% of the blue whales that enter into the Gulf of California remain in the area between 2–20 days. Accordingly, the estimated biomass required for 57 blue whales using the area on a single day will be ~21 t day^{-1}, which represents ~20% of the ~104 t day^{-1} originally estimated.

5.5. Discussion

5.5.1. Main factors influencing estimations of prey biomass demand

The main goal of the present chapter was to illustrate the influence of changes in krill population structure and seasonal variation in their energy content on the estimates of prey biomass demand of large whales. Although this goal was accomplished, estimations were also influenced by other factors whose effects, in some cases, were even more relevant than those produced solely by changes in population structure and energy content of prey. Based on the results of the present study, the ratios between *AMR* or *FMR* and *BMR*, have the strongest influence in the final estimations of prey biomass demand (see Appendix 5-1). Whale species and sex were also important in the estimated biomass demand as consequence of the influence of length and mass of whales. The apparent digestive efficiency had a lower influence in the estimates, whereas the procedure to estimate the winter-spring daily rates needs further confirmation and can be improved with information on occupancy of whales.

Similarly, the estimations of minimum swarm density are dependent of all those factors plus the added effect of engulfing volume (a size dependent feature) and of the different speeds and times used to perform feeding activities, traveling or resting.

5.5.2. Mass of blue and fin whales

Table 2-1 shows weight (mass) values for *B. musculus* and *B. physalus* published between 1904 and 1997 based either on measurements of whales at whaling stations or from empirical equations obtained with previously published mass and length data. As expected, data change according to whale ground, age and sex. Accordingly, it is highly advisable to select length or mass data from as close as possible to the studied region.

Notably, estimations by Trites & Pauly (1998) represent the heaviest masses reported for both species (Table 5-7). Compared with the present study, those masses are 22% and 35% larger for female and male fin whales, and 80% and 73% larger for female and male blue whales. Such difference seems to be related to the origin of the data. Their model was developed using data originally published by (Lockyer, 1981b) with Antarctic whales, but southern-hemisphere Balaenopterid whales are frequently larger than their northern-hemisphere counterparts (Urbán-Ramírez, 2000). In the present work, lengths used for both sexes of *B. musculus* were consistent with those reported by Jefferson *et al.* (1993) for the species in the North Pacific. In the case of *B. physalus*, a strong discrepancy in mass occurs for specimens in the North Atlantic: Kenney *et al.* (1985) reported smaller values for the east coast of the U. S. than those reported by Víkingsson *et al.* (1988) for Iceland. Fortunately, the masses reported by Tomilin (1957) in the North Pacific are closer to those recorded by Merrick (1997) in the Bering Sea (Table 5-1).

5.5.3. Effect of seasonal changes in energy content and krill population structure

Mårtensson *et al.* (1996) have shown that small changes in energy content of prey can increase annual estimates of consumption by several tonnes. However, this author does not report uncertainty on the parameters used for that study. In the present study, once that uncertainty was considered, the effect of changes in whale mass or seasonal changes in the energy content of prey were negligible. Still, seasonal variations in the energy content of regional euphausiids (mostly *N. simplex*) can alter the estimates of maximum biomass removal rates by whales. For fin whales, a seasonal variation of ~18% in the energy content of a prey represented a 22% increase in the maximum biomass required, *i.e.* twice the differences in biomass demand observed between male and female fin whales. This represents an extra of ~21 t day^{-1} in the biomass demand for the whole population of fin whales in the Gulf of California.

Although blue whale and fin whale have relatively well-known feeding habits in certain regions (Del Angel Rodríguez, 1997; Jiménez-Pinedo, 2010; de Voss *et al*., 2018), no consensus exists in the energy value of their prey. Some authors assume 1.3 kcal g^{-1} (Steimle & Terranova, 1985) when fin whales feed on fish, and Lockyer (1987) used a value of 0.93 kcal g^{-1} when feeding on small zooplanktonic crustaceans. This represent a range of 1.3/0.93 = 1.4, *i.e.* slightly less than the 1.7 range in energy content due to swarm structure on a dry weight basis (Table 2-8), but higher than the 1.11 range observed for swarms on a wet weight basis (Table 5-2). Still, even a small difference can produce an important increase in the maximum biomass demand of both blue and fin whales in the Gulf of California. Such differences highlight the importance of accurate evaluation of energy content and wet weight of prey and the need of further studies on swarm structure.

5.5.4. Consumption rates during winter-spring

Evidence suggests that blue whales must feed during winter migration. Apparently, the lack of sufficient food does not threaten their survival. However, it seems clear that pregnant, and more importantly, lactating blue whales will require extra amounts of energy in order to feed their calves (Lockyer 1981b). Appropriate calculations are required to estimate the extra amount of energy required by lactating blue whales in the area in order to estimate the minimum threshold (in terms of krill density) needed to satisfy such requirements.

5.5.5. Importance of the area for blue and fin whales

Wade & Gerrodette (1993) estimated that there are 1,415 (*C. V.*= 24%) individuals of *B. musculus* in the Eastern Tropical Pacific, whereas Calambokidis & Barlow (2004) estimated 2,994 (*C. V.*= 0.14) blue whales in the Eastern North Pacific. Thus the 576 individuals estimated

for the Gulf of California and adjacent waters (Gendron, 2002) will represent 17–41% of the total, and the 283 individuals that enter the Gulf of California (Gendron, 2002; Ugalde de la Cruz, 2008) will represent 9–20% of the whole population in the Eastern North Pacific. In contrast, fin whales in the North Pacific range from 14,620 to 18,630 individuals (Braham, 1991), and the 300 individuals in the Gulf of California (Urbán-Ramírez, 1996) represent only 1.6–2.1% of such a population. Still, the Gulf of California represents a permanent residence area for fin whales and serves as a winter-feeding ground and nursery area for blue whales that migrate from the Oregon-California region. This feeding behaviour has been observed in other blue-whale nursery areas (Hucke-Gaete *et al*., 2004), suggesting that metabolic requirements are not fulfilled in summer feeding grounds. Lockyer (1981b) estimates that fasting mammals lose 0.25% of their mass daily, and if whales were completely fasting during the 8 months of winter migration they will lose around 45% of mass. This figure is unrealistic for most animals, and the fact that in reality they only lose 25% of their mass suggests that feeding in "winter" grounds can be critical for the survival of Balaenopterid whales. This seems particularly important for lactating and pregnant females. For instance, Busquets-Vass (2017) demonstrated, using stable isotopes from baleens, that blue-whale males do not migrate south every year and can stay in California for several years, whereas females do migrate every year. Such regular behavior seems consistent with a higher demand for food in pregnant and lactating blue whales, which can be provided in winter grounds. This should be taken into consideration in the face of local and regional stressors. For instance, increasing whale-watching activity may disrupt daytime feeding and diving behavior of those whales, and might be conducive to an abandonment of the area if disturbed. In terms of climate change, *N. simplex* does not cope well with high temperatures (Brodeur,1986) and hypoxic habitats (Tremblay *et al*., 2010), and here the prediction is that *N.*

simplex will be restricted to northern colder habitats (Brodeur,1986; Gómez-Gutiérrez *et al*., 2012) forcing blue and fin whales to adapt to spatial and temporal changes in prey availability.

5.5.6. Feeding strategies for both whale species

At the end of spring and beginning of summer both whale species prey on the subtropical krill *N. simplex* in the Gulf of California, where productivity is high but does not peak as much as in the feeding grounds from high latitudes (California-Washington region). The results provided in the present study still need to be compared with secondary production data of *N. simplex* in the Gulf of California to estimate if whales decrease substantially the available prey biomass in the area. It is common to observe that under natural conditions, species with similar ecological requirements coexist in an apparently stable form even when resources are limited (Broadhead & Wapshere, 1966).

Evidence exist that the winter-spring diet of blue and fin whales in the Gulf of California is comprised mainly of *N. simplex* swarms (Del Angel-Rodríguez, 1997; Jiménez-Pinedo, 2010). For blue whales, the maximum required prey densities (41–51 g m^{-3}) seem to be met at least with some of the observed swarm densities in the field (32–88 g m^{-3}; Gendron, 1992; Ladrón de Guevara *et al*., 2008). It is expected that lower abundance of prey or lower prey density will trigger migration of blue whales to more profitable areas, explaining their restricted use of the gulf. For fin whales, the maximum required density of *N. simplex* swarms in winter time needs to be 47–62 g m^{-3} and 59–75 g m^{-3} in spring. Such results imply that in general the maximum observed swarm densities in the Gulf of California (88 g m^{-3}) merely satisfy the estimated daily consumption of *B. physalus* during spring. Gendron *et al.* (2001) suggested that sardine-like fishes can be important part of the diet of *B. physalus* in this area. According to this, as conditions become warmer, *B. physalus* might be feeding on fishes and other organisms in

several areas of the Gulf of California to satisfy their annual metabolic demands. The smaller size and lower energy requirements of *B. physalus* can explain a higher tolerance to habitats with lower prey quality (i. e. lower energy content). This can be compensated by an extensive use of the area, by alternative food sources in other seasons, or alternative food sources in the same season when food density of the preferred prey is below the required limit.

The present work provides abundant elements to re-evaluate the contribution of winter whale grounds as feeding areas, and to carry on future projects on feeding ecology of Balaenopterid whales in tropical and subtropical areas. In order to evaluate the impact of the proposed prey biomass demands in the Gulf of California it is also important to generate a more comprehensive evaluation of *N. simplex* swarms in the area, including density estimations (using hydroacoustic methods and different plankton gears); spatial and temporal distribution of those swarms; frequency of occurrence; and the net content of food provided by each swarm as a function of size and density. It is also important to analyse the rates of predation on *N. simplex* by other vertebrates such as birds and fishes, and the mortality caused by parasites and parasitoids (Gómez-Gutierrez *et al*., 2015a, b). Finally, the geographic, bathymetric and temporal segregation among species that consume this euphausiid, and the effect on different species preying different stages of this euphausiid should be determined.

5.6. References

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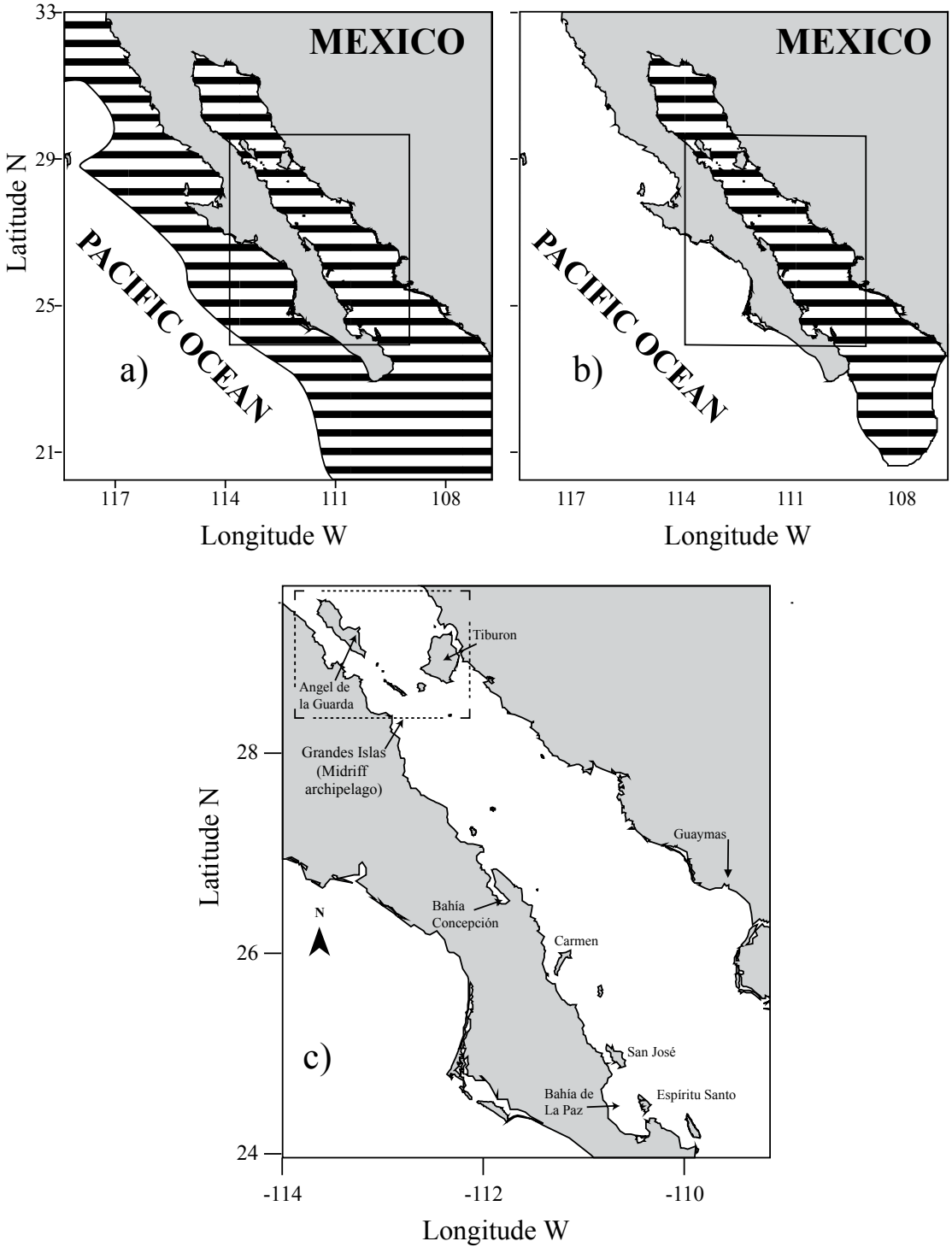

Figure 5-1 Approximate distribution of blue and fin whales in the Gulf of California and adjacent waters. a) Blue whale distribution based on historical sightings and satellite-monitored radio tracked animals (Mate *et al*., 2000). b) Fin whale distribution based on historical sightings. c) Detail of the central part of the Gulf of California for location of places used in text.

Table 5-1 Reported weights for blue whale (*B. musculus*) and fin whale (*B. physalus*).

Species	Weight (kg)	Author	Comments
B. musculus	69, 235	Lockyer (1976)	Average of males and females Antarctic
	102,736	Trites & Pauly (1998)	Average of males and females (Worldwide)
	110,126	Trites & Pauly (1998)	Females, calculated from L_{max} (Worldwide)
	95,347	Trites & Pauly (1998)	Males, calculated from L_{max} (Worldwide)
	61,280	Calculated from L_{max}	Females L_{max} = 26.8 m (True, 1904)
	55,142	Calculated from L_{max}	Males L_{max} = 25.46 (assuming that 95 % of female length)
B. physalus	45,000	Tomilin (1904)	North Pacific Females
	35,000	Tomilin (1904)	North Pacific Males
	30,000	Kenney *et al.* (1985)	Males and females, from east USA
	42,279	Víkingsson *et al.* (1988)	Males and females from Iceland
	49,000	Merrick (1997)	Males and females from Bering Sea
	55,590	Trites & Pauly (1998)	Average of males and females (Worldwide)
	59,819	Trites & Pauly (1998)	Females, calculated from L_{max} (Worldwide)
	51,361	Trites & Pauly (1998)	Males, calculated from L_{max} (Worldwide)
	47,298	Calculated from L_{max}	L_{max} = 24 m (Jefferson *et al*.,1993)
	42,645	Calculated from L_{max}	Males L_{max} = 22.8 (assuming 95 % of female length)

Weight values appear ordered by date of publication for each species

Table 5-2 Proportions of different age classes and relative energy contribution in *N. simplex* swarms, in four hypothetical scenarios with different proportions of male and female adults, and different proportions of each sex (Gendron, 1992). .

	Percentages of each life stage			
Case	E-1	E-2	E-3	E-4
Proportion Female: Male	1:1	2:1	1:1	2:1
Mature animals within females (%)	43	43	4	4
Mature animals within males (%)	72	72	4	4
Juvenile	14.2	15.8	32.0	32.0
Juvenile Females	12.7	16.0	21.3	9.5
Adult-sized females	9.5	12.7	16.0	21.3
Ovigerous females with eggs	10.8	14.3	1.0	1.3
Spent Females	10.8	14.3	1.0	1.3
Juvenile Males	4.7	3.1	16.0	10.7
Adult-sized Males	16.0	10.7	4.7	3.1
Males with spermatophores	36.0	24.0	2.0	1.3
Individuals kg^{-1}	156,112	155,033	251,662	244,369

	Energy (kJ kg^{-1})							
	February				May			
	E-1	E-2	E-3	E-4	E-1	E-2	E-3	E-4
Juvenile	187	207	682	663	154	170	560	544
Juvenile Females	166	220	450	583	136	180	370	479
Adult-sized females	448	594	1218	1577	368	488	1000	1295
Ovigerous females with eggs	737	976	111	143	605	802	91	118
Spent Females	562	744	84	109	462	611	69	90
Juvenile Males	108	72	598	387	89	59	492	318
Adult-sized Males	227	150	1255	812	187	123	1031	667
Males with spermatophores	1745	1155	156	101	1433	949	128	83
Total (kJ kg^{-1})	4181	4118	4555	4375	3434	3383	3741	3594

Total energy values are referred to a 1-kg (wet weight) swarm of *N. simplex*.

Table 5-3 Individual winter-spring daily rate (*WDR*) in (kJ ind^{-1} day^{-1}) of male and female blue (*Balaenoptera musculus*) and fin whales (*B. physalus*).

Species	Sex	Mass (kg)	Mean WDR (kJ ind^{-1} day^{-1})	Lower limit -95% CI (kJ ind^{-1} day^{-1})	Upper limit +95%CI (kJ ind^{-1} day^{-1})
B. musculus	Male	**55,142**	892,974	230,706	1,550,148
		69,235	978,329	249,292	1,770,976
		95,347	1,089,632	257,418	2,199,596
		102,736	1,163,192	265,838	2,299,343
	Female	**61,280**	956,392	260,580	1,657,414
		69,235	1,004,075	266,032	1,794,107
		102,736	1,123,514	278,006	2,263,252
		110,126	1,209,053	284,133	2,429,395
B. physalus	Male	30,000	571,301	182,067	1,021,584
		35,000	610,974	186,013	1,130,061
		37,000	624,869	190,569	1,161,975
		42,279	653,748	194,001	1,219,882
		42,645	671,651	197,880	1,253,937
		49,000	696,776	200,299	1,331,948
		51,361	718,530	202,993	1,389,673
		55,590	744,036	206,512	1,477,155
	Female	30,000	571,180	179,821	1,024,404
		37,000	616,959	186,269	1,151,016
		42,279	659,625	193,715	1,255,321
		45,000	694,492	196,043	1,323,161
		47,298	721,817	199,935	1,353,797
		49,000	737,828	205,034	1,384,909
		55,590	763,211	209,923	1,454,047
		59,819	787,404	214,319	1,518,372

Estimates are based on mass from Table 5-1 and estimated via Monte Carlo simulations from Equation (1). Bold values represent the most likely mass for whales in the Gulf of California

Table 5-4 Individual winter-spring biomass demands of prey (*WBD*) of *B. musculus* and *B. physalus* during winter-spring in the southeastern Gulf of California (kg ind^{-1} day^{-1}) estimated from different methods

Species	Month	Mass (kg)	Mean *WBD* (kg ind^{-1} day^{-1})	Lower limit -95% CI (kg ind^{-1} day^{-1})	Upper limit +95%CI (kg ind^{-1} day^{-1})
B. musculus	February	**55,142**	**207**	55	**360**
		61,280	**215**	57	**373**
		69,235	225	59	399
		95,347	245	60	486
		102,736	261	62	530
		110,126	276	63	560
B. physalus	February	30,000	134	41	240
		35,000	142	43	260
		37,000	147	43	270
		42,279	153	44	285
		42,645	**157**	45	**295**
		45,000	161	46	305
		47,298	**165**	46	**313**
		49,000	169	47	321
		51,361	172	48	328
		55,590	176	49	337
		59,819	180	50	351
B. physalus	May	30,000	162	50	294
		35,000	172	52	313
		37,000	177	53	328
		42,279	185	54	344
		42,645	**191**	55	**357**
		45,000	196	56	368
		47,298	**201**	57	**382**
		49,000	206	58	391
		51,361	211	59	404
		55,590	216	59	417
		59,819	220	60	429

All estimates are adjusted for prey assimilation and winter demand ratio. Variation in energy content of *N. simplex* swarms correspond to types depicted in Table 5-3. Bold numbers depict mass, average and maximum biomass demand for whales with a mass likely to be found in the Gulf of California.

Table 5-5 Maximum winter-spring biomass demand of prey (*WBD*) (kg ind^{-1} day^{-1}), maximum required densities of *N. simplex* swarms (g m^{-3}) and estimated time invested on feeding (h day^{-1}) according to sex and whale species for winter-spring in the southeastern Gulf of California.

Species		*B. musculus*		*B. physalus*			
Month		February		February		May	
Sex		Males	Females	Males	Females	Males	Females
M (kg)		55,142	61,280	42,279	47,298	42,645	47,298
Average *WBD* (kg day^{-1})		207	215	153	165	191	201
Maximum *WBD* (kg day^{-1})		360	373	285	313	357	382
Filtered volume (m^3)		28.24	23.28	21.16	17.72	21.16	17.72
Filtering rate (m^3 h^{-1})		367.3	302.8	252.8	211.8	252.8	211.8
Mean required krill density (g m^{-3})		23.5	29.6	25.1	32.5	31.5	39.6
Max. required krill density (g m^{-3})		40.8	51.3	47.0	61.7	58.9	75.1
Examples of krill density (kg m^{-3})		**Expected consumption rate (kg h^{-1})**					
Schoenherr, 1991	0.0045	1.7	1.4	1.1	1.0	1.1	1.0
Schoenherr, 1991	0.0150	5.5	4.5	3.8	3.2	3.8	3.2
Gendron 1992	0.0326	12.0	9.9	8.2	6.9	8.2	6.9
Ritz & Hosie, 1982	0.0783	28.8	23.7	19.8	16.6	19.8	16.6
Ritz & Hosie, 1982	0.0848	31.1	25.7	21.4	18.0	21.4	18.0
Ladrón de Guevara *et al*., 2008	0.0880	32.3	26.6	22.2	18.6	22.2	18.6
Croll et al., 2005	0.1500	55.1	45.4	37.9	31.8	37.9	31.8
Examples of krill density (g m^{-3})		**Estimated time invested on feeding (h day^{-1})**					
	4.5	218	274	251	329	314	400
	15.0	65	82	75	99	94	120
	32.6	30	38	35	45	43	55
	78.3	**13**	**16**	**14**	**19**	**18**	**23**
	84.8	**12**	**15**	**13**	**17**	**17**	**21**
	88.0	**11**	**14**	**13**	**17**	**16**	**20**
	150.0	**7**	**8**	**8**	**10**	**9**	**12**

Selected mass for each species and sex corresponds to whales likely to be found in the Gulf of California. Expected consumption rate (kg h^{-1}) over *N. simplex* was estimated from filtering rates of whales and available krill density. Time invested on feeding was estimated from expected consumption rate and maximum *PBD*. Bold numbers depict times below 24 h day^{-1}.

Appendix 5-1 Steps involved to calculate the ratio between Active Metabolic Rate (AMR) or Field Metabolic Rat (FMR) and Basal Metabolic Rate (BMR) as suggested by Leaper et al., 2007.

Species	Weight (kg)	Kleiber (1975) (6) (kJ d^{-1}) M (kg) a=293.1 b=0.75	Boyd (2002) (11) (kJ d^{-1}) M (kg) a=2529.2 b=0.524	Nagy (2005) (9) (kJ d^{-1}) M (g) a=4.82 b=0.734	Lockyer 1981b (8)[b] (kJ d^{-1}) M (kg) a=110 b=0.783	Innes et al. (1986) (10) kg d^{-1} M (kg) a=0.42 b=0.67	Corrected *AMR* or *FMR*				FMR/BMR ratio			
							(11) *FMR*[c]	(9) *FMR*[c]	(8) *AMR*[c]	(10) *AMR*[d]	LL			UL
B. musculus	69,235	1,251,008	869,589	2,740,603	2,837,654	735	1,086,986	3,425,754	3,547,067	5,006,959	0.9	2.7	2.8	4.0
	102,736	1,681,930	1,069,365	3,661,436	3,865,122	957	1,336,706	4,576,795	4,831,402	6,522,438	0.8	2.7	2.9	3.9
	110,126	1,771,876	1,109,005	3,852,957	4,081,165	1,003	1,386,256	4,816,197	5,101,456	6,833,167	0.8	2.7	2.9	3.9
	95,347	1,590,362	1,028,348	3,466,238	3,645,707	911	1,285,435	4,332,798	4,557,134	6,204,282	0.8	2.7	2.9	3.9
	61,280	1,141,576	815,715	2,505,757	2,579,021	677	1,019,643	3,132,197	3,223,777	4,613,806	0.9	2.7	2.8	4.0
	55,142	1,054,697	771,827	2,318,970	2,374,462	631	964,784	2,898,713	2,968,077	4,298,818	0.9	2.7	2.8	4.1
B. physalus	45,000	905,577	693,851	1,997,584	2,025,115	551	867,314	2,496,980	2,531,394	3,751,528	1.0	2.8	2.8	4.1
	35,000	750,010	608,240	1,661,089	1,663,373	465	760,300	2,076,362	2,079,216	3,170,162	1.0	2.8	2.8	4.2
	30,000	668,124	561,041	1,483,386	1,474,247	420	701,301	1,854,232	1,842,809	2,859,085	1.0	2.8	2.8	4.3
	42,279	864,190	671,541	1,908,194	1,928,590	528	839,426	2,385,243	2,410,738	3,597,985	1.0	2.8	2.8	4.2
	49,000	965,301	725,514	2,126,429	2,164,751	583	906,893	2,658,037	2,705,938	3,971,798	0.9	2.8	2.8	4.1
	55,590	1,061,117	775,107	2,332,784	2,389,553	634	968,883	2,915,980	2,986,942	4,322,187	0.9	2.7	2.8	4.1
	59,819	1,121,102	805,465	2,461,767	2,530,751	666	1,006,832	3,077,209	3,163,439	4,539,814	0.9	2.7	2.8	4.0
	51,361	999,979	743,627	2,201,162	2,246,003	602	929,534	2,751,452	2,807,504	4,099,022	0.9	2.8	2.8	4.1
	37,000	781,929	626,211	1,730,243	1,737,346	483	782,764	2,162,804	2,171,682	3,290,417	1.0	2.8	2.8	4.2
	47,298	869,796	674,581	1,920,307	1,941,653	531	843,227	2,400,384	2,427,066	3,618,827	1.0	2.8	2.8	4.2
	42,645	940,046	712,199	2,071,967	2,105,656	569	890,249	2,589,958	2,632,070	3,878,837	0.9	2.8	2.8	4.1

Numbers between parenthesis correspond to formulae in text. Lower limit (LL) and upper lipid (UL) were estimated from corrected *FMR* from Boyd (2002) and corrected *AMR* from *R* (Innes *et al*., 1986)

[a] Mass in kg was transformed into g

[b] Originally reported as kcal d^{-1}, but transformed into kJ d^{-1} using an equivalence of 4.184 kJ $kcal^{-1}$

[c] Energy demand is calculated for an assimilation efficiency of 80%

[d] Energy demand is calculated for a prey calorific value of 5,450 kJ kg^{-1} and an assimilation efficiency of 80%

Chapter 6. **Summary and conclusions**

Evidence shows that both *Balaenoptera musculus* and *B. physalus* breed and feed in the Gulf of California. However, it is not clear if the food supply supports the energy requirements needed for breeding. To estimate biomass intake by predators, it was necessary to understand and estimate the effect of development, reproduction, season and location on the energy content of krill.

6.1. Reproduction in *Nyctiphanes simplex*

Large increases in weight coupled with large increases in energy content for reproduction (via lipids) of a polar species are expected where high production events (spring and summer) occur for short periods, with no reproduction the rest of the year. In contrast, a low weight increase with moderate increase in energy for reproduction is consistent with an environment with a continuous food supply where *N. simplex* can reproduce throughout the entire year.

The ecological function of this neritic, subtropical euphausiid as a pivotal food source for several resident and migratory pelagic and epibenthic predators in the Gulf of California, does not rely solely on high abundance or density, since adults in breeding condition appear to provide a substantial source of essential fatty acids, and since ovigerous females carrying eggs can constitute a considerable energy supply to upper trophic levels.

6.1.1. Sexual dimorphism

The effect of *TL* on *DW* followed a power function and significant differences in the slopes (allometric exponents) confirmed sexual dimorphism on *Nyctiphanes simplex* (Chapter 2 & Chapter 3). Lower, or less sustained weight gain in females can be attributed to higher energy

expenditure during reproduction. Accordingly, year-round increased weight gain at higher rates in males is consistent with year-long reproductive events for *N. simplex*.

In early-winter months males exhibited lower allometric exponents than the annual mean, with a slight increase at the end of winter. Allometric exponents for females followed a similar trend, but were always lower than in males. A slower *DW* gain during colder months, when primary productivity and reproductive activity peaks, suggests stalled growth as consequence of the use of energy for reproduction.

A stalled weight gain in males during colder months can explain why proteins and energy content by mass unit (J mg^{-1}) were lower in colder months than in warmer months (Chapter 4), as seasonal variation in energy content by mass unit was driven by protein contribution to *DW*. Protein gain was more associated with somatic growth whereas lipid played a larger role during oogenesis and reproduction. As energy provided by proteins is not necessarily available for krill reproduction or other activities, it contributed mostly to the nutritional value of krill as prey.

6.1.2. Biochemical changes before and after reproductive maturity

During somatic growth, females increased *DW* 3.8 times before reaching adult size, whereas males increased *DW* an average 2.8– 6.1 times (from J_m stage to SA_m and A_m stages respectively). For both sexes, somatic growth was driven mainly by proteins and this was followed by polar and neutral lipids. As *TL* increased, polar lipid content (mg ind^{-1}) increased at similar rates to the *DW*. Consequently, the percentage of polar lipids remained relatively constant during somatic growth. Neutral lipids increased at lower rates than polar lipids or *DW*, decreasing their relative contribution. During stages preceding maturity, most of the assimilated energy via lipids is devoted to growth as the relative contribution of total lipid content to *DW* decreased >50% from juvenile forms into post-juvenile non-mature forms.

Once individuals reached sexual maturity, lipid storage became more relevant. The relative contribution of total lipids increased 1.2–2.3 times compared with post-juvenile non-mature forms, with a concomitant increase of ~20% in energy per mass unit. During egg production, concentrations of neutral and polar lipids of females increased ~1.75 and ~2.5 times respectively. As expected for a subtropical species with continuous food supply, lipid storage was most relevant for the reproductive processes, as post-juvenile non-mature forms did not accumulate large amounts of lipids during growth.

Changes in protein and lipid content had a significant effect on the energy content that *N. simplex* can provide as prey at different life stages and between sexes. During somatic growth, the energy content of females increased 2.7 times, mainly due to increase in *DW* via proteins. During gonad maturity and egg production the estimated energy content of mature females increased 1.2–1.8 times depending on brood size, mostly via lipids and without significant increases in *DW*. Males increased their energy content 2.1–2.4 times during somatic growth but no significant changes occurred in energy content or *DW* during gonad maturity (development of spermatophores). Apparently, energy required for spermatophore production is very low and does not demand substantial lipid storage. It is unclear if this feature can be generalized to all euphausiid species or if this is a unique feature of subtropical and perhaps tropical species.

6.1.3. Changes in fatty acids during maturity

Polar lipids increased at high rates during somatic growth and even at higher rates during gonad maturity of females. Despite those quantitative changes, fatty acid profiles of polar lipids did not exhibit strong changes, suggesting that composition of structural lipids was more stable throughout both development and maturity. In contrast, neutral lipids increased substantially during gonadal maturity of females and experienced significant changes in fatty acid

composition. At least three essential fatty acids seem to be key components for the gonadal maturity of this subtropical krill although their contribution differed depending on life stage and sex. The transition from differentiated juvenile forms into adult-sized forms was marked by a notable increase in the relative contribution of 20:4ω6 (ARA) in both sexes. For males, strong increases in 20:5ω3 (EPA) and gradual increases of 22:6ω3 (DHA) during development accounted for most of the variation in essential fatty acids, whereas for females, the relative content of 22:6ω3 increased almost two fold during gonad maturity. In crustaceans, those fatty acids are commonly required during reproduction and are also considered essential (none or very low *de novo* synthesis), and therefore they should be obtained via their prey. Accordingly, availability of specific food sources that provide those fatty acids can be a conditioning factor towards maturity and reproduction.

6.2. Fasting

Fasting experiments were useful to calibrate some morphometric indices as indicators of unfavourable feeding conditions that might occur naturally. Morphometric indices were better correlated with fasting conditions when they did not depend on sex, or when sex differences were taken into account. The earliest sign of fasting was a decrease in krill stomach-fullness (>30%) after 40 h, followed by a decrease in size of the digestive gland (~7%) and a strong decrease in total lipid content (>60%) after 57 h. Finally, a decrease in the observed dry weight (< 9%) compared with the expected weight for animals of a given size occurred after 86 h fasting. Unfortunately, I did not test for protein loss, and therefore I cannot confirm if water and proteins also decreased after longer fasting times. Still, differential responses in morphometric parameters under laboratory conditions were useful to approximate fasting times in wild animals.

Compared with polar species adapted to light-limited primary productivity, *N. simplex* has evolved in areas with constant food supply, and does not have enough lipid storage to deal with fasting times >86 h, this species requires continuous food inputs and survival rates are expected to decrease if productivity in the Gulf of California decreases as consequence of either global warming or intense el Niño events.

6.2.1. Body condition and protein content

In general *BC* followed protein content more closely than lipid content, and therefore body condition was not considered a strong proxy of recent feeding conditions. I proposed the use of a combined set of morphometric indices or a combination between biochemical and morphometric indices to detect not only unfavourable feeding conditions in the field but the time frames for recent feeding events and type of food.

This approach was particularly useful to explain apparently contradictory results. For instance, male krill specimens collected in October 2001 had relatively low lipid content (close to the threshold of 21.8 μg mg^{-1} for fasting animals), but relatively high body condition (>1.3) and high protein content. Such high values in *BC* and protein content, together with a higher 18:1ω9/18:1ω7 ratio, suggest high krill uptake of animal-prey during October 2001. However, as response rate of lipids to fasting conditions was faster than response rate of *BC*, it is possible that at the time of being captured, individuals were subject to short fasting conditions (<60 h) as evidenced by lower lipid content.

6.2.2. Hepatosomatic index and lipid content

Among the morphometric indices, *HSI* was not only sensitive to short-term changes in trophic condition, but also had the strongest negative correlation with fasting time, suggesting

that, at least for subtropical krill, *HSI* can be confidently used to compare food availability at short time scales (< 5 days).

During fasting, almost 70% of the lipid content of *N. simplex* was consumed within 3 days, producing a decrease of 4.6% *DW* via lipid consumption. These large changes over a short period confirm that this subtropical species is adapted to a continuous food supply, and therefore is more sensitive to short-term temporal and geographical changes in food availability compared to temperate and polar species.

As *HSI* had a faster response to fasting conditions than total lipid content, then the combined use of both indices can be useful for discerning between recent and previous fasting events. Here I propose that only animals feeding continuously will exhibit both high *HSI* and high lipid content, whereas high lipid content, but low *HSI* will indicate recent unfavorable feeding conditions, but prior favorable feeding conditions or better prior food quality. In contrast, large *HSI* and low lipid content will only indicate very recent feeding activity.

In euphausiids, shrinkage occurs only after molting, and *N. simplex* has an intermolt period of 3–6 days. Apparently, more than one molting event is required to evidence shrinkage in terms of the eye: total length index as during short fasting periods (< 5 days) there was no evidence of shrinkage in krill.

6.2.3. Fatty acids

Most fatty acids exhibited significant decreases (40–60%) in concentrations after animals fasted for 57 h, and an even higher decrease (> 80%) after 124 h. Nonetheless, the relative ratios of some fatty acids commonly used as diatom (16:1ω7/16:0) and dinoflagellate markers (22:6ω3/20:5ω3), did not show significant differences during the 137-h fasting period, confirming that they are consistent trophic markers. Conversely, other ratios increased during

fasting as consequence of differential fatty-acid decrease rates (Σ ω6/ω3 FAs, C_{16}/C_{18} PUFA, 16:1ω7/18:4ω3, and the zooplankton marker 18:1ω9/18:1ω7). These results highlight the need to evaluate overall feeding (or fasting) conditions before assessing and using fatty acid ratios as food biomarkers.

6.3. Biochemical composition and environmental effects: Regional scale

Following previous conclusions (Chapter 2 and Chapter 3), sex, stage and overall feeding conditions were considered before evaluating the environmental effects on the biochemical composition of *N. simplex*. During March 2010 only mature-sized individuals were compared for environmental effects at regional scales. Similarly, non-fasting conditions were tested before comparing biochemical composition and fatty acids among sampled stations.

Spring hydrographic conditions allowed *N. simplex* to access diverse food items in quantities sufficient to minimize fasting periods. For wild conditions, average fasting periods were < 2.5 days, regardless of oceanographic conditions. Short fasting times for specimens collected in spring indicate good generalized feeding conditions. The biochemical analysis suggests that, depending on the hydrographic conditions, *N. simplex* can switch from diatoms into alternative food sources. Likely, sources such as detritus, copepods, and cyanobacteria or Chlorophyceae are consumed either directly or indirectly, and can satisfy short-term requirements as shown by morphometric indices. In the long term, all those different food sources guarantee a continuous food supply for this krill species

Feeding strategies of krill were modified by hydrographic conditions, and morphometric indices and fatty acid profiles were useful to infer such effects. For instance, krill distributed along the east coast, close to the upwelling region, had higher proportions of diatom biomarkers. Here I observed differences between the north and east portion of the upwelling region: There

was indirect evidence of continuous primary production in the northern section of the east coast (high concentrations of C_{16} PUFA, 16:1ω7, phytanic acid, the largest total fatty acid concentrations observed in this study, high *HSI*, high concentration of chlorophyll-*a*). The dynamic conditions in the southern section of the upwelling region restricted the retention of food for longer periods. Here, krill fed actively (high stomach content) but feeding conditions were below optimum (*HSI* below values observed in the north, and low total lipid concentration, plus low molting rates reported in literature).

Compared with other regions, krill from the warm southwest region had a small, but significant increase in 18:2ω6 and 18:3ω3, and low proportions of 20:5ω3. While ubiquitous in several microalgae groups, it is still possible to propose that either Chlorophyceae or cyanobacteria play an important trophic role for *N. simplex* in warmer regions, far from the influence of coastal upwelling events. Both groups have large proportions of 18:2ω6 (10–20%), and 18:3ω3 (13–25%), and lower proportions of 20:5ω3 (6–8%) than diatoms, and unlike dinoflagellates, they presumably have negligible proportions of 22:6ω3 (0.3–0.53%). Furthermore, eurythermal adaptations have been reported for some cyanobacteria inside the Gulf of California, and Chlorophyceae can also exist in warm waters.

In order to acquire better information on the timescale and the ecological relevance of the associations between key plankton species, like *N. simplex*, and their alternative food sources further studies should focus on both subtropical and tropical habitats, especially during summer conditions, when upwelling is weaker, and circulation patterns are different.

6.4. Environmental effects: seasonal scale

As in the previous chapter (Chapter 3), hydrographic conditions allowed *N. simplex* to access diverse food items, which is consistent with the euryphagous habits reported for this subtropical

krill species. The use of alternative food sources can explain survival in stressful conditions, particularly during warm and less productive periods.

Higher chlorophyll *a* concentrations in colder months were consistent with the winter-spring diatom blooms promoted by frequent upwelling conditions. Larger average total lengths of *N. simplex* and the accumulation of storage lipids during colder months suggest favourable feeding conditions. In general, energy provided by lipids in colder months is readily available for krill reproduction and other activities and, as with many invertebrates, lipid accumulation might promote gonad maturity and trigger the intense reproductive period. This additional energy also contributes to the nutritional value of krill as prey.

There were also substantial differences between the beginning and the end of the colder period. In winter (February), relative abundance of typical diatom biomarkers was not as high as that observed during the winter-spring transition (May). Still, the high levels of C_{16}PUFA observed in winter suggest the presence of diatoms at the exponential phase of growth. By the end of the diatom bloom and during the winter-spring transition (May) large-sized males in Bahía de La Paz region exhibit low *DW* and consequently low *BC*. This decrease was mostly an effect of low protein content, consistent with a non-carnivorous diet, and suggests a direct intake of diatoms, probably at the stationary phase of growth, as suggested by the high proportions of 14:0 >4% and 20:5ω3 >20% observed in storage lipids.

During warmer months, when high temperatures, a strong stratification, low phytoplankton densities, and lower chlorophyll *a* values prevailed, average length of *N. simplex* decreased, suggesting overall poor feeding conditions, particularly in summer (July). Still, lack of adequate food is probably not the only reason for body shrinkage in this subtropical species. Summer high temperatures are considered physiologically unfavourable (oxidative stress) for *N. simplex*, and

some other krill species can also shrink if exposed to high temperatures, even when food supply is abundant (e.g. *E. pacifica*). Accordingly, I proposed (Chapter 4) that decreases in body size for the overall population of *N. simplex* in Bahía de La Paz is a physiological response that allows survival in stressful summer conditions, these being either food scarcity or high temperatures or a combination of both.

During the summer-winter transition (October), when chlorophyll *a* values decreased and total phytoplankton abundance declined, males exhibited small sizes and low neutral lipid content in both absolute and relative terms. However, energy content was higher and body conditions reached their highest values as a consequence of the increase in dry weight via the increase in protein content. In fact, specimens collected in summer had high energy content (> 18 J mg^{-1}). This suggests the use of alternative food sources during the transitional period, which is consistent with the euryphagous habits reported for *N. simplex*. Biochemical evidence and literature information suggest that alternative food sources can be different from year to year or among regions, and can include dinoflagellates, silicoflagellates, and nanoplankton (as those groups increase their abundance in warmer months), or Cryptophyta and autotrophic prokaryotes such as cyanobacteria (inferred in other studies by pigment correlation with krill abundance).

6.5. Energy content of *Nyctiphanes simplex*: nutritional value as prey

In the present study seasonal variations in the energy content of the subtropical euphausiid *N. simplex* did not alter significantly the average estimates of biomass removal rates by large whales. However, a decrease of ~18% in the energy content of krill from February to May represented a 22% increase in the maximum estimated biomass demand for fin whales (Chapter 5). This represents an extra of ~21 t day^{-1} in the biomass demand for the whole population of fin

whales in the Gulf of California. Accordingly, it was important to estimate if krill densities in the area were enough to support those increases in demand.

For blue whales, the maximum required densities of *N. simplex* swarms of ~51 g m^{-3} can be met during winter in the Gulf of California (32–88 g m^{-3}). It is expected that lower prey density will trigger migration of blue whales (particularly lactating females) to more profitable areas, consistent with a differential use of the area. For fin whales, the required density needs to be >62 g m^{-3} in winter and >76 g m^{-3} in spring. Here, the observed swarm densities merely satisfy the estimated daily consumption of *B. physalus*. As the water becomes warmer, *B. physalus* might be feeding on fishes and other organisms in several areas of the Gulf of California to satisfy their annual metabolic demands. The use of habitats with lower prey quality can also be compensated by an extensive use of the area, by alternative food sources in other seasons, or alternative food sources in the same season when food density of the preferred prey is below the required threshold. On the other hand, higher energy requirements of *B. musculus* and stenophagous habits (feeding on a limited variety of prey), may explain its restricted use of the area, making it advantageous to move to more profitable areas, once the abundance of krill decreases.

Apparently, metabolic requirements of some Balaenopterid species are not fulfilled in summer feeding grounds, and estimated weight loss for fully fasting whales is almost twice the real weight loss observed. This suggests that feeding in “winter” grounds can be critical for the survival of such whales. Here, I stressed the physiological and ecological importance of winter feeding grounds for the survival of the species and the effect that some external factors such as ocean warming or intensive whale watching might exert on feeding patterns of whales in the area, with adverse effects on whale populations.

The results obtained in the present work show the importance of regional estimates of prey energy and biochemical content, and the effect of population structure and seasonal variations on the consumption rates of predators. Although the trophic significance of such changes for whale populations seem minor compared with other sources of variation such as the ratio between *AMR* or *FMR* and *BMR*, the ecological effects of variations in prey energy still seem relevant.

There are, however, several issues that must be resolved to obtain more realistic estimates of the trophic impact of Balaenopterid whales in the Gulf of California. For instance, in order to evaluate the impact of the proposed prey biomass demands in the Gulf of California and adjacent waters it is also important to estimate the abundance of *N. simplex* swarms in the area; the spatial and temporal distribution of those swarms and the frequency of occurrence; and the net content of food provided by each swarm as a function of size and density. In order to achieve this knowledge, we need a better understanding of population structure of krill swarms and of the factors driving this structure.