Investigating the effects of long chain omega-3 fatty acids on primary school achievement

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Kerry Maree Lee

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Abstract

Background: All parents are keen to support their child to learn and grow. A variety of studies have identified benefits to children's cognitive development with omega-3 (ω-3) PUFA supplementation. The majority of these studies however have involved children with learning or behavioural difficulties and have generally utilised highly specific cognitive tests. Few studies have involved healthy normally-achieving mainstream children and even fewer have used classroom tests to identify academic rather than cognitive changes.

Aim: The aim of this study was to investigate whether supplementation with ω-3 PUFA (fish oil) affected the academic achievement of 8-13 year old general classroom children. Whether these children, their parents and teachers could detect changes in learning and behaviour attributed to this supplementation was also investigated.

Methods: A double-blind randomised placebo controlled study over a 15 week period was undertaken with 209 children. Randomisation was stratified for age and gender. These were healthy normally-achieving mainstream children who attended the same school. Every school day the active group consumed 900 mg of omega-3 whilst the placebo group consumed 900 mg of vegetable oil. Changes to academic ability was investigated using the Thurstone Word Fluency Tests (testing fluency and spelling), the NZ generated asTTle reading test and maths basic facts tests. The daily consumption of foods enriched in ω-3 PUFA was assessed using food frequency questionnaires at baseline and recording the child's intake of these foods every day at school for the duration of the study. Possible changes in behaviour and attitude were investigated using children, parent and teacher questionnaires.

Findings: The food frequency questionnaire and intake records identified a low consumption of ω-3 PUFA rich foods. Fish oil treatment did not affect fluency and reading compared to placebo treatment. Significant improvements were identified with fish oil compared to placebo in subgroups of 8-9 year olds for an aspect of spelling and in highly numerate and literate children for division. Parents and teachers did not identify any significant differences between treatment groupings when completing the behaviour questionnaire. Children consuming fish oil reported at 4 and 15 weeks

significant improvements related to getting along with the others compared to children in the placebo group. This trend was also reflected in the teacher questionnaires regarding child behaviour.

Conclusions: Despite some significant improvements being evident, because of the fact that these were only in subgroups and potentially the result of multiple calculations, the notion that omega-3 can influence academic achievement cannot be accepted. These findings however strongly highlight the need for additional research.

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All photographs used as chapter headings in this thesis are images taken by the researcher in the process of completing this study.

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Abbreviations

Abbreviation	Term
AA	Arachidonic Acid
ABC	Aberrant Behaviour Checklist
ADHD	Attention-Deficit Hyperactivity Disorder
AFC	Anterior Frontal Cortex
AI	Average Daily Intake Level
ALA	Alpha-Linolenic Acid
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
asTTle	Assessment Tools for Teaching and Learning
CI	Confidence Interval
CNS	Central Nervous System
COWA	Controlled Oral Word Association
CPRS	Connors Parents Rating Scales
CTRS	Connors Teachers Rating Scales
CVLT	California Verbal Learning Test
DCD	Developmental Coordination Disorder (Dyspraxia)
DGLA	Di-homo-gamma-linolenic acid
DHA	Docosahexaenoic Acid
DLFC	Dorsolateral Frontal Cortex
DPA	Docasapentaenoic Acid
EFA	Essential Fatty Acids
EPA	Eicosapentaenoic Acid
ETA	Eicosatetraenoic Acid
FADS	Fatty Acid Deficiency Symptoms
FFQ	Food Frequency Questionnaires
fMRI	Functional Magnetic Resonance Imaging
FSANZ	Foods Standards Australia New Zealand
GLA	Gamma Linolenic Acid
HVLT	Hopkins Verbal Learning Test
HUFA	Highly Unsaturated Fatty Acids
IRT	Item Response Theory
KABC	Kaufman Assessment Battery for Children
LA	Linoleic Acid
LC PUFA	Long-Chain Polyunsaturated Fatty Acid
LTM	Long-Term (secondary) Memory
MABC	Movement Assessment Battery for Children
MRI	Modern Magnetic Resonance Imaging
MUFA	Monounsaturated Fatty Acids
NEPSY	Neuropsychological Assessment
NZ	New Zealand
PET	Positron Emission Tomography
PPVLT	Peabody Picture Vocabulary Test
PUFA	Polyunsaturated Fatty Acids
RAVLT	Rey Auditory Verbal Learning Test
RBC	Red Blood Cell

ROS	Rostock-Oseretzky Scale,
RCT	Randomised Controlled Trials
RDI	Recommended Daily (Dietary) Intake
SA	Stearidonic Acid
SD	Standard deviation
SDT	Suggested Dietary Target
SFA	Saturated Fatty Acids
SNAP	Swanson, Nolan & Pelham Rating Scale
SOLO	Structure of Observed Learning Outcomes
TEA-ch	Test of Everyday Attention for Children
TOVA	Test of Variables of Attention
TWFT	Thurstone Word Fluency Test
UL	Tolerable Upper Intake Limit
VLFC	Ventrolateral Frontal Cortex
WAIS	Wechsler Adult Intelligence Scales
WIAT	Wechsler Individual Achievement Test
WISC	Wechsler Intelligent Scale for Children- Third Edition
WFT	Word Fluency Tests
WJ	Woodcock-Johnston Psycho-educational Test Battery
WM	Working Memory
WRAT	Wide range Achievement Test
ZPD	Zone of Proximal Development
α	Alpha
ω-3	Omega-3
ω-6	Omega-6

Chapter 1

Introduction

Chapter 1: Introduction

Scientists, for many years have researched the old adage 'you are what you eat' by investigating the effect diet plays on the body's functions including that of the brain. In the last three decades there has been an increasing interest in the effects of polyunsaturated fatty acids (PUFAs) on behaviour and learning, with a main focus being on the effects of omega-3 (ω-3) fatty acids, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from fatty fish and fish oil. The brain is a fat rich organ, with ω-3 PUFA playing a fundamental role in its structure and function (Bourre, 2006). Traditionally studies in this area have involved animals, fed diets lacking ω-3 PUFA, with results indicating that this lead to significant reductions in DHA levels in brain lipids and dramatic changes in brain function such as changes in learning and memory, depression and aggression (Sinclair, Begg, Mathai, & Weisinger, 2007).

More recently the majority of ω-3 PUFA cognitive research has focused on the elderly or children with learning and behaviour difficulties, with very little research having been undertaken into the effects on the learning of typically achieving children. More particularly, there has not been a randomised, double-blind, placebo controlled study carried out in New Zealand (NZ), and only a handful internationally, on the effects of ω-3 PUFA on the academic achievement of 'general' school children. To date no studies have used assessment measures routinely used in classrooms rather than the experimental laboratory tasks frequently used in this type of research.

The aim of this study was to investigate the effects of diet supplementation with long-chain (LC) ω-3 PUFA on the academic achievement of 'general' classroom children. This chapter will outline why this research is needed as well as providing an overview of the study.

Importance of this Investigation

Parents are constantly bombarded with claims of the nutritional benefits of dietary supplementation yet many of these claims originate from marketing promotional material rather than scientific findings (Baglione, Tucci, & Stanton, 2012). The few scientific publications that are quoted to support supplementation in the general

population are in fact studies of small numbers of people such as those with specific learning or behavioural difficulties e.g. dyslexia. Whilst there have been significant findings reported by some of these trials, at the time of the development of this study there was no evidence of the effect of ω-3 supplementation on mainstream children. This study aimed to address that. The results of this study, whether supportive of beneficial effects or not, will be of interest and value to parents and schools, as well as to the general public as discussed below.

There is "good evidence that the deficiency of some micronutrients and omega-3 fatty acids influence the cognition and behaviour of children" (D. Benton, 2008b, p. 39). If evidence is found to show that supplementation with ω-3 PUFA enables children to increase their potential by positively influencing behaviour and academic achievement, then this could have a profound effect on education. Schools could start reallocating funding to provide ω-3 PUFA to children to help reduce the already large 'education gap' identified between achievement of low and high decile children in NZ (McNaughton & Lai, 2009; UNICEF Innocenti Research Centre, 2002). Other variables, such as demographic, socio-economic, health and social factors have been shown to influence academic achievement, yet unlike providing ω-3 PUFA supplementation, these are often unable to be addressed by the classroom teacher. Pressure could be brought to bear on the government to assist with this supplementation, similar to that brought to the UK government, when in 2006 it announced a controversial proposal to supplement children *en masse* across the educational system with ω-3 PUFA (D. Kennedy et al., 2009).

"Nutrition is a modifiable factor, which can influence cognitive development in children" (Bryan, 2004, p.302). In the complex education environment it is one of the few things over which parents have the majority of control. Findings from this study will add information to the pool of knowledge that will assist parents to make informed dietary choices for their children. Parents of children with behaviour and/or learning difficulties may first turn to supplementing their children's diets with ω-3 PUFA, rather than to drugs such as Ritalin.

The impact of changing parental demands could have a pronounced effect on the food industry. Demand and the subsequent cost of foods high in ω-3 PUFA may increase markedly. "If further research points to increased health and behaviour benefits of

increased omega-3 levels and supplementation for children, then the sources of supplementation need to be considered. With dwindling fish stocks, obtaining recommended levels of DHA and EPA may become an issue" (Kirby, Woodward, & Jackson, 2009, p. 29). Novel animal feed strategies are already being developed with the intention of increasing the ω-3 PUFA content of eggs, dairy, fish, poultry, pork and beef products (Uauy & Valenzuela, 2000). Although animal products enriched with ω-3 PUFA have existed on the world market for many years (Born, 1998; Uauy & Valenzuela, 2000), fortification debates, similar to those which occurred in NZ in 2009, over fortification of bread with folic acid (New Zealand Food Safety Authority), may occur as parents and communities start lobbying for subsidised or easy access to products containing or enriched with ω-3 PUFA.

Failure to find a strong association between ω-3 PUFA consumption and educational benefits would also be significant. A systematic review undertaken in 2007 stated there was "insufficient evidence to either confirm or refute the hypothesis for the effects of omega-3 and fish oil on the behaviour, cognition and educational outcomes of normal children" (Taylor & Connock, 2007, p.23). The findings from this review highlight the need for randomised controlled trials (RCTs). They stated that "until such trials report their findings, there is no clear basis for changing the current recommendation of the consumption of at least two portions of fish per week" (Taylor & Connock, 2007, p.23).

The following section provides the reader with background information to key aspects of the study. It is not anticipated the reader will have in-depth knowledge on all aspects related to this study which include educational, scientific, food as well as the nutritional theory and latest research findings. For this reason the following section will provide a brief overview to explain relevant aspects of the brain and how it functions and influences academic achievement and behaviour. This will be followed by information on ω-3 and its influence on behaviour and academic achievement, followed by the methodology chapter, which will outline and justify the methods utilised. The summarised results will be presented in the results chapter and then explained and related to other research findings in the discussion chapter. Recommendations for future studies will be compiled from the findings of this study and the areas where research is identified as lacking or contradictory. The key findings from this study and their implications for children, parents, teachers and the wider community will be woven together in the conclusion chapter.

Chapter 2

Literature Review

Chapter 2: Literature Review

In order to interpret the findings of this study one must first understand how the brain is structured and how the brain's ability to function changes as it develops. Nutrients are vital to this development and therefore an understanding of their role and their impact is also needed. This study investigates how these nutrients (ω-3 PUFA in particular) affect children's ability to learn. It is therefore necessary to understand how cognition and academic achievement are commonly measured. The following literature review is divided into two main sections. The first section focusses on the brain, its structure and function as well as the tools used to assess academic achievement and behaviour. The second section focusses on ω-3 PUFA. This will include information on what ω-3 PUFA is, its role in brain structure and function as well as behaviour. This will be followed by the influences of supplementation with ω-3 PUFA on children's academic achievement in areas such as verbal fluency, spelling, maths and behaviour.

The Brain

The brain is a specialised organ made up of approximately 100 billion nerve cells called neurons. The role of these neurons is to gather and transmit electrochemical signals across relatively long distances. These neurons are made of a cell body (containing the nucleus), an axon (long cable like projection which carries the electrochemical message) and the dendrite or nerve ending which connects the cell to other cells (as shown in Figure 1). Axons are frequently covered with a myelin sheath, which is composed of 70-80% lipids (fats) and 15-30% proteins, and develops over time, by a process called myelination (Paus et al., 1999). Essential fatty acids (EFAs) play an important role in the synthesis of myelin. The myelin sheath helps to prevent the electrochemical message leaving the axon by decreasing the capacitance across the cell membrane and increasing the electrical resistance (Bryan et al., 2004; Paus et al., 1999; Reiss, Abrams, Singer, Ross, & Denckla, 1996).

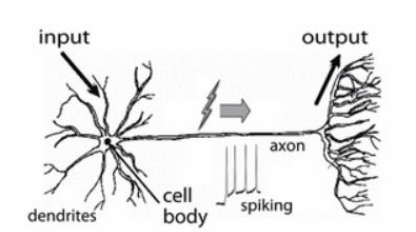


Figure 1: Schematic diagram of a neuron (Jezzard & Toosy, 2005, p. 94)

During the postnatal period if there is a deficiency in these EFAs, a major delay in the myelination process will occur accompanied by impaired learning (C. Chang, Ke, & Chen, 2009). As children mature, and myelination occurs, information is processed more quickly (Galotti, 2011). These myelinated axons are white in appearance, hence the term "brain white matter" (Valentine & Valentine, 2012), 24% of which consist of phospholipids (Stevens et al., 2003; Uauy & Dangour, 2006). Essential fatty acids also have a crucial role as 'messengers' and are involved in the synthesis and function of brain neurotransmitters (C. Chang et al., 2009). Omega-3 plays an important role in neural growth as well as the development of synaptic processing of neural cell interaction. Key to this development and functioning is ω-3 PUFA, and any reduction in these levels whilst the brain is still developing may have profound effects on its structure and function (C. Chang et al., 2009; Innis, 2007; Uauy & Dangour, 2006).

Research has shown the positive influence of ω-3 PUFA supplementation for those up to the age of two when the brain is still rapidly growing (D. Benton, 2008a; Bryan et al., 2004; Cheatham, Colombo, & Carlson, 2006; Hughes & Bryan, 2003; J. McCann & Ames, 2005; Thatcher, 1991; Waber et al., 1981). This is believed to be due to the fact that the first two years of life are critical periods of rapid brain growth (B. Casey, Giedd, & Thomas, 2000), when nutritional deficiencies will have a marked effect on the child's development, especially that of the brain (D. Benton, 2008a; Bryan et al., 2004; Dalton et al., 2009; Hughes & Bryan, 2003; Muldoon et al., 2010). In more recent years the research focus has moved to those people in cognitive decline (ageing) (Beydoun, Kaufman, Satia, Rosamond, & Folsom, 2007; Bragin et al., 2005; AD Dangour et al., 2009; Kalmijn et al., 2004). As yet very little research has occurred on school-aged children (Pollitt, 1996), possibly because many regions of the brain are close to being fully developed. There are however a few regions of the brain which are slower to develop and may therefore be suitable for further research. The following section will

outline when these regions develop and what they are responsible for. It is only then the reader can understand why specific tests are needed in order to activate specific regions of the developing brain.

Brain Development

In the first third of a human pregnancy the child's brain is developing rapidly and this continues until the age of two (D. Benton, 2008a; B. Casey et al., 2000). The brain weight is 10% of the bodyweight for a new-born compared with only 2% for an adult. By the time the child is two years old the brain has grown to about 80% of the adult weight, however not all parts of the brain are fully developed at this age. The frontal lobes, which make up a quarter to a third of the mass of the cerebral hemispheres (Passler, Isaac, & Hynd, 1985), are one of the slower areas of the brain to develop. As a growing brain requires more ω-3 PUFA it is important to identify which areas are fully developed and which are growing at set ages (and therefore likely to be affected by ω-3 PUFA supplementation). What constitutes fully developed or fully matured is however hotly debated. In 1966 Luria wrote that the prefrontal regions of the cortex did not mature until between 4 and 7 years of age, whereas other researchers relate neurological development to myelinization and dendrite growth and suggest this continues until the age of 12 or beyond (D. Kennedy, Makris, Herbert, Takahashi, & Caviness, 2002; Passler et al., 1985; Plaisted, Wilkening, Gustavson, & Golden, 1983). Brain weight continues to increase until around 11-14 years of age when maximum weight is achieved (D. Benton, 2008a; Nolte, 2009). It is therefore difficult to clearly state when maturation occurs. Previous studies have focussed on the volume of the brain; this too however has proven to be problematic. The total cerebellum volume peaks approximately 2 years later than the cerebral volume (B. Casey et al., 2000; Mackie et al., 2007) adding to the confusion of what constitutes the brain and its maturation.

Modern magnetic resonance imaging (MRI) has also highlighted the complex and dynamic nature of brain maturation. There is an initial overproduction of grey (cortical) matter, which slowly decreases (is 'pruned') in volume whilst at the same time there is an increase in white matter (myelinated neural coatings) (B. Casey et al., 2000; Reiss et al., 1996; Sowell et al., 2003; Sowell, Thompson, Tessner, & Toga, 2001) throughout childhood and well into adulthood (Caviness, Kennedy, Richelme, Rademacher, & Filipek, 1996; Giedd et al., 1999; Sowell et al., 2003). The media often refers to this

action as ‘hard wiring’ (Giedd et al., 1999; Spano, 2003). This increase appears to be regional by nature, for example there is an increase in white matter in the dorsal prefrontal cortex but not the ventral prefrontal regions (B. Casey et al., 2000; Reiss et al., 1996; Sowell et al., 2001). Many believe that the slowest area to fully myelinate is the frontal cortex, commencing at approximately 6 months of age and continuing into adulthood (Bryan et al., 2004; NEMO Study Group, 2007; Reiss et al., 1996; Sowell et al., 2001; Spano, 2003; Youdim & Yehuda, 2000).

Whilst measurable parameters behave in a spurt-like fashion there is conflicting research suggesting that the underlying development is essentially continuous (Stuss, 1992). Supporting arguments have identified synaptogenesis appearing to be simultaneous in multiple areas (Rakic, Bourgeois, Eckenhoff, Zecevic, & Goldman-Rakic, 1986) and neurotransmitter receptors maturing at the same time (Lidow, Goldman-Rakic, & Rakic, 1991). “There is substantial support for a stepwise model of development, rather than a gradual progression, with convergent evidence that growth spurts occur in early infancy, again around 7-10 years of age, with a final spurt during adolescence” (Anderson, 2001, p. 122). Key regions of the brain can be seen in Figure 2

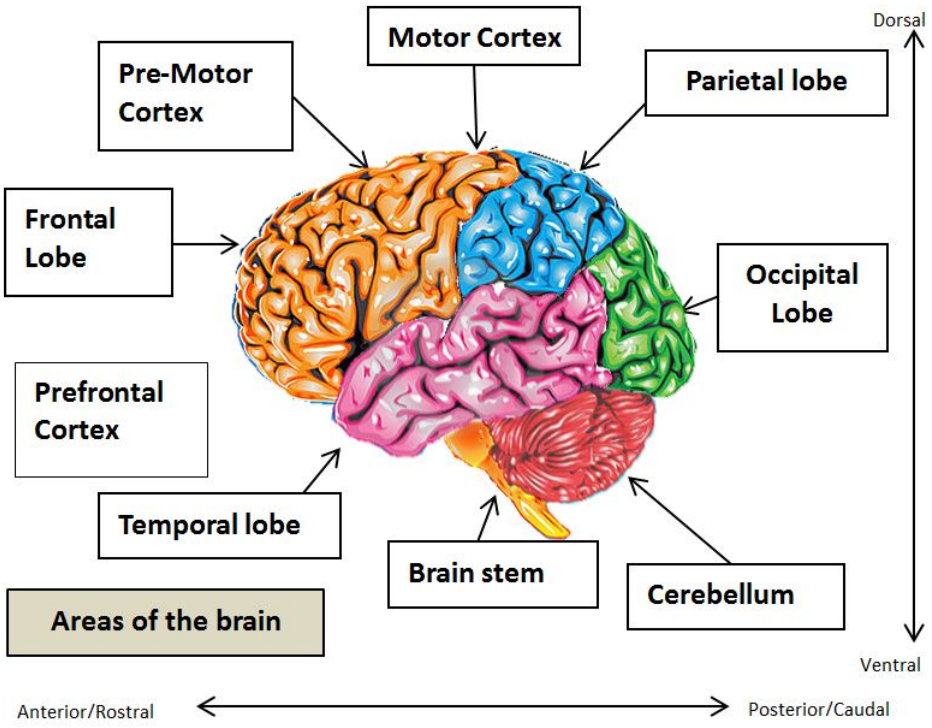


Figure 2: Areas of the brain

Growth spurts in frontal lobes occur between birth and 2 years, from 7-9 and again in the mid-teens (H. Epstein, 1974; Hudspeth & Pribram, 1990; Thatcher, 1991, 1992). These are critical times when optimal nutrition has a major impact on cognition, and any intervention may have the greatest impact (D. Benton, 2008a; Cheatham et al., 2006; Scott, Stewart, & De Ghett, 1974). For this reason ideally ω-3 PUFA research involving school-age children should include 7-9 year olds or 14-16 year olds.

Regions of the Brain

As stated above the brain is divided into many regions each with a differing role, and these regions develop at different rates, with the slowest thought to be the frontal cortex (Bryan et al., 2004; NEMO Study Group, 2007; Reiss et al., 1996; Sowell et al., 2001; Spano, 2003; Youdim & Yehuda, 2000). In order to understand how nutritional supplementation could affect a child's learning and behaviour, it is important to comprehend which aspect of learning or behaviour is controlled or influenced by each region of the brain.

The largest part of the brain is the cerebrum, which is divided into four different lobes. These cerebral lobes are folded into gyri (ridges) and sulci (grooves/fissures). Fissures are the large sulci (grooves) which divide the lobes. Each lobe (as seen in Figure 2) is responsible for a different cognitive function. The temporal lobe (on the side of the head) processes auditory information as well as information from the senses of taste and smell; the parietal lobe (underneath the top rear part of the skull) processes sensory information from the body, visuospatial processing and knowledge of number; the occipital lobe (at the back of the head) processes visual information and the frontal lobe (underneath the forehead) controls fine motor skills (including speech and voluntary movements) and cognitive functions (Nolte, 2009). There are three main divisions of the frontal lobes: the *motor and pre-motor cortex*, which are involved with planning movements, and the *pre-frontal cortex*, which is responsible for executive functioning (planning, making decisions, implementing strategies, inhibiting inappropriate behaviours and using working memory to process information) (B. Casey et al., 2000; Galotti, 2011).

Areas of the pre-frontal cortex have been shown to be activated during tasks which rely on working memory (B. Casey et al., 1995). The exact function of the frontal lobe however is not clear, and various theories exist to explain its role with regards to

executive functions and working memory (Alvarez & Emory, 2006). The single-process theories suggest a single process or system being responsible for a number of different executive or dysexecutive symptoms (P. Burgess & Alderman, 2004). The multi-process theories propose a frontal lobe executive system consisting of a number of components that work together in everyday actions (heterogeneity of function), whilst the construct-led theories assume that most, if not all frontal functions can be explained by one construct (homogeneity of function) such as working memory or inhibition (P. Burgess & Alderman, 2004).

Memory

The notion of memory is extremely complex. Memory forms the basis of all learning as people must take in (encode) and store information, and then use (retrieve) it, at an appropriate time (J. Fletcher, 1985; Strauss, Sherman, & Spreen, 2006). As learning cannot occur without some form of memory it is important to understand what memory is, how it is measured and where in the brain it occurs.

When people use the term 'memory' in everyday language they are usually referring to declarative or explicit memory (Squire, 2004). In 1890, James stated that short-term (primary) memory was qualitatively different from long-term (secondary) memory (LTM). In 1968, Atkinson and Shiffrin developed a 'multi-store model' where different forms of information (visual, acoustic etc.) formed short-term memory, and rehearsal was seen as the only mechanism for this information to reach long-term storage (Atkinson & Shiffrin, 1968). This model was seen as too simplistic and, in 1974, Baddeley and Hitch developed a 'multi-component' working memory theory, with separate subsystems for different forms of information (Jonides et al., 2008). The term short-term memory was replaced with the term 'working memory' (WM), and "refers to a mental workspace in which multiple sources of information are manipulated in order to perform complex problem-solving tasks" (P. Fletcher & Henson, 2001, p. 852). A figure summarising the complexity of these ideas is provided on the following page.

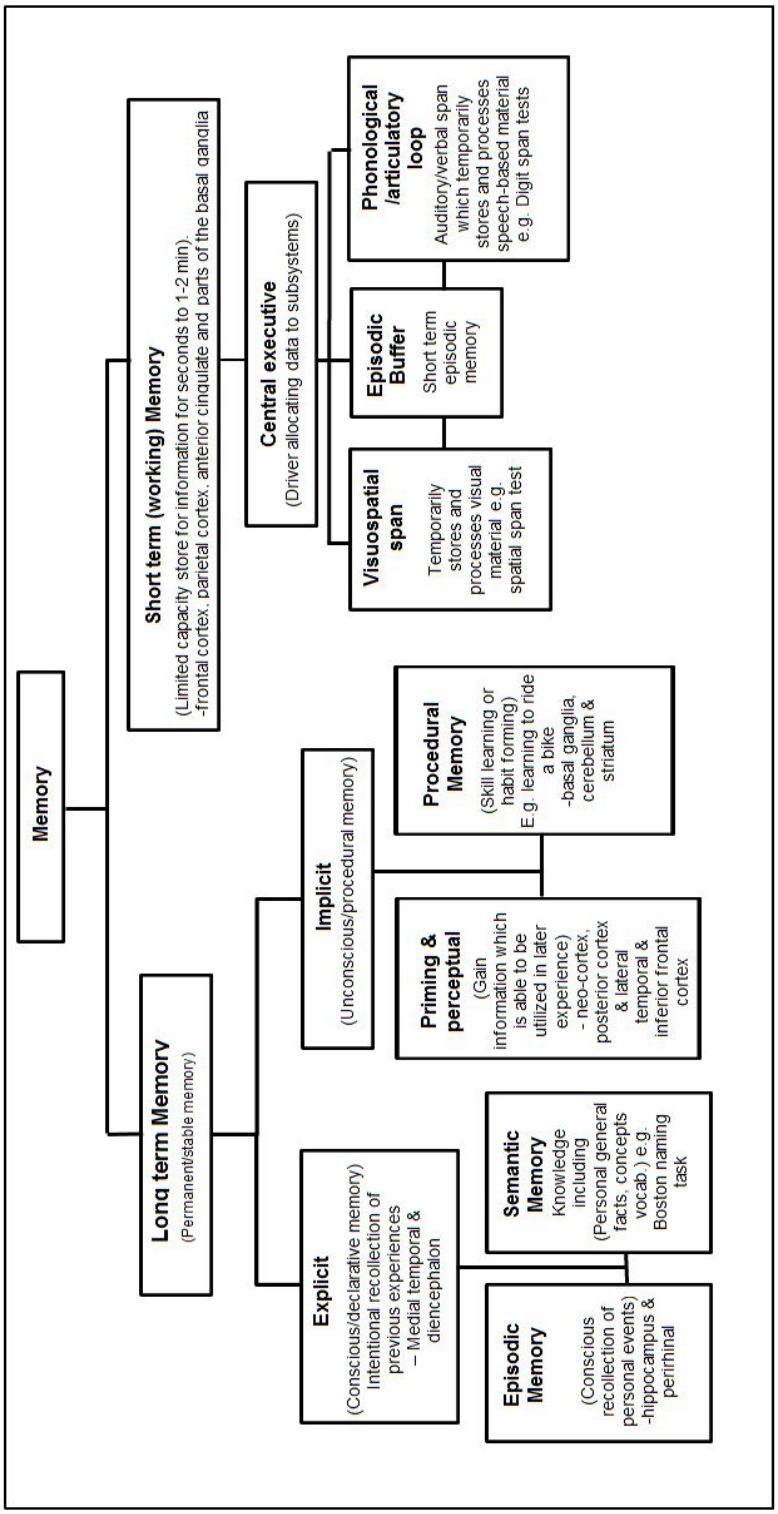


Figure 3: Memory Model developed from compilation of ideas and models by Baddeley & Hitch (1974, as cited in Jonides et al., 2008), Moscovitch (2004), Schacter (2000), Squire (2004) and Strauss, Sherman & Spreen (2006).

This 30 year old theory has gradually evolved and currently postulates that the central executive (located in the prefrontal lobes) channels information to three component processes: the phonological loop, which stores verbal-acoustic information; the visuospatial sketchpad, which stores visual and spatial information; and the episodic buffer, which links information across these sub-systems.

The visuospatial sketchpad is believed to assist with eye tracking and aspects of grammar, whilst the phonological loop is believed to play a key role in language acquisition (Alloway, Gathercole, Willis, & Adams, 2004). This loop has been shown to be very important during native language learning, and may also influence behaviour (Baddeley, 2003a). Memories in this verbal storage system are subject to rapid decay, however sub-vocal rehearsal process can be used to restore them (Alloway et al., 2004).

The episodic buffer is a relatively new concept and is concerned with the storage of information. It is capable of binding together information from a number of different sources into 'episodes' or chunks, whilst being heavily dependent on executive processes (Baddeley, 2000). The episodic buffer is believed to provide direct imports into episodic LTM (Alloway et al., 2004).

The central executive relies heavily on the frontal lobes (Baddeley, 2003b). The central executive is a flexible system responsible for the attentional control of WM, temporary activation of LTM (Bull & Scerif, 2001), co-ordination of multiple tasks, shifting between tasks or retrieval strategies (Baddeley, 1996) and selective attention and inhibition (Alloway et al., 2004). WM performance increases with increased myelination of the frontal lobe, whereas reading ability is correlated with myelination of the left temporal lobe (Nagy, Westerberg, & Klingberg, 2004). Moscovitch (2004) suggested that "the frontal lobes, control the information delivered to the medial temporal and di-encephalic system and encode, initiate and guide retrieval, monitor, and help interpret and organize the information that is retrieved…(and) act as *working-with-memory* structures" (p.8). It is believed that ω-3 PUFA accumulates in the frontal lobes, as identified in various animal (rat) studies (Carrié, Clément, de Javel, Francès, & Bourre, 2000a; Xiao, Huang, & Chen, 2005). Prenatal DHA deficiency studies have shown that the greatest reduction has been observed in the frontal cortex and it is the last region to fully recover normal DHA concentrations following dietary fortification (McNamara & Carlson, 2006). Further research has identified that DHA

supplementation has resulted in increased activation of the pre-frontal cortex during a sustained attention task (McNamara et al., 2010). For these reasons it is believed that changes to ω-3 levels may therefore affect cognitive function associated with this part of the brain.

According to *schema theory*, knowledge is stored in LTM in schemata (Sweller, 1994). These schemata categorise information elements according to how they will be utilised. A schema can hold a large amount of information yet be processed as a single unit in WM. "Schemata can integrate elements and production rules and become automated, thus requiring less storage and controlled processing…Schemas can also reduce working-memory load" (Kirschner, 2002, p. 3). When a learner adds to their prior knowledge (schema) they are able to reduce the WM load.

Knowledge and intellectual skill based on knowledge, is heavily dependent on schema acquisition (Sweller, 1994). Intellectual skill is acquired gradually and incrementally rather than an all-or-none fashion. "When a complex intellectual skill is first acquired, it may be usable only by devoting considerable cognitive effort to the process. With time and practice, the skill may become automatic to the point where it may require minimal effort for its operation. It is only then that intellectual performance can attain its full potential" (Sweller, 1994, p. 298). Schema acquisition and automation both substantially reduce WM load.

Cognitive Load Theory, developed by Sweller, assumes that a limited WM is connected to an unlimited LTM. Working memory load is affected by the inherent nature of the material and by the manner in which the material is presented (Kirschner, 2002). "The construction of adequate and rich schemata is especially important in complex learning tasks where it will require more effort, because the elements contained in the to-be-learned material is highly interconnected" (Kirschner, 2002, p. 4). In problem-solving, access to written recording systems can also help students to store information, but mental working space is the critical constraint (Ministry of Education, 2008b).

The majority of WM and LTM research involves observing patients with illnesses or head injuries to see what they are/aren't capable of doing and remembering (Alvarez & Emory, 2006; Strauss et al., 2006), as well as participants completing tasks whilst undergoing positron emission tomography (PET) or functional magnetic resonance imaging (fMRI). This enables researchers to see which parts of the brain are being

activated whilst the activity is being undertaken (Gabrieli, 1998). Using functional imaging in order to investigate functional specialization in the frontal cortex, is problematic especially when tasks are complex and sites of activation numerous (P. Fletcher & Henson, 2001). Each cortex has many functions, and singling out one activity which focusses solely on one of these functions is almost impossible.

At present there appears to be no one agreed understanding of what memory is and which parts of the brain are devoted to it (Squire, 2004). As well as this it is extremely difficult to devise a 'memory' test where there is no crossover between implicit and explicit/declarative memory, as participants are often building on prior experiences (Gabrieli, 1998), and hence responses and regions of the brain which are activated vary (Hikosaka, Miyashita, Miyachi, Sakai, & Lu, 1998). In order to store ideas the posterior regions of the brain are engaged, these include the parietal, temporal and occipital lobes, where-as rehearsal utilises a network of regions that include the ventrolateral frontal cortex (VLFC) (P. Fletcher & Henson, 2001). The left VLFC (as shown in Figure 4) is primarily concerned with the maintenance of verbal information and the right VLFC with the maintenance of spatial information. The VLFC is activated when 'selecting' information from LTM in order to use it in WM in some way. The dorsolateral frontal cortex (DLFC) is activated when 'selecting', manipulating and monitoring information that is already active in the WM. The anterior frontal cortex (AFC) is less well known as it is believed to be activated when selecting processes, goals and sub goals (P. Fletcher & Henson, 2001). Regions of the brain regularly linked with memory are shown in Figure 4.

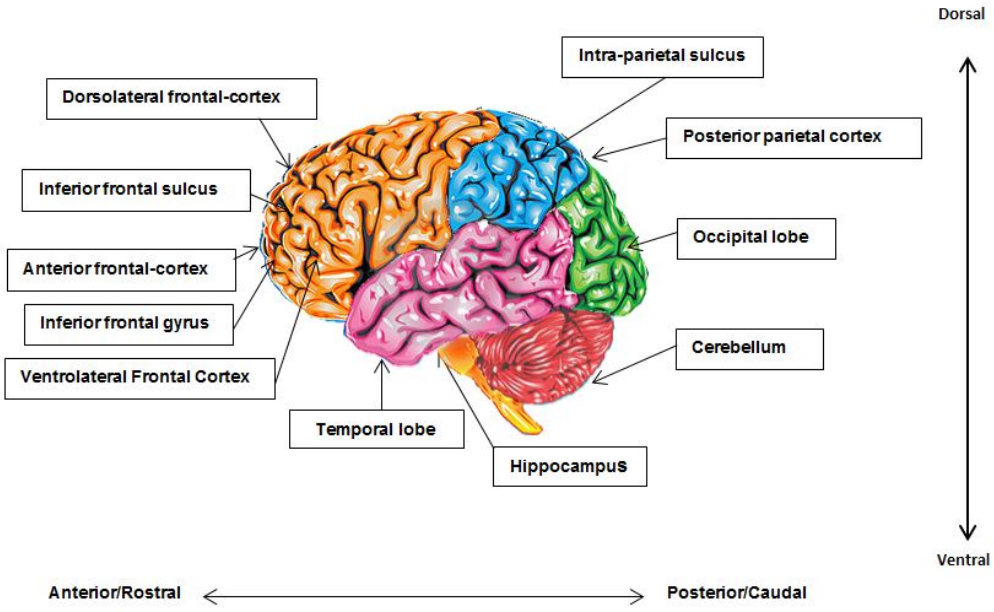


Figure 4: Specific regions of the brain which have been shown to be linked with memory

Little is known about the neural bases of cognition in normally developing children (B. Casey et al., 2000). It is believed that the period between the ages of 5 and 7 is critical for cognitive development (Bernstein, 1989; Riva, Nichelli, & Devoti, 2000). During this period, children are taught the components of language (Riva et al., 2000) which are strictly related to cognitive development. As children age their attention span increases as does their ability to focus their attention and shut out distractions (executive functioning) (Galotti, 2011). Their WM increases and they are more likely to plan ahead, use strategies and undertake planning of problem-solving tasks (Galotti, 2011).

The majority of research investigating the brain's organisation of cognitive abilities is based upon contemporary Western, urban middle class and literate brain-damaged individuals. “Cultural and linguistic diversity is an enormous, but frequently, overlooked moderating variable” (Ardila, 1995, p. 144). It is believed that the degree of brain lateralisation of language depends on literacy and in general, on the verbal training histories. Language usage differs according to the cultural background and strongly

correlates with the subject's educational level. Educational levels represent yet another influential variable of neuropsychological test performances (Ardila, 1995).

In a UK study, the relationship between WM and national curriculum achievement was investigated with 7 and 14 year olds. Close associations were found between national curriculum assessment and WM scores, showing that children who failed to achieve national expected levels had poor WM function. At 7 years of age, children with high abilities in English and maths scored better on WM measures than children of low or average ability. At 14 years of age, WM scores were strongly correlated with achievements in maths and science but not English (S. Gathercole, S. Pickering, C. Knight, & Z. Stegmann, 2004). Utilising the contents of WM involves a wide range of processes that may be generalised under the heading of executive processes (P. Fletcher & Henson, 2001). Yet recent functional imaging has highlighted the inadequacies of recent understanding of the range of 'executive functions' subserved by frontal cortex regions (Alvarez & Emory, 2006; P. Fletcher & Henson, 2001).

Executive Functioning

As ω-3 PUFA is believed to influence executive functioning (Aman, Mitchell, & Turbo, 1987; Berr et al., 2009; Bragin et al., 2005; A. Dangour et al., 2006; Dullemeijer et al., 2007; Kalmijn et al., 2004; Muldoon et al., 2010; Peet & Horrobin, 2002a; Sinn, Milte, & Howe, 2010; Van de Rest et al., 2008) it is important to understand what executive functioning is and how it relates to learning, behaviour and brain function.

Executive functioning is a popular psychological construct (first developed in the 1960's), which tends to be a catch-all phrase that equates with higher order cognitive activity (A. Benton, 1994; Hooper, Swartz, Wakely, de Kruif, & Montgomery, 2002). In order for a task to be a true measure of executive function it must be novel, effortful and should have some element of maintenance in WM (Bull & Scerif, 2001).

Executive function has been linked with the frontal lobes, which have rich connections with all other parts of the brain, with feedback and feed forward connective loops (Barkley, 1997b; Hooper et al., 2002; van der Sluis, de Jong, & van der Leij, 2007). Bull, Johnston and Roy (1999) identified that the functioning of the frontal lobe in children showed its greatest developmental increase between the ages of 7 and 10, whilst children aged around 10 had reached adult performance levels for many

executive function tests. Limited central executive may affect literacy, vocabulary and mathematics which then has the flow on effect on college entrance scores and occupational success (S. Gathercole et al., 2004). Executive functioning may be related to normal reading and writing development (Altemeier, Abbott, & Berninger, 2008) and is correlated with writing tasks and handwriting in normally developing populations.

Developing sensitive and specific measures of executive functioning has proved very difficult (Alvarez & Emory, 2006; Anderson, Fenwick, Manly, & Robertson, 1998; Hughes & Bryan, 2003; van der Sluis et al., 2007). "One difficulty has been the tendency to define executive function in terms of elusive 'meta' constructs such as planning, organisation, and problem-solving … such measures tap many subordinate cognitive processes that are not specific to executive functioning" (Archibald & Kerns, 1999, p. 116).

Welsh, Pennington and Groisser (1991), and Daigneault, Braün and Whitaker (1992) identified numerous components of executive functioning however Denckla (1996) reduced these down to four global terms: *initiating* (organisation, planning, strategy, fluency, efficiency and WM), *sustaining* (attention-driven behaviours), *inhibiting/stopping* (regulation of behaviour), and *set shifting* (problem-solving efficiency, cognitive flexibility, self-monitoring).

Baddeley (1996) listed four main functions of the central executive, these being: the ability to coordinate performance on two separate tasks and to switch between them, the ability to use retrieval strategies, the ability to attend selectively to one stimulus and inhibit the disrupting effect(s) of others, and the ability to hold and manipulate information in long-term memory. Barkley (1997a) identified inhibition and WM as the two fundamental components, whilst Pennington (1997) included not only inhibition and WM but also set shifting. Miyake et al. (2000) built on Denckla, Baddeley, Barkley and Pennington's models and identified the three main components as inhibition, shifting and updating/monitoring. These components are most commonly identified in the majority of literature and research relating to executive function.

Seigler (2005), on the other hand, developed an overlapping wave model, which is commonly applied to NZ classroom teaching, and is based on the premise that children's thinking is highly variable when selecting strategies to undertake a task. Children gradually change towards a faster and more accurate performance in at least

four ways: (1) introduction of new more advanced strategies, (2) increasing use of the more advanced strategies from among those that are already known, (3) increasingly effective execution of strategies, and (4) more adaptive choices among strategies. When problems become more difficult a backup (easier) strategy is more likely to be utilised.

'Inhibition' is often seen as the primary executive function that precedes, supports and allows development of other executive functions, including those which require switching attention from tasks (Carlson & Moses, 2001; Miyake et al., 2000). In order to problem-solve, one first needs to inhibit automatic responses so that strategic processes can be undertaken (Altemeier et al., 2008). Inhibition first occurs around 3-4 years of age and continues to develop through adolescence. Lack of inhibition and poor WM has been linked to lower mathematical ability (Bull & Scerif, 2001; van der Sluis et al., 2007). Children with Attention-Deficit Hyperactivity Disorder (ADHD) also have difficulties with inhibition tasks (Bull & Scerif, 2001). These children also have been shown to exhibit deficiencies in EFAs (Arnold et al., 1989; J. Burgess, Stevens, Zhang, & Peck, 2000).

Preschool boys (who have much higher rates of ADHD) (Breen, 1989; Gershon, 2002; Richardson, 2006; Richardson & Puri, 2000) are believed to require more EFA's (I. Colquhoun & Bunday, 1981; Pudelkewicz, Seufert, & Holman, 1968) and show less response inhibition than girls (Carlson & Moses, 2001). For this reason one might expect to find gender differences in any ω-3 PUFA research involving inhibition and mathematics, however this is a very poorly researched area as will be outlined in the ω-3 section of this chapter. Boys have also been found to outperform girls on oral fluency tasks, whilst girls outperform boys on written fluency tasks (Berninger & Fuller, 1992). To contrast this, two studies of typical development found no main effects for gender on executive tasks, nor interactions between age and gender in children under 12 years old (Altemeier et al., 2008; Welsh et al., 1991). Studies with less skilled readers found they have no difficulty activating relevant information, but do have difficulty suppressing the activation of irrelevant information (Bull & Scerif, 2001).

"Set shifting is the ability to flexibly shift attention as task demands change" (Altemeier et al., 2008, p. 489). It involves both inhibition and attention switching mechanisms (Miyake et al., 2000). This is believed to be involved in arithmetic performance by supporting changes between strategies. Children with arithmetic deficits have been

shown to display poorer shifting ability (van der Sluis et al., 2007); they tend to stay with a learned strategy rather than switching to a new strategy (Bull & Scerif, 2001). Bull and Scerif (2001) also found that more irrelevant information gained access to WM for children of lower mathematical ability, whilst children with difficulties in reading produced similar shifting responses to those able readers (van der Sluis et al., 2007).

Updating focuses on the ability to monitor and code incoming information and to update the content of memory by replacing older items with new more relevant information. For this reason updating is concerned with the dynamic, goal directed manipulation of memory content (van der Sluis et al., 2007). Few studies have investigated updating ability and its relation to reasoning, arithmetic or reading (van der Sluis et al., 2007). WM tasks and updating share the requirements to store information, and to revise the content of memory in light of new information (van der Sluis et al., 2007). Updating may also be a prerequisite for successful multi-task arithmetic calculations (Geary, 2004).

Mathematical ability has been shown to be significantly correlated with all measures of executive functioning (Bull & Scerif, 2001). It is believed that once children are able to retrieve arithmetic facts directly from LTM, executive processes may not play such an important role as the skill has become more automatic (Bull & Scerif, 2001). For this reason any ω-3 PUFA research would need to be undertaken prior to this automated response, however it must be noted this has not yet been well researched. Existing research will be outlined in the ω-3 PUFA section of this chapter, and will support the justification for this study's methodology.

In order for a child to learn and utilise their WM they need to focus on the task at hand, decide what is to be done, ignore any distractions, utilise the appropriate strategy and then change to a new one when needed. People find some of these tasks easier than others and devising tests to evaluate changes in these abilities is the key to investigating executive function. The preceding section has highlighted links between executive functioning and abilities in numeracy and literacy.

The following section will provide additional background material into literacy and numeracy development and neurological links. Only when these notions are fully introduced and explained will the links between literacy, numeracy and ω-3 PUFA be discussed.

Literacy and Language

Executive functions require an activity to be novel and challenging and have been shown to be correlated with writing tasks (Hooper et al., 2002), handwriting (Berninger et al., 2006), written out-puts (Hooper et al., 2002), and some integrated reading writing tasks (Altemeier et al., 2008). As stated later in this chapter, once literacy skills are highly developed and more automated they require less cognitive load and executive function. For this reason any ω-3 PUFA research involving children may find more pronounced results with children who have not yet attained this sophisticated literacy ability, although there is very little research to support this. The following section provides information relating to the formative development of these literacy skills and abilities, with the specific detail relating these to ω-3 PUFA research, being provided in the following ω-3 PUFA section.

Literacy and language are not single entities as they include listening, speaking, reading and writing. Each is a complex functional system that draws on common as well as unique brain processes to achieve different goals (Luria, 1973). The brain needs to identify letters and sounds, organise these in some form and then produce them. Speech has both a phonetic level (producing vowels and consonants) and a phonological/phonemic level (organising the speech and sound patterns in order to make sense) (Bowen, 2002).

Phonological awareness refers to a person's awareness of, and access to the sound structure of language. Two types of tasks have been developed to investigate phonological awareness. 'Phonological analysis' involves the ability to identify sounds within words whilst 'phonological synthesis' involves being able to blend speech segments into syllables or words (Wagner & Torgesen, 1987). "The same WM resources that are theoretically linked to math computation problem solving are presumably used to perform phonological awareness tasks" (Hecht, Torgesen, Wagner, & Rashotte, 2001, p. 196). Phonological processing has been linked with reading skills such as word-level reading and reading comprehension (Hecht et al., 2001).

Early spelling development relies on the analysis of speech sounds of spoken words (phonological word form) and proceeds from phonetic to partially phonemic to fully phonemic spelling in representing speech with alphabet letters (Berninger et al., 2006). Spelling does not rely solely on visual cues rather orthographic representations of words

are created by mapping them onto their spoken word counterparts. An autonomous orthographic lexicon is then constructed, where orthographic word forms are represented independent of phonological word forms; these are linked to spoken words and meaning, and retrieved automatically (Steffler, Varnhagen, Friesen, & Treiman, 1998). This is followed by a morphological stage during which children learn to spell the morphological variants of base words and the spelling rules for transforming letters at the beginning and end of base words into prefixes and suffixes (Carlisle, 1994). Although children progress from phonological through orthographic to morphological stages, they are, at the same time, also learning to coordinate phonological, orthographic, and morphological information (Carlisle, 1994).

It is believed that expert spelling requires both reflective and automatic processing (Berninger & Richards, 2002). Initially a written word is stored in temporary representations while the learner analyses the constituent letters and letter patterns and then relates the orthographic information to other aspects of language such as phonological, morphological and semantic knowledge. After the connections are developed and the word is encountered numerous times a more permanent word-specific spelling can be stored in long-term memory. In rats ω-3 PUFA has been shown to concentrate in the frontal lobes (Carrié, Clément, de Javel, Francès, & Bourre, 2000b; Delion et al., 1994). In humans optimal DHA concentrations are important to the cognitive processes supported by the frontal lobes of the brain (Bryan et al., 2004; Martinez, 1992). Frontal lobes are believed to be responsible for executive functioning and WM. Therefore before a child has competent spelling which is memorised and automatic, one could assume they would have an increased cognitive load utilising their WM rather than their LTM. Different effects of supplementation might be evident and measurable with these children. The few ω-3 PUFA research studies undertaken using spelling have been outlined in the ω-3 PUFA spelling section of this chapter.

The relationship between semantic processing and memory encoding is sometimes referred to as the ‘depth of processing’ effect (Craik & Lockhart, 1972). Those tasks which produce better recall often emphasise the meaning of items rather than the surface features. Anterior and posterior regions of the VLFC have been associated with this deep encoding (Gabrieli, 1998; Kapur et al., 1994) as well as generating words rather than just reading them (Petersen, Fox, Posner, Mintun, & Raichle, 1989). For this

reason ω-3 PUFA research ideally needs to focus on deep encoding rather than activities which utilise surface features.

WM tests have been shown to be good comprehension predictors (Daneman & Merikle, 1996). The "general processing hypothesis" states that a poor comprehenders' processing deficit is not specific to linguistic material but can be seen with a number of ways such as numerical and visuo-spatial materials (Engle, Cantor, & Carullo, 1992). However when these variables were matched, poor comprehenders were shown to have shorter listening spans than good comprehenders. Longer listening span participants are believed to be not only quicker to process linguistic material but also make greater use of strategies. This performance is affected by an increase in memory load, unlike those participants with a low listening span, who continue to utilise a strategy even when it is not efficient (DeBeni, Palladino, Pazzaglia, & Cornoldi, 1998). Less skilled readers have also been shown to have less effective suppression mechanisms than highly skilled readers (Gernsbacher, 1993). "Poor comprehenders more easily maintain active, and therefore intrusive, irrelevant information that has been processed" (DeBeni et al., 1998, p. 318). It is believed that general intellectual capacity could be a co-variant when investigating WM and comprehension (DeBeni et al., 1998).

The inferior frontal gyrus has been linked to language since the advent of neuropsychology (Broca 1861, as cited in Costafreda et al., 2006). This part of the frontal lobe has been linked with word comprehension and production: phonology (processes linked with the sounds of words) and semantics (processes associated with the meaning of words) (Costafreda et al., 2006; Gabrieli, 1998).

Studies investigating differences in neural activation between children and adults, have shown minor differences but in general there are not major changes in the regions which are activated in order to undertake fluency and arithmetic processes (Dowker, 2006; Gaillard et al., 2003; Kawashima et al., 2004). Some studies however have found a greater activation on the right inferior frontal lobe in younger children (Gaillard et al., 2000), and the superior frontal and parietal cortices between ages 9 to 18 whilst being correlated with WM performance (Dowker, 2006; Klingberg, Forssberg, & Westerberg, 2002; Rivera, Reiss, Eckert, & Menon, 2005). At times there may be no substantial or noticeable difference in behaviour or strategy use between children and adults but this does not confirm that the same regions are being activated in the brain. Rocha has

shown that children and adults use different strategies for solving arithmetic calculations. It is believed that learning enriches the arithmetic knowledge by increasing the number of available strategies (F. Rocha, Rocha, Massad, & Menezes, 2005).

"Phonological awareness tasks are sensitive predictors of emerging individual differences in mathematical computation skills because both domains substantially require resources devoted to phonological memory in the central controlling executive" (Hecht et al., 2001, p. 197). Few studies have investigated the relationship between performance in mathematics and levels of phonological ability (Holmes & Adams, 2006; Passolunghi, Vercelloni, & Schadee, 2007).

In a study investigating gender links between numeracy and literacy, differences between males and females on the verbal processing task tended to be smaller at the high-end of the distribution than at the low end, with females tending to have the advantage (Royer, Tronsky, Chan, Jackson, & Marchant, 1999). Boys have been shown to be less able and more reluctant readers (R. Mitchell, Murphy, & Peters, 2008; M. Smith & Wilhelm, 2009; Watson, Kehler, & Martino, 2010; Wheldall & Limbrick, 2010). Females on verbal tests have an overall advantage, with those at the bottom end of the distribution showing a wide gap in attainment, with dis-engaged boys struggling to match more engaged female counterparts (J. Fletcher, 1985).

Mathematics

It is very important to distinguish between arithmetic and mathematics. A common phrase is "arithmetic is to mathematics as spelling is to writing". Arithmetic deals with addition, subtraction, multiplication, division and the use of number calculations, whereas mathematics is the study of relationships among numbers, shapes and quantities, and includes arithmetic and algebra, calculus and geometry and trigonometry (Cobb, 1988; Grattan-Guinness, 2004).

Debate exists as to the relationship between mathematical and language abilities. Some research suggests that common processes underlie arithmetic and grammar (Baldo & Dronkers, 2007). Studies that focus on people with brain injuries have shown some patients to have a severe language impairment, yet relatively preserved mathematical skills, other patients show a reverse disassociation and yet others show an impairment to

both (Butterworth, 1999). It is believed that verbal skills play a role in arithmetic ability and/or common processes subserve both abilities (Baldo & Dronkers, 2007; Gullick, Sprute, & Temple, 2011). This notion is further supported as mathematical and reading disabilities are comorbid in many children (Geary, Hoard, & Hamson, 1999; Räsänen & Ahonen, 1995). Between 5 and 8% of school-aged children have some form of memory or cognitive deficit that interferes with their ability to learn concepts or procedures in one or more mathematical domains (Geary, 2004). In a 1995 study investigating 80 children with arithmetic difficulties, it was found that the only type of arithmetic error connected to reading performance was the error in single digit multiplication (Räsänen & Ahonen, 1995). Bull, Johnston and Roy (1999) on the other hand found a significant correlation between maths and reading abilities when working with 44, 7 year olds with regular ability. They also found that maths performance levels were linked with executive functions skills even when statistically controlling for reading ability, IQ scores and short term memory.

Difficulties in mathematics learning and understanding, strongly impair not only school achievement but also everyday life, yet few studies have made a systematic investigation of the factors involved in mathematics learning, especially in early primary school children (Dowker, 2006; Geary et al., 1999). This contrasts markedly with the large number of studies dedicated to reading and comprehension (Holmes & Adams, 2006; Passolunghi et al., 2007). To understand and solve early arithmetic problems, one must be able to monitor new information and understand words, phrases and sentences in order to develop a meaningful interpretation of the task (Navarro et al., 2011). Those people with disabilities in maths do not share one core deficit but rather such functions as poor maths achievement, reading-related, memory, visuospatial skills, and/or executive skills (Mazzocco & Myers, 2003).

The importance of gender differences in cognitive abilities has been identified and highlighted by many authors (M. Casey, Pezaris, & Nuttall, 1992; J. Fletcher, 1985; Geary, 1996; F. Rocha et al., 2005; Royer et al., 1999). On mathematics tests males at the top end of the distribution performed better than females however females outperform males because of their engagement in academics. These differences tend to counter each other unless results are analysed separately (Royer et al., 1999).

It is commonly stated that “males from selected populations receive better scores on standardised maths achievement tests than females” (Royer et al., 1999, p. 181). The “Rote versus Autonomous Learning Hypothesis” developed by Kimball (1989) stated that the boys approach to maths learning enabled them to generalise mathematical knowledge to new and unfamiliar problems, girls on the other hand, took a rote learning approach that translated into good performance on maths tests which left them at a disadvantage with unfamiliar problems. “Boys significantly outperformed girls on measures of number series, numeration, problem-solving, calculation, number knowledge, and word problems (Royer et al., 1999, p. 183). The above findings have been attributed to socialization experiences, varying learning styles as well as pronounced biases against females, in the selected tests as girls have also been shown to outperform boys in primary and middle school (Hyde, Fennema, & Lamon, 1990), especially with computation tasks (Royer et al., 1999). Differences may be due to test solving speed as males develop the ability to retrieve maths facts faster than females (A. Rocha, Massad, & Pereira, 2005), with 10-11 year old and college aged males showing a significant advantage in math-fact retrieval (Royer et al., 1999). Males are more likely to develop the ability to rapidly and automatically retrieve correct answers to addition, subtraction and multiplication problems. High maths ability males were shown to be faster than their female counterparts, however slow males were slower than slow females (Royer et al., 1999). These small differences in retrieval time for individual problems can add up over the course of a long test and can be important in situations where students do not finish the test because of time constraints. Gender differences in favour of males may also be due to strategy flexibility (A. Rocha et al., 2005) as males quickly apply knowledge to novel situations whereas females flourish in a classroom environment when test procedures are clear and content can be rehearsed and practised within ample timeframes (J. Fletcher, 1985). “The use of these automated skills frees up cognitive capacity that can be used to think about the problems to be solved and perhaps to assist in learning additional maths content” (Royer et al., 1999, p. 262).

It seems just as many researchers believe and can verify that girls outperform boys (Kimball, 1989), this does however depend on the method of data gathering. One researcher reported on the high school grades and awards given over a five-year period for 1000 enrolled students. Between 60 and 94% of the award recipients for maths went

to females and yet the average male score for one of those years (1996) was 548 compared to 478 for females (Royer et al., 1999).

Gender differences were identified in a study which investigated links between left-hand use and spatial ability whilst problem-solving. Right-handed people with right-handed parents were believed to have a greater dependence on analytical rather than spatial strategies for problem-solving. The study showed that left-handed girls and those who were right-handed girls but who had left-handed parents showed a much closer resemblance to the spatial ability of boys than to those girls who were right-handed but had right-handed parents (M. Casey et al., 1992). For this reason Casey and colleagues suggested avoiding taking gender as a unitary group.

Arithmetic Strategies

Children use a variety of strategies to solve arithmetic problems.

The focus in NZ primary schools is on learning basic facts, and therefore any deficit in arithmetic fact retrieval is very important. The NZ Numeracy Development Project identifies seven strategies or mental processes students use to estimate answers and solve operational problems with numbers (Ministry of Education, 2008b). Progress through the stages indicates an expansion of knowledge and an expansion of the range of strategies that students have available to use. When students experience an unfamiliar problem or when the mental load gets high they may revert to a previous strategy. The seven strategies are as follows:

- Stage zero (Emergent) -students are unable to consistently count the number of objects.
- Stage one (One-to-one counting) - students can count and form a set of objects up to 10, but cannot solve simple problems that involve joining and separating, e.g. 4 + 3.
- Stage two (Counting from one on materials) - students rely on counting physical materials e.g. fingers. They count all the objects in both sets to find an answer.
- Stage three (Counting from one by imaging) - students count all the objects and can join and separate simple problems. At this stage students can imagine visual patterns in their minds and count them.

- Stage four (Advanced counting) - counting on. Students understand that the end number in a counting sequence measures the whole set. Rather than counting all objects they can count on.
- Stage five (Early additive part-whole) - students recognise numbers are abstract units and can be partitioned and recombined, e.g. 10 + 6= 16 so 9 + 6 =15.
- Stage six (Advanced additive/early multiplicative part-whole) - students choose appropriately from a repertoire of part-whole strategies to solve addition and subtraction problems, e.g. 63-29 = same as 63-30+1.
- Stage seven (Advanced multiplicative/early proportional part-whole) - students choose appropriately from a repertoire of part-whole strategies to solve multiplication and division problems, e.g. 4 x 16 = same as 8 x 8 = 64.
- Stage eight (Advanced proportional part-whole) - students choose appropriately from a repertoire of part-whole strategies to solve fractions, proportions and ratio problems (Ministry of Education, 2008a).

Children in their first year of schooling focus on stages, one, two, and three of the NZ Number Framework, which have an addition and subtraction focus to, and with, five (as explained in the literature review section). After two years at school, children (7 year olds) should be able to instantly recall all stage 4 (curriculum Level 1) basic facts, which include addition and subtraction facts to 10, and the 'ten and' facts such as 10 and 4, and 10 and 7. As a pre-cursor to learning their 2x tables, children at stage 4 of the Number Framework are also being introduced to doubles to 20 and the corresponding halves. At stage five (curriculum Level 2) it is hoped that children (around 7-8 years old) will have mastered all addition facts to 20, subtraction facts to 10, and the multiplication facts for the 2, 5 and 10 times tables and their corresponding division facts. It is not until children are in Year 6 (9-10 year olds), when it is hoped that they will be working at stage six (curriculum Level 3) in the NZ Number Framework, that they will have instant recall of all addition and subtractions facts to 20, and have mastered all the multiplication facts up to the 10 times table and the corresponding division facts. Children at intermediate level (stage seven and approximately 10-13 years old) are then expected to be able to identify factors of numbers, and common multiples of numbers. These basic facts are critical so that children can apply "their available strategies with proficiency and fluency across all the numbers and problem types that they may encounter" (Ministry of Education, 2008a, p.14).

This contrasts with a more simplistic view of only four main strategies to solve addition problems, namely counting fingers, verbal counting, retrieval and decomposition (S. Cho, Ryali, Geary, & Menon, 2011; Geary, Hoard, Byrd Craven, Nugent, & Numtee, 2007). Initial counting procedures are both time consuming and effortful (S. Cho et al., 2011; Geary, Hoard, Byrd-Craven, & Catherine DeSoto, 2004). Utilising finger counting, in the process of arithmetic, appears to greatly reduce the WM demands of the counting process (Geary, 1990), and is believed to activate different neural regions (Butterworth, 1999; Geary, 2006; Gruber, Indefrey, Steinmetz, & Kleinschmidt, 2001). In a US study 100 7-9 year olds were divided into groups because they either used counting or retrieval strategies to solve single digit addition problems. Performance differences were not pronounced yet distinct patterns of neural activity were observed between these groups (S. Cho et al., 2011). This was partially attributed to the fact that these children had not fully automated the retrieval process and therefore it was still effortful and time-consuming compared to adults, while counting was a well-practised skill that could be executed quickly and accurately. Initially as children transition from counting to retrieval processes, differences in the neural activation may not be substantial. Counting at this point is a well-practised skill that can be executed quickly and accurately whereas those relying on retrieval strategies can be slower and more error prone (S. Cho et al., 2011). Findings indicated that the medial temporal lobe played a critical role during the transition from procedural to memory-based problem-solving and its activation declined with expertise. These findings also suggest that counting relies on greater prefrontal cortex resources and that those children who utilise retrieval strategies rather than counting have a greater activation of the LVFC. This indicates that the shift to memory-based processes may be more effortful than previously assumed (S. Cho et al., 2011). Children with reading and/or maths learning difficulties commit more counting errors and use developmentally immature processes e.g. counting all rather than counting on, more frequently and for more years than do their peers.

Neurological Links, Working Memory and Mathematics

Children with poor mathematical ability also show problems with WM (Geary, 2004; Mazzocco & Myers, 2003; Passolunghi et al., 2007). Working memory has been shown to be related to a variety of numerical and mathematical abilities used for counting, which underlie the solution of simple addition and subtraction problems (Adams &

Hitch, 1997; Alloway & Passolunghi, 2011; Rivera et al., 2005; van der Sluis et al., 2007). More specifically "inhibitory processes, central executive, phonological awareness, and naming speed are considered to be related to early math learning" (Navarro et al., 2011, p. 581). Children with mathematical difficulties often have problems retaining information in WM while engaged in other processes such as counting (Geary et al., 1999).

In 1985 McCloskey and colleagues developed a componential model of the physiological process of arithmetic (McCloskey, Caramazza, & Basili, 1985). In 1988 an "encoding complex theory" was developed to challenge this (Campbell & Clark, 1988). This model was based on the premise that operations were not carried out in a separate relatively autonomous module but rather information-processing led to an interaction of different areas. Both models evoked a great deal of research (Neumärker, 2000) and subsequently the majority of the studies utilised the multi-component model developed by Baddeley which explored the role of cognitive processing and mathematical problem-solving (Hecht et al., 2001; Swanson, Jerman, & Zheng, 2008).

The phonological loop is believed to be associated with counting, mental arithmetic (Adams & Hitch, 1997), mathematical algorithms, addition, subtraction, multiplication and retaining problem information (Dehaene, Spelke, Pinel, Stanescu, & Tsivkin, 1999; Holmes & Adams, 2006), as well as supporting the retrieval of number facts from LTM (Bull et al., 1999; Dehaene & Cohen, 1997; Hecht et al., 2001).

Phonological processing has been associated with computational skills in children aged 7-10 years old, with poor mathematical ability (Hecht et al., 2001). The same phonological processing abilities that are considered to influence reading also appeared to contribute to growth in general computation skills (Hecht et al., 2001). Phonological WM has been demonstrated to be a significant predictor of mathematics skills in children. The central executive is believed to be important for the acquisition of new solution strategies and for switching between learned solution strategies. For this reason activities which utilize both these skills may prove to be very useful in ω-3 research.

Research findings show that children with poor mathematics ability are less able to utilise executive functions and switch to new strategies. "Higher mathematics ability is related to a higher working memory span….higher mathematics ability is associated

with a lower amount of interference from irrelevant information" (Bull & Scerif, 2001, p. 281).

Those children with both reading and maths learning difficulties "do not show the shift from procedure-based problem-solving to memory-based problem-solving that is commonly found in typically achieving children, suggesting difficulties in storing arithmetic facts in or accessing them from, long-term memory" (Geary, 2004, p. 8). Understanding what, why and when strategies are typically used whilst undertaking arithmetic tasks, is key to understanding neural and cognitive changes and links.

Previous studies have shown that the left prefrontal cortex assists children and adults, with the retrieval of arithmetic facts (Passolunghi & Siegel, 2004), the selection of appropriate retrieval strategies and the inhibition of procedural strategies (Geary, 2006). For this reason these strategies may prove useful in ω-3 research although this has not yet been investigated.

Counting abilities are considered to be important prerequisites in children for the acquisition of addition and subtraction skills (Ashcraft, Yamashita, & Aram, 1992; von Aster, 2000). Children rely on counting during early mastery of addition facts and continue to use counting strategies as a backup process in the event of retrieval difficulties (Ashcraft et al., 1992). Forward counting is the basis of automatic cognitive processes, whereas the production of backward sequence of number words (utilised in subraction) is controlled by the WM system (von Aster, 2000). Repeated use of counting procedures develops LTM associations between problems and answers (Geary et al., 1999). This development of an automatic/rote response is most evident around the age of 7-8 years old (S. Cho et al., 2011).

Between 7 and 9 years old is an important period for acquiring knowledge of arithmetic facts (S. Cho et al., 2011). Children with a higher mathematical ability have a higher speed of performance (Bull & Scerif, 2001). "As the strategy mix matures, children solve problems more quickly because they use more efficient memory-based strategies and because, with practice, it takes less time to execute each strategy" (Geary, 2004, p. 7). Of the four arithmetic operations, division is often the last to be introduced and is often considered the most difficult as it typically has the largest number of prerequisite skills (Foley & Cawley, 2003). As division is the hardest arithmetic operation, demanding a higher cognitive load, it may therefore be a suitable test for investigating

ω-3 PUFA supplementation however minimal research has been undertaken using basic facts as objective measures.

When undertaking ω-3 PUFA research, it is important to identify which region of the brain is used for each task, as this study involved basic facts tests the following section has outlined the neural involvement with these activities, whilst Figure 5 has identified where in the brain these regions are located.

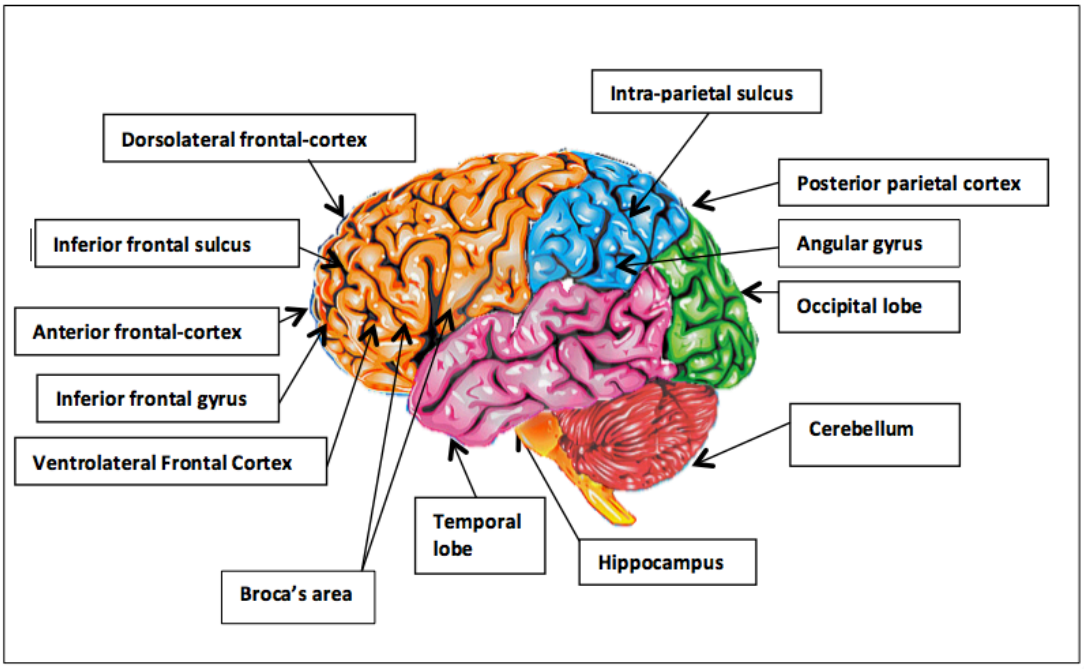


Figure 5: Specific regions of the brain which influence mathematics

While children at around 7-8 years old start to rely on memory retrieval, counting procedures and other reconstructive strategies for solutions, it is not until they are adults that they rely solely on their memory for these solutions (Campbell, 2008; Rivera et al., 2005). In 1995, Ardila commented on the lack of research investigating mathematical procedures and cognitive activity in neuropsychology and a decade later Rivera and colleagues were repeating this call, whilst emphasising the need to investigate children rather than focusing solely on adults (Dowker, 2006; Geary & Brown, 1991; Rivera et al., 2005). There is now a gradual increase in research related to how the brain processes arithmetic information, unfortunately the focus has remained on adults rather than children. Research has focused on tasks of varying complexity and using different arithmetic operators such as asking participants to focus solely on one operation e.g. addition or on multiple operations such as when undertaking calculations (Arsalidou & Taylor, 2011; Baldo & Dronkers, 2007; Fehr & Herrmann, 2007). Research has

demonstrated that educated adults do not rely exclusively on direct retrieval for simple addition, multiplication, division or subtraction, rather they use procedural strategies based on counting and transformation for simple arithmetic (Campbell, 2008; Rivera et al., 2005).

In 1992 Dehaene proposed a 'triple-code-model" where distinct regions of the brain, both in function and location, are associated with number processing. The parietal lobes are utilised when participants use numbers, number comparisons, approximations and/or estimation procedures. The occipital regions are believed to be responsible for the processing of written Arabic numerals, and the left peri-sylvian regions are utilised when numbers are written or spoken. This latter region is believed to access arithmetic facts that have been memorised to structures in the basal ganglia (Dehaene, 1992). It is believed the angular gyrus is associated with verbal manipulation of number and retrieval of verbally-stored maths facts, especially with highly skilled participants (it is, however, activated less with simple addition problems) (De Smedt, Swillen, Verschaffel, & Ghesquiere, 2009; Dehaene, Piazza, Pinel, & Cohen, 2003; Gullick et al., 2011; Menon et al., 2000). It is an interesting aside to note that Albert Einstein's brain (when dissected 7 hours after his death) showed an extensive development of the posterior parietal lobes, (Neumärker, 2000), which have been noted for their links with exact calculations and verbal retrieval. The relationship between brain function and mathematics achievement may depend on the complexity and tasks being performed: more skilled participants may be able to recruit the angular gyrus for taxing computations, and less angular gyrus activity for active performance on simple problems" (Gullick et al., 2011, p. 645).

When working with two adult patients with acalculia (acquired impairment to perform simple mathematical tasks), Dehaene and Cohen (1997) found rote arithmetic ability (e.g. multiplication tables) was associated with the left subcortical structures while numerical manipulation e.g. subtraction and division problems were associated with bilateral inferior parietal pathways. In 2003 multiplication was believed to be the key supporter of both division and multiplication facts (Mauro, LeFevre, & Morris, 2003). Simple addition, subtraction and multiplication were later seen to activate the frontal, inferior temporal, lateral occipital and intra-parietal regions. On analysis quantitative processing e.g. subtraction, was found to be mediated by the bilateral intra-parietal sulci, verbal fact knowledge e.g. multiplication tables, activated the left peri-sylvian

regions and spatial attention e.g. number line comprehension, activated the superior parietal cortex (Dehaene et al., 2003). The peri-sylvian region, which is often linked with language, has recently been shown to be negatively correlated with maths achievement. Participants with higher maths ability scores showed lower activity in this region compared with those with lower maths ability scores, possibly indicating greater efficiency and processing and less reliance on linguistic retrieval coded material (Gullick et al., 2011). Baldo and Dronkers (2007) differentiated this further by identifying no significant foci for addition and multiplication, but the superior parietal cortex, pre-central gyrus and the inferior frontal gyrus for subtraction and the inferior parietal cortex and inferior frontal cortex for division.

The left and right hemispheres are believed to influence cognition. The left hemisphere is believed to focus on verbal strategies and the right hemisphere on spatial strategies (M. Casey et al., 1992). The position of the above key regions can be seen in Figure 5. The basal ganglia are connected to and surround the thalamus. They are a group of nuclei situated in the forebrain deep within the cerebral hemispheres (‘inside’ the brain), and act as a relay station linking cortical and sub-cortical regions (Arsalidou & Taylor, 2011; Neumärker, 2000). For this reason they have not been shown in Figure 5. The peri-sylvian region encompasses the sylvian fissure (lateral sulcus) which divides the frontal, temporal and parietal lobes. As this is a large region encompassing 3 cortices it too has not been shown in Figure 5

Numerous discrepancies arise as to which neural regions are activated when undertaking arithmetic operations (Arsalidou & Taylor, 2011; Dowker, 2006). As recent studies have involved a variety of activities from simple single digit calculations only involving one strategy e.g. addition, to complex calculations which involve multiple operations, direct comparisons of findings have proven inconclusive (Dowker, 2006; Kawashima et al., 2004). There is a growing consensus that the pre-frontal, intra-parietal, occipital, and occipito-temporal cortices are activated during each of the arithmetic operations (Gruber et al., 2001; Kawashima et al., 2004). The inferior parietal region is believed to be responsible for the transformation of numerical symbols into quantities, and the representation of relative number magnitudes. The prefrontal lobe is believed to be responsible for sequential ordering of successful operations, control over their execution, error correction, as well as such things as inhibition of verbal responses (F. Rocha et al., 2005). It is believed that adults rely more on the parietal cortex when

solving arithmetic problems (Dehaene et al., 2003), whereas children appear to rely on the hippocampus and prefrontal cortex. As adults gain proficiency with recently learned arithmetic facts, the prefrontal cortex and intra-parietal sulcus are activated less and there is an increase in activation of angular gyrus (S. Cho et al., 2011). Children utilise their prefrontal cortex when undertaking these tasks (Rivera et al., 2005) and this area of the brain is believed have a high DHA content (Carrié et al., 2000b; Xiao et al., 2005), for this reason it is suggested that ω-3 PUFA supplementation influence proficiency with arithmetic facts, although little research has yet been undertaken in this area. The few studies recorded are elaborated upon in the ω-3 PUFA maths section in this chapter.

Summary of the above literature related to the brain

In summary the brain is a very fatty organ dependent on EFA including ω-3 PUFA. The brain is divided into many regions which develop and mature at different rates. Between the ages of birth and 2, 7 and 9 and again between 14 and 16 the body as well as the brain is undergoing rapid growth where nutritional requirements are increased. If these nutritional requirements are not met abnormalities and differences in cognition and behaviour can be observed. For this reason many research studies involve children within these ages.

Learning and memory are complex tasks which cannot be specifically related to one region of the brain. Memory is divided into LTM and WM (short term memory). The central executive is believed to be key to WM and is responsible for controlling a person's attention, coordination of multiple tasks, shifting between tasks or retrieval strategies, selective attention and inhibition as well and as temporarily activating LTM. Executive functioning has been linked with the frontal lobes and is influenced by ω-3 PUFA. It is believed to affect literacy, vocabulary and mathematics. Isolating a sensitive and specific measure of executive functioning is extremely difficult as it involves ‘meta’ constructs such as planning, organisation and problem-solving. Inhibition is often seen as a primary executive function and has been linked with many tasks including problem-solving and mathematical ability. The frontal lobe, which is believed to be responsible for executive functions such as inhibition, is known to require large amounts of ω-3. It is no surprise then to note that children with ADHD, who frequently have deficiencies in EFA's also have difficulties with inhibition tasks. Boys show less inhibition responses than girls, are more likely to suffer ADHD (Breen, 1989; Gaub &

Carlson, 1997; Gershon, 2002; Richardson, 2006; Richardson & Puri, 2000) and appear to be at a disadvantage for the ALA/EPA/DHA conversion process (Burdge, Jones, & Wootton, 2002; Burdge & Wootton, 2002; Innis, 2007). They also tend to score lower on written fluency tests, especially with lower ability children (Berninger & Fuller, 1992). However there is still debate about this as some studies have shown no gender effect on executive tasks in children under 12 years old (Altemeier et al., 2008; Welsh et al., 1991).

Another executive task is the ability to shift attention from one task to another which is known as set shifting. This involves both inhibition and attention switching mechanisms and has been linked with arithmetic and some reading performance.

Ability at maths is significantly correlated with all measures of executive functioning, when a task is not completed automatically. Once children are able to retrieve arithmetic facts directly from LTM the executive processes play a reduced role.

Executive functions have also been correlated with writing tasks and some reading-writing tasks, with comprehension and listening being correlated with WM. Spelling also utilises WM, however only until the word has been encountered numerous times, at this point LTM is used. Verbal processing efficiency and reading comprehension have also been shown to be linked. Poor listeners, readers and comprehenders have been found to stay focused often on irrelevant information. When investigating WM and comprehension, general intellectual capacity could be a covariate.

Verbal fluency and arithmetic (not involving writing) have been consistently linked with the frontal lobes, and executive tasks, and debate exists whether children and adults use the same regions of the brain, and the same strategies, when undertaking these tasks. Many variables including culture, language and educational levels affect which region and how the brain is activated.

Links between frontal lobe activity, executive processing and the completion of literacy and arithmetic tasks have been identified in the above section. In the last decade education has placed a heavy emphasis on numeracy and literacy whilst using terms such as ‘back to the basics’. Parents are now keen to see improvements in reading, writing, spelling and arithmetic, rather than the complex tasks which incorporate these aspects.

The brain and its resulting actions are also regulated by the consumption of nutrients. The following section mainly focusses on one energy source, that being PUFA (more specifically ω-3). In order to understand how ω-3 PUFA effects learning one must first understand how it is obtained and what it does in the body, more specifically its chemical composition, metabolism and subsequent links to child development. This will be followed by detail of research into the effects of ω-3 PUFA on child development (both behavioural and cognitive).

Omega-3 PUFA

Omega-3 is one of many dietary fatty acids. Fats are energy sources used to keep the body warm and to protect against mechanical shock. In addition to this, dietary lipids transport the fat soluble vitamins A, D, E and K and are necessary for their absorption (Australian National Health and Medical Research Council and New Zealand Ministry of Health, 2006). They have important functions in the regulation and transportation of substances through membranes and an influence on metabolic processes, determining growth and development, brain development and a variety of body functions (Bryan & Luszcz, 2000; Hughes & Bryan, 2003). Dietary fatty acids consist of hydrocarbon chains and are classified according to their degree of saturation (number of double bonds). There are saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and PUFAs.

Saturated fats contain no double bonds and are fully saturated with hydrogen. They can be made by the body and therefore are not essential in the diet (Australian National Health and Medical Research Council and New Zealand Ministry of Health, 2006). Polyunsaturated fatty acids on the other-hand contain two or more double bonds between carbon atoms (Gadoth, 2008). They are divided into omega-6 (ω-6) or ω-3 depending on the position of the first double bond nearest the methyl (CH_3) end of the carbon chain (Vorster, Nell, Kumanyika, & Tee, 2004). The notation system for these fatty acids consists of the number of carbons, the number of double bonds, and the location of the first double bond in relation to the last carbon from the methyl (CH_3) end of the chain. This carbon is always labelled 'omega' (ω) after the last letter of the Greek alphabet, and the first carbon is always labelled 'alpha' (α) (Gadoth, 2008; B. Ross, Seguin, & Sieswerda, 2007). An example of this is EPA, which is a carboxylic acid with a 20-carbon chain and five double bonds; the first double bond being on the third carbon

from the omega end (hence receiving the term ω-3), thus giving it a physiological name of 20:5 (ω-3). It must be noted however that this is not the only type of ω-3, for example DHA is also a carboxylic acid but has a 22-carbon chain and six double bonds; again the first double bond being on the third carbon from the omega end, thus giving it a physiological name of 22:6 (ω-3). Other ω-3 PUFAs, include α-linolenic acid (ALA) 18:3ω-3, stearidonic acid (SDA) 18:4ω-3, ω-3 eicosatetraenoic acid (ETA) 20:4ω-3 and ω-3 docasapentaenoic acid (DPA) 22:5ω-3 (Plourde & Cunnane, 2007).

Mammals cannot produce their own ω-6 and ω-3 PUFA and must therefore gain these from their diets (Gadoth, 2008; Innis, 2008). The human body can synthesise all but two fatty acids. These being linoleic acid (LA) ω-6 and ALA ω-3, which have subsequently been called EFAs (Wolmarans & Oosthuizen, 2001). These EFAs have 18 carbon atoms with double bonds and in the liver these are elongated and de-saturated (additional double bonds inserted) to form highly unsaturated LC PUFAs which have a higher number of carbon atoms and double bonds (Schachter et al., 2005). These longer highly unsaturated fatty acids are the most important for brain development and function (Freeman et al., 2006). Figure 6 shows how people obtain EPA and DHA through the food they eat via the conversion of ALA, consumed in products such as vegetable oils, which is then metabolised into EPA and finally into DHA. The rate of DHA synthesis from ALA is slow and inefficient, especially with the desaturation of EPA to DHA (Pawlosky, Hibbeln, Novotny, & Salem, 2001). In humans about 5-10% of ALA is converted into EPA and 1-5% of EPA is converted into DHA (Burdge et al., 2002; Goyens, Spilker, Zock, Katan, & Mensink, 2005), with the conversion rate of ALA to DHA being stated at less than 1% (Arterburn, Hall, & Oken, 2006) and even as low as less than 0.5% (Plourde & Cunnane, 2007). Because of this slow conversion rate, and the importance of LC PUFAs, humans need to consume foods rich in these important compounds (Schachter et al., 2005). North American and European agencies have recently regulated that infant formula include Arachidonic acid (AA) and DHA (Plourde & Cunnane, 2007). This is because the brain of a breast-fed infant acquires 50% more DHA than a formula fed infant receiving no DHA (Das, 2003; Farquharson, Jamieson, Logan, Cockburn, & Ainslie Patrick, 1992; Makrides, Neumann, Byard, Simmer, & Gibson, 1994).

Males appear to be at a disadvantage for the ALA/EPA/DHA conversion process (Burdge et al., 2002; Burdge & Wootton, 2002; Innis, 2007), and at times this

conversion process of ALA to DHA is so slow in males, it is undetectable (Arterburn et al., 2006). Females on the other hand are believed to need more DHA during puberty. During puberty Dutch girls were shown to increase from 14.8 to 25.5% of body weight while males' fat decreased from 10.5 to 9.3% (Boot, Bouquet, De Ridder, Krenning, & de Muinck Keizer-Schrama, 1997). This increase has been partially attributed to the need to store adipose tissue (high in DHA) in preparation for pregnancy and lactation when the foetus requires large amounts of DHA for brain development (Clandinin et al., 1980a, 1980b; Haggarty, 2004).

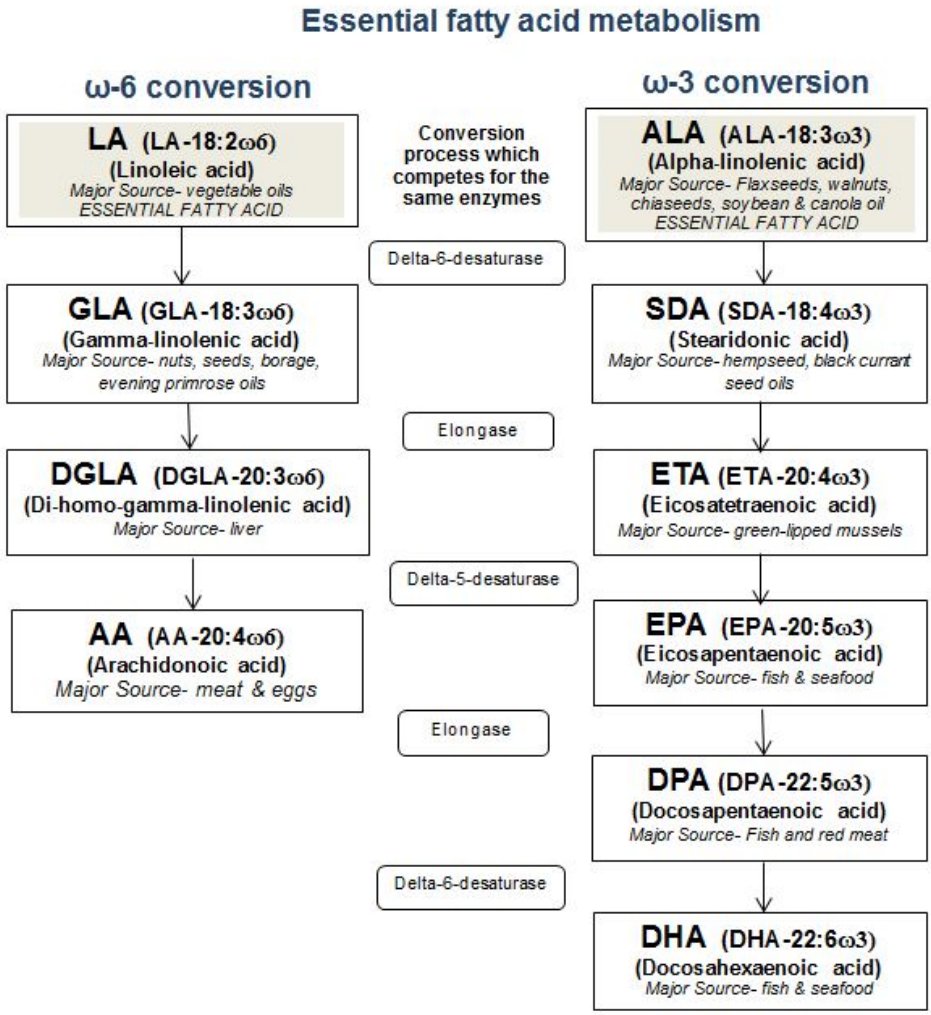


Figure 6: Metabolism of key essential fatty acids, from commonly consumed food sources. Developed from figures produced by Neuringer, Anderson and Connor (1988); Das (2003); Vorster et al (2004); Arterburn (2006) and Schachter et al., (2010).

It is believed the enzymes required in the metabolism of ω-3 and ω-6 PUFAs are common to both processes (cascades) as shown in Figure 6. This means that competition occurs for the same enzymes, which can become limiting factors in the conversion process (Harnack, Andersen, & Somoza, 2009; Hibbeln, Nieminen, Blasbalg, Riggs, & Lands, 2006).

Saturated levels of these enzymes are also believed to limit synthesis of DHA (Muhlhausler et al., 2010). Higher intake levels of ω-6 fatty acids are therefore believed to influence the availability of DHA and EPA resulting in negative effects on cognition (Agostoni et al., 1997; Neggers et al., 2009; Novak, Dyer, & Innis, 2008; Whalley, Fox, Wahle, Starr, & Deary, 2004). This has reflected more strongly in girls possibly due to their increased intake requirements during puberty (Lassek & Gaulin, 2011).

The optimal ω-6 to ω-3 PUFA ratio is controversial but believed to be around 1-2:1, however Western diets have a ratio of around 10-25:1 (Dalton, 2006; DeFilippis & Sperling, 2006; Kirby et al., 2009; Simopoulos, 2000, 2002a). A ratio of 1:2.3 derived from a dietary intake of seafood (Hibbeln et al., 2006) is believed to be more reflective of a 'natural diet'.

Individuals differ in their ability to convert EFAs to LC-PUFAs. Factors that affect and or interfere with this conversion include: genetic, environmental, stress hormones, excessive alcohol consumption, deficiency of vitamin and mineral cofactors (especially zinc deficiency) and high saturated fat, hydrogenated fat and ω-6 PUFA diets (Lassek & Gaulin, 2011; Richardson & Puri, 2000; Uauy & Valenzuela, 2000). It has been suggested that this lack of ability for conversion may affect at least some individuals with learning and/or behaviour problems such as ADHD (I. Colquhoun & Bunday, 1981; Vorster et al., 2004). This helps to explain the favourable response in individuals with ADHD to the Feingold Diet, which excludes fruits rich in salicylates (these inhibit the conversion to DHA) (I. Colquhoun & Bunday, 1981).

The most commonly available dietary source of EPA and DHA is cold water oily fish such as salmon or mackerel. Lean fish store lipids in their liver whilst fatty or 'oily' fish store it in their flesh (Calder & Yaqoob, 2009). Consuming EPA and DHA directly, from fish, seafood or supplementation, for example, results in higher levels of DHA and

EPA in the blood (Innis, 2007, 2008). The major sources of EPA and DHA are seafood and marine oils e.g. salmon, sardines, fish oil and algal oils (as shown in Table 1), whilst walnuts, sunflower, soybean and canola oil are plant sources rich in ALA, and seed oils (e.g. corn) are high in ω-6 PUFA (Plant and Food Research, 2010).

Fish sources containing >1000mg ω-3/100g	Fish sources containing 500-1000mg ω-3/100g	Fish sources containing <500mg ω-3/100g
Red/pink Salmon–canned in brine	Tuna- canned in spring water	Fresh/frozen snapper
Canned mackerel	Fresh/frozen kahawai	Fresh/frozen hoki
Canned herring	Fresh/frozen trevally	Fresh/frozen tarakihi
Canned sardines	Calamari & squid	Takeaway fish e.g. fish & chips
Fresh/frozen salmon	Green lipped mussels	Fresh/frozen fish fingers
Fresh/frozen tuna	Oysters	scallops

Table 1: Variety of fish and seafood commonly eaten in NZ (Plant and Food Research, 2010; Quigley, Burlingame, Milligan, & Gibson, 1995; Stonehouse et al., 2011)

DHA is the most abundant ω-3 PUFA in cell membranes and is present in all organs, whilst only minute quantities of ALA and EPA are usually present in tissues. Generally DHA exceeds EPA 5- to 30-fold in most organs, and is several hundred-fold more abundant than EPA in the brain and retina (Arterburn et al., 2006). It comprises over 97% of ω-3 PUFA in the brain (Lauritzen, Hansen, Jørgensen, & Michaelsen, 2001) and 95% in the retina (Kuratko & Salem Jr, 2009). The liver is considered to be the primary site for biosynthesis of DHA, although recent research has also demonstrated it can also be synthesised in the brain (Barceló-Coblijn & Murphy, 2009). It becomes readily available to the brain via the circulating blood stream (H. Kim, 2007). DHA accrues most rapidly in areas of high neural growth (Hibbeln et al., 2006) and the human brain turns over approximately 4mg per day of DHA (Rapoport, 2006) with an estimated half-life of DHA in the brain of 2.5 years (Umhau et al., 2009). DHA and AA are rapidly incorporated into the nervous system, retina and brain during the brain's growth spurts (Martinez, 1992).

An increased intake of LC ω-3 PUFA is believed to alter the physical properties of the cell membrane (which influences the activity of membrane proteins including receptors, transporters, ion channels and signalling enzymes). As a result of these effects, transcription factor activation is altered and gene expression modified (Calder & Yaqoob, 2009). DHA is a vital nutrient for the optimum development of the central

nervous system, and its deficiency disrupts serotonin, norepinephrine and dopamine transmission across cellular membranes (Su, 2009). Effects of dietary deficiencies cannot always be rectified by supplementation. A South African study involving children deficient in iron, DHA and EPA showed they performed worse in cognitive tests after supplementation compared to baseline levels and those in placebo groups (Baumgartner, Smuts, Malan, Kvalsvig, et al., 2012). These results are similar to those found in research involving rats where WM was not reversible but long term memory improved with repletion of iron, DHA and EPA (Baumgartner, Smuts, Malan, Arnold, et al., 2012).

Biomarkers of ω-3 PUFA intake and levels in the brain

Assessing nutrient intake can be very problematic. Although diet records are commonly thought to be the 'gold standard' method of dietary assessment, a 1992 American study identified similar correlations between the fatty acid composition of adipose tissue and estimates of intake from the food frequency questionnaire and from diet records. This led the researchers to suggest that both these dietary assessment methods have similar validity in the measurement of polyunsaturated fatty acid intake (Hunter et al., 1992). A recent systemic review demonstrated that food frequency questionnaires (FFQs) gave acceptable values for total ω-3 PUFA, EPA and DHA (Serra-Majem, Nissensohn, Øverby, & Fekete, 2012).

Data gathered via FFQ and diet records is also not easily applied to other countries. One of the reasons why international comparisons are problematic is because of the diversity in the amounts of ω-3 PUFA available to domestic animals (grass-fed animals compared with corn fed animals can have very different ω-3 PUFA levels). These data are rarely adjusted for country-specific differences in nutrient compositions of foods (Hibbeln et al., 2006). Blood concentrations EPA and DHA have been shown to be associated with dietary intake (Arterburn et al., 2006; Lucas, Asselin, Mérette, Poulin, & Dodin, 2009; Lucas et al., 2010). Tissue compositions of ω-3 PUFA are believed to be closer determinants of the risk of morbidity and mortality due to ω-3 PUFA-related chronic illnesses than un-adjusted dietary intakes (Hibbeln et al., 2006). Biomarkers are seen as more accurate than different dietary assessment methods to rank individuals, as they can reflect past PUFA consumption (Godley et al., 1996) and therefore are able to reflect an increase in nutrient status such as ω-3 PUFA in populations over time periods (Serra-

Majem et al., 2012) and in doing so reduce biological and analytical variability (Harris & Thomas, 2010). Some of the most commonly used biomarkers of ω-3 status are blood components such as total plasma, plasma phospholipids and red blood cell (erythrocyte) ω-3 levels (Harris & Thomas, 2010; Kuratko & Salem Jr, 2009; Serra-Majem et al., 2012).

Plasma phospholipid concentrations of DHA increase rapidly in a dose dependent manner after supplementation (Arterburn et al., 2006; Conquer & Holub, 1998; Katan, Deslypere, Van Birgelen, Penders, & Zegwaard, 1997; Kuriki et al., 2003; Subbaiah, Kaufman, & Bagdade, 1993). In a study which supplemented adults with 864 mg/d DHA the plasma phospholipid DHA increased 1.9% per gram of dietary DHA over the 8 weeks with the majority occurring in the first 4 weeks (Cao, Schwichtenberg, Hanson, & Tsai, 2006), whilst a 1997 study identified a rise in red blood cell (RBC) DHA of 1% per gram ingested from fish oil (Katan et al., 1997). Those with a low initial baseline level incorporate DHA faster than those with a high baseline level (Cao et al., 2006; Vidgren et al., 1997). In a meta-regression does-response analysis of the effect of DHA supplementation, dosages as low as 126 mg/day for 4 weeks produced significant differences in both RBC and plasma phospholipids compared to baseline levels (Arterburn et al., 2006). Saturation with DHA can occur in plasma phospholipids within 1 month with high dose supplementation however it can take 4-6 months to reach this steady state in RBC (Arterburn et al., 2006; Katan et al., 1997). At the conclusion of supplementation there is a rapid decline in RBC levels for the first couple of weeks and then a gradual decline (Cao et al., 2006) with these levels taking longer than 18 weeks to obtain baseline (Brown, Pang, & Roberts, 1991). It has been suggested that RBC DHA is a better index for long-term ω-3 PUFA intake, whereas the plasma phospholipids appear more sensitive to short-term change (Cao et al., 2006).

For DHA, the plasma phospholipids can be around 1.9%, in healthy US children (Holman, 1981) whilst RBC DHA levels can be 2.2% (Stevens et al., 1995). Generally these plasma phospholipid DHA and RBC DHA biomarker levels correlate with ω-3 PUFA intake and brain tissue levels (Kuratko & Salem Jr, 2009). How to best raise these phospholipid and RBC levels is unclear. Some research has shown eating fish is more effective than supplementation (Elvevoll et al., 2006; Visioli, Risé, Barassi, Marangoni, & Galli, 2003) and other studies have shown that there is no difference between these methods (Stonehouse et al., 2011).

PUFA Intake Levels

In order to undertake research in this area it is vital to know how much LC PUFA is being consumed as well as how much ought to be consumed. The Australian and NZ governments have been providing nutrition advice for over 75 years. Reviewing the recommendations is an on-going process as food and lifestyle patterns of the community constantly change and more scientific evidence becomes available (Baghurst, 2005). New Zealand nutritional guidelines are comparable to international dietary guidelines (Theodore et al., 2009). Numerous researchers have identified the variance between international guidelines (Garg, Wood, Singh, & Moughan, 2006; Harris, 2007; Harris, Kris-Etherton, & Harris, 2008; O'Sullivan, Ambrosini, Beilin, Mori, & Oddy, 2011; Pate et al., 2006).

Understanding dietary guidelines can be confusing for many people as recommendations frequently use differing terms (key terms are explained below). A term commonly used to address the issue of chronic disease prevention is the 'Suggested Dietary Target' (SDT) which is a daily average from food and beverages for certain nutrients that may help in prevention of chronic disease (National Health Medical Research Council, 2006b). The RDA refers to the average daily dietary nutrient intake level sufficient to the meet the nutrient requirement of nearly all (97 to 98%) healthy individuals in a particular life stage and gender group. The AI is used when an RDA cannot be determined. It refers to the recommended average daily intake level based on observed or estimates of nutrient intake by an apparently healthy group of people that are assumed to be adequate. The 'Tolerable Upper Intake Limit' (UL) refers to the highest average daily nutrient intake level that is likely to pose no risk of adverse effects to almost all individuals in the general population (A Report of the Panel on Macronutrients, Subcommittees on Upper Reference Levels of Nutrients and Interpretation and Uses of Dietary Reference Intakes, & Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 2005; National Health Medical Research Council, 2006a).

Table 2 shows the AIs for LA and ALA which were based on the median intakes of any gender-related age groups taken from an analysis of the 1995 National Nutrition Survey of Australia (Australian Bureau of Statistics: Commonwealth Department of Health and Aged Care, 1998). For ω-3 PUFA the AI was based on the median intake for all adults

of the relevant gender. The AIs do not necessarily reflect optimal intakes but are values found in populations with no apparent EFA deficiency (National Health Medical Research Council, 2006b). The adjusted SDT (aSDT) values were deducted from the SDT values based on the age and sex specific energy intakes for each age group. The SDT values are applicable to individuals 14 years and older and no SDT has been set for children younger than 14 years. These values were generated by Meyer and Kolanu (2011) using National Health Medical Research (2006b) data.

	Average daily intake level			**Suggested & Adjusted Suggested Dietary Targets**		**Tolerable Upper Intake Limit**
	LA g/day	**ALA** g/day	**Total LC ω-3 PUFA** **(DHA+ EPA + DPA) mg/d**	**aSDT** **mg/d**	**SDT** **mg/d**	**Total LC ω-3 PUFA** **(DHA+ EPA + DPA) mg/d**
Boys 9-13 years	10	1.0	70	510		3000
Girls 9-13 years	8	0.8	70	410		3000
Adult- males	13	1.3	160		610	3000
Adult-females	8	0.8	90		430	3000

Table 2: Nutrient Reference Values for Australia and NZ, based on National Health Medical Research Council (2006) data and calculations using this data (Meyer & Kolanu, 2011).

Australian data were utilised as there is no official data available regarding the ω-3 PUFA intake of NZ children. When data from the Australian Social Science Data Archive were compared with data gathered 13 years earlier the LC ω-3 PUFA intake levels had not markedly changed (Meyer & Kolanu, 2011). The median (interquartile range) LC ω-3 PUFA intakes were 88mg (46-159) for 9-13 year olds, indicating that only 50-60% of children met the AI for LC ω-3 PUFAs, whilst only 6% of children met the aSDTs for LC ω-3 PUFA per day (Meyer & Kolanu, 2011). The median and mean values varied as the LC ω-3 PUFA intakes were not normally distributed (National Health Medical Research Council, 2006a).

Fish and seafood are the richest source of ω-3 PUFA per gram ingested (Meyer et al., 2003). However the Australian data recorded that fish and seafood were not commonly

eaten by children who consumed on average only 13 g per day (and a median intake of zero). Almost 8-9 children out of 10 did not eat fish or seafood. In contrast children consumed 106g of meat per day, which explained why 33% of their ω-3 PUFA came from meat (National Health Medical Research Council, 2006a). A 2002 nutrition survey for NZ children aged 5 to 14, reported that fish and seafood contributed twice the proportion of protein to Pacific Island males (8%) than NZ European males (4%) and was consumed weekly by 37% of NZ children (Parnell, Scragg, Wilson, Schaaf, & Fitzgerald, 2003).

The majority of the general public when reading research would be unable to distinguish a high ω-3PUFA intake from a low intake if it was only referred to in terms of mg/day of EPA and DHA. Previously the 2003 Australian Dietary Guidelines (National Health Medical Research Council, 2003) focussed on how much of certain nutrients should be consumed, however the more recent 2012 Australian Dietary guidelines focussed on the food choice recommendations (National Health Medical Research Council, 2013). This helps parents to know how much of one food type to feed their children rather than specific daily nutrient levels. The UK Scientific Advisory Committee on Nutrition (2004) recommends a minimum of 2 portions of fish (one oily) per week (approximately 450mg/d EPA & DHA) (Harris et al., 2008), whilst the World Health Organization (2003) recommends up to 2 fish meals per week (approximately 400-1000mg/d of LC PUFA) (Garg et al., 2006; Yashodhara et al., 2009). The Australian Heart Foundation has recommended that children should follow adult recommendations and consume 500mg/day EPA and DHA (D. Colquhoun, Ferreira-Jardim, Tuesday, & Eden, 2008).

The Foods Standards Australia NZ (FSANZ) introduced food standards to assist consumers in their purchasing of foods high in ω-3 PUFA. A food cannot be termed a ‘source’ of ω-3 PUFA unless it contains no less than 200mg of ALA or 30mg of total EPA and DHA per serving, whilst a food termed a ‘good source’ of ω-3 PUFA must contain no less than 60mg of total EPA and DHA per serving (Food Standards Australia New Zealand, 2000).

Many children, in both developed and developing countries, are consuming low intakes of ω-3 PUFAs (Hibbeln et al., 2006). Research has tended to focus on people with diets deficient in ω-3 PUFA as effects are believed to be more pronounced compared with

those with diets containing adequate ω-3 PUFA levels. Omega-6 is widely consumed in an average "western" diet, however it is believed that ω-3 PUFA is often lacking (Hibbeln et al., 2006). Research has also involved low socio-economic groups as it is believed these groups will have a diet with a low ω-3 PUFA intake (Darmon & Drewnowski, 2008; Thompson et al., 2009). This belief has not been supported by the findings of a 2007 study, which found minimal differences between verbal learning and memory with both well-nourished Australian and undernourished Indonesian children (NEMO Study Group, 2007).

However in countries where malnutrition is severe enough to lead to stunting of growth, ω-3 PUFA supplementation has reduced school absentee rates. In a six month period school absentee rates were significantly lower for those 6-9 year old South African children who consumed 191 mg/d DHA compared with those children in the placebo group (Dalton, 2006). In a 6 month Thai study, 8-12 year olds in the ω-3 PUFA group also showed lower absenteeism (Thienprasert, Kheovichai, Samuhaseneetoo, Sukanand, & Hamazaki, 2002). Similar results were found in a large 3-month Indonesian study, with 233 8-14 year olds (K. Hamazaki et al., 2008). These results suggest that those children who don't have a diet containing fatty fish may be at risk of not fulfilling their full potential at school.

The amount and type of intakes of ω-6 and ω-3 PUFA has been shown in animal studies to affect the accumulation of DHA in the brain (Bourre, Durand, Pascal, & Youyou, 1989; Neuringer, Connor, Lin, Barstad, & Luck, 1986). Plasma lipids levels of DHA are low in most terrestrial animals suggesting the brain has particular mechanisms to concentrate DHA (Farooqui, Horrocks, & Farooqui, 2000). The exact role of ω-3 PUFA on the brain however is unclear. It is believed ω-3PUFA may influence brain function by reducing oxidative stress and exerting anti-inflammatory effects as well as neurotransmission, membrane fluidity, ion channel and enzyme regulation and gene expression (Innis, 2003; van de Rest et al., 2012).

Neurodevelopmental Benefits of ω-3 PUFA Supplementation

In the mammalian brain DHA is the most abundant PUFA (Uauy & Dangour, 2006). There is a high content of DHA in the cerebral grey matter, cortex and hippocampus and once the stores of DHA are depleted in these areas they are very difficult to restore (Boyle, Yuhas, Goldberg, & Lien, 1998; McNamara & Carlson, 2006). The traditional

way to examine the role of DHA on the function of the brain is to examine animals which have been fed diets lacking ω-3. A review of these studies reveals that when there is a reduced level of DHA in the brain, there are also changes to; the size of the neurons, learning and memory, auditory and olfactory responses to stimuli and nerve growth factor levels (Sinclair et al., 2007). In a review of animal studies, Davis-Bruno and Tassinari (2011) report that deficiency was linked with adverse developmental outcomes whilst supplementation did not demonstrate a long term benefit on the neurologic development. The remainder of this section will outline links between neurodevelopmental disorders and ω-3 supplementation.

Following the discovery that deficiencies in EFAs affect behaviour and neural function (Caldwell & Churchill, 1966), the majority of initial research focused on animal models. Studies on monkeys deficient in ω-3 PUFA noted changes in temperament (Reisbick, Neuringer, & Connor, 1996). Essential fatty acid deficiency symptoms include excessive thirst, frequent urination, dry hair and skin, dandruff, brittle nails and kidney damage (Sinn, 2007; Stevens et al., 2003). By the early 1980s, researchers identified links between EFA deficiency symptoms and hyperactive children (Rudin, 1981).

Mental health has been linked with people's ability to cope with everyday stresses and one in four people may suffer from mental illness during their lifetime (World Psychiatric Association, 2000). Nutrition has an important role to play in mental health as the brain relies on nutrients for its development and functioning. Frequently recent research is identifying LC ω-3 PUFA as a potentially modifiable risk factor for mental health (Dauncey, 2009).

In a study of over 240 children, Berninger & Colwell (1985) found that physical impairment in brain development (neuro-developmental findings) contributed more to classroom disabilities than to the diagnosis of learning disabilities for specific academic subjects. They also found that performance disabilities interfered with memory retrieval and production of oral or written language, whilst learning disabilities interfered with creation and representations in memory.

The majority of the children with neuro-developmental disorders present behavioural or learning challenges for a classroom teacher. These disorders have been linked with low blood and/or dietary levels of ω-3 PUFA and the majority of findings indicate that many of the symptoms may be alleviated by ω-3 PUFA supplementation (Ward, 2000).

Research undertaken on school-aged children has mainly focussed on the effects of ω-3 PUFA consumption on neuro-developmental disorders such as ADHD, dyslexia (specific reading difficulties), dyspraxia (developmental coordination disorder – DCD) and the autistic spectrum (Johnson, Ostlund, Fransson, Kadesjo, & Gillberg, 2009; NEMO Study Group, 2007; Richardson & Montgomery, 2005). The following paragraphs will provide a brief description of ω-3 PUFA research which has been associated with these disorders.

Depression and Bipolar Disorders Numerous international studies showed that rates of major depression, postpartum depression and bipolar disorders were inversely proportional to countries' fish consumption (Freeman et al., 2006; Hibbeln, 1998, 2002; Silvers & Scott, 2002; Tanskanen, Hibbeln, Hintikka, et al., 2001). Treatment with ω-3 PUFA has shown benefits in unipolar depression (Nemets, Stahl, & Belmaker, 2002; Peet & Horrobin, 2002b; Su, Huang, Chiu, & Shen, 2003). It is believed DHA taken without EPA may not be sufficient to improve major depression, whilst EPA on its own may sometimes be of benefit (Kidd, 2007). The amount however of EPA is believed to be important as an improved effect was noted with a dose of 1 g but not with a higher dose of 2 or 4 grams per day (Peet & Horrobin, 2002b). This may indicate a 'ceiling effect' or maximum effective dose for EPA. Although not conclusive a review of 13 randomised control trials investigating the effects of treating depression with ω-3 PUFA's, reported only 7 trials showing some improvements in depressive symptoms (Sinn et al., 2010). The majority of the above research has involved adolescents and/or adults as there is very little ω-3 PUFA research involving young children suffering from depression.

Although findings are inconsistent (Freeman et al., 2006), a number of studies have shown significant reductions in negative behaviours associated with bi-polar disorder when treated with ω-3 (Stoll et al., 1999) Other studies have shown improvements in symptoms measured by the Mania Rating Scale (Frangou, Lewis, & McCrone, 2006; Wozniak et al., 2007), whilst another study involving a high dose of 6 grams per day of EPA showed no treatment effect (Keck et al., 2006). There are educational implications in this research since it has been estimated that as many as one third of all children with a diagnosis of ADHD may, in fact, be suffering from the early onset of bi-polar disorder (Richardson & Ross, 2000).

Schizophrenia A review of 18 studies found suggestive evidence that RBC levels of DHA was decreased in schizophrenic patients (antipsychotic-naïve and those on antipsychotic medication). Levels of LA were also decreased in medicated patients but not the antipsychotic-naïve group, whilst levels of AA were decreased in the antipsychotic-naïve group (Hoen et al., 2012). Reviews of research have indicated that schizophrenic symptoms are reduced, although not eliminated, when patients increase their intake of ω-3 PUFA (Freeman et al., 2006; Peet & Stokes, 2005; Sinn et al., 2010). It is believed that AA (a competitor with ω-3 EPA for enzymes) levels may improve negative symptoms but worsen the positive symptoms for schizophrenia (Peet & Horrobin, 2002a). In a study with similar findings the authors suggested the cortical and prefrontal dopamine systems contributed to the negative symptoms whilst the subcortical and limbic dopamine systems caused the positive systems (Sethom et al., 2010). Some trials which have only used EPA (and not combined it with DHA) have shown significant improvements in symptoms (Emsley, Myburgh, Oosthuizen, & van Rensburg, 2002; Peet & Horrobin, 2002a) whilst another study found no improvement with supplementation (Fenton, Dickerson, Boronow, Hibbeln, & Knable, 2001). The US Office of Dietary Supplements in the National Institute of Health reviewed 86 studies involving ω-3 PUFA fatty acid supplementation on patients with all types of psychiatric disorders and symptoms. The subsequent 400 page report suggested that short term intervention using LC ω-3 PUFA fatty acids had "potential" for people with schizophrenia (Schachter et al., 2005).

Aggression and Antisocial Behaviour Numerous studies have shown links to antisocial behaviour and lower ω-3 PUFA dietary intake levels (D. Benton, 2007; D. Benton & Gesch, 2003; Bourre, 2005; Dani, Burrill, & Demmig-Adams, 2005; Gesch, Hammond, Hampson, Eves, & Crowder, 2002; K. Hamazaki et al., 2008; Iribarren et al., 2004; Vaddadi, 2006; Zanarini & Frankenburg, 2003). These include studies involving participants such as young adult prisoners (Gesch et al., 2002; Zaalberg, Nijman, Bulten, Stroosma, & van der Staak, 2010), university students undertaking exams (T. Hamazaki et al., 1996), university staff (T. Hamazaki et al., 2002) and men tackling substance abuse (Buydens-Branchey & Branchey, 2008). As aggression and antisocial behaviour can have a profound influence on classroom dynamics and children's learning a more detailed explanation of some of these findings relating to children has been provided below.

Changing behaviour in childhood can influence a child's future, for example modifying antisocial and aggressive behaviour in childhood can reduce criminality and substance abuse in later life especially in males (D. Thomas, Bierman, & Powers, 2011). Eron (1990) has suggested that aggressive antisocial behaviour crystallises around 8 years old however Kellam, Rebok, Ialongo and Mayer (2006) believe that this is malleable and teacher child interactions can have a strong influence on future actions. If supplementation is able to modify the behaviour of even one child in a classroom then it is possible that the resulting changes to the classroom dynamics and learning environment may also impact on the learning of others.

The specific action of ω-3 PUFA with regards to behaviour is not clear but is believed to involve the activation of the serotonergic neuron system as this has been shown to reduce depression and aggression (K. Hamazaki et al., 2008). There is the possibility however that increased ω-6 PUFA levels or an altered ω-6: ω-3 PUFA ratio may do the opposite and enhance aggression as found in a Japanese study where physical aggression in 9-12 year old girls increased in the placebo group (taking 50% soybean and 50% rapeseed oil providing 600mg/d LA ω-6 PUFA) but not with those taking 514mg/d DHA and 120mg/d EPA. Significant results in the girls (*n*=85) were demonstrated using the Hostility-Aggression Questionnaire for Children but not for the boys (*n*=83) in this group. The changes in physical aggression scores over time and those of the ratio EPA:AA in RBC were significantly correlated in girls who agreed to blood collection (Itomura et al., 2005). It must be noted however that aggression against others (extraggression), which was assessed by a picture frustration test, did not change in the control group but increased significantly in the fish oil group (who had higher DHA RBC levels but similar EPA levels to the control group). The researchers thought this extraggression could be partly attributed to low baseline levels for extraggression in the control group.

Another study undertaken by some of these researchers, this time in Indonesia, involved 231 school children, who were 9-12 years old. The placebo group consumed soybean oil whilst the active group consumed 650mg/d DHA and 100mg/d EPA. Using the Hostility-Aggression Questionnaire for Children no significant changes in physical aggression were demonstrated, whilst unlike the earlier Japanese study extraggression was not assessed. Another strong contrast between the two studies was that in this study the ratio EPA:AA in RBC did not show a marked increase even after DHA

supplementation, which could account for the marked difference in results (K. Hamazaki et al., 2008).

Whilst yet another earlier study undertaken by some of these same researchers involved seventeen female and five male Japanese university students, who consumed 1500-1800mg/d DHA (depending on participant's weight). These students did not show an increase in extraggression, at times of mental stress (university exams), unlike the twelve female and seven male students in the control group (taking 97% soybean oil) who after three months showed a significant increase in extraggression (T. Hamazaki et al., 1996). Extraggression in this study which involved adults was assessed using dementia-detecting tests, a picture frustration test and an adapted Stroop test, whilst no testing for physical aggression was undertaken.

This study was repeated, however this time with a start time in the summer holidays and no exams within thirty days of any testing checkpoints (therefore the study involved no/less stress). A picture frustration test and a Cook and Medley hostility scale was used to assess forty-six Japanese university students (T. Hamazaki et al., 1998). In the previous study DHA was seen as the stabilising effect during the thirteen-week stressful timeframe. This study was considered less stressful and no significant differences for extraggression were found between groups.

The DHA may have a calming, aggression-stabilising effect at times of stress as well as preventing arrhythmias (T. Hamazaki et al., 1998), which all might account for some of the above results. The AA:EPA ratio may also influence aggression, hostility and anti-social behaviour (Schuchardt, Huss, Stauss-Grabo, & Hahn, 2010; Simopoulos, 2002a).

Dyspraxia (DCD) is usually defined in terms of difficulty with planning and motor coordination (Richardson & Ross, 2000). Dyspraxia disproportionately affects males and frequently occurs with (co-morbid) dyslexia (Stordy, 2000) which may be a reason why it is often undiagnosed (Richardson & Montgomery, 2005). Few RCTs of fatty-acid treatments in dyspraxia have been undertaken (Richardson, 2004a; Stordy, 2000).

In a study in the UK, where no placebo was used and therefore everyone knew what they were taking, eleven boys and four girls (aged 5-12) were provided with 480mg DHA, 35mg AA, 96mg ALA, 80mg vitamin E and 24mg thyme oil each day. This supplementation of ω-3 and ω-6 did not include EPA and was for a period of 4 months.

The children's motor skills were assessed using the Movement Assessment Battery for Children (MABC) and total impairment scores and checklist scores showed a significant improvement (Stordy, 2000).

Perhaps the most well-known ω-3 intervention study is the 'Durham Study'. This was a three-month study using a supplement containing 174mg/d DHA, 558mg/d EPA and evening primrose oil (60mg/d GLA) verse a placebo and involving 110 children with diagnosed but untreated DCD. It was one of the first studies which clearly demonstrated links between ω-3 supplementation and improvements in reading, spelling and behaviour (Richardson & Montgomery, 2005). Children were assessed using the Movement Assessment Battery for Children, the Wechsler Objective Reading Dimensions, and Connors' Teachers Rating Scales. After this a one-way cross-over from placebo to active treatment was undertaken for an additional three-months where similar changes (improvements) were seen in the placebo-active group.

Autism usually appears in the first two years of life and involves social and communication problems. The three main criteria which are essential for its diagnosis include a disturbed social interaction, lack of communication and restriction of the normal variation on behaviour and interests (Meguid, Atta, Gouda, & Khalil, 2008). Autism is about 3 to 4 times more common in males than females and prevalence estimates range from 1 to 15 per 10000 children (Richardson & Ross, 2000). The impact of autism is extensive with an annual societal cost for the UK estimated to exceed 1 million pounds. (Järbrink & Knapp, 2001).

Children with autism tend to have higher ω-6 PUFA levels in their RBC membranes compared with controls (Bell et al., 2004; Vancassel et al., 2001). When the diet of patients with Aspergers's syndrome and Autism was supplemented with fish oils there was an increase in the levels of EPA (~200%) and DHA (~40%) and a significant reduction in the level of AA (~20%) (Bell et al., 2004). Patients with classic autism/Asperger's syndrome had significantly higher concentration levels of phospholipase A^2 (PLA^2) compared to controls. Phospholipase A2 are enzymes which release fatty acids such as AA (Murakami & Kudo, 2002) which competes with EPA for enzymes(Simopoulos, 2002b). However with supplementation with EPA these PLA^2 concentrations reduced compared to unsupplemented patients (Bell et al., 2004).

A brief six-week Austrian study involved thirteen autistic boys between the ages of 5-17 years consuming 700mg/d DHA and 840mg/d EPA. The Aberrant Behaviour Checklist (ABC) was used as the assessment tool however no clear significant differences were noted. There was a trend indicating a reduction in disobedience, distractibility and impulsivity and improved stereotype and hyperactivity by ω-3 PUFA group although this was not at a level of significance (Amminger et al., 2007).

In an open non-controlled, three month Egyptian study, 30 autistic children (18 boys and 12 autistic girls) between the ages of three and 11 years old were provided with 250 mg/d DHA and 5 mg/d EPA. The Child Autism Rating Scale (CARS) was used as the assessment tool, and 20 of these children showed a significant improvement in their autistic behaviour compared with baseline levels (Meguid et al., 2008).

Dyslexia has only recently been accepted as a neurological syndrome (K. Taylor & Richardson, 2000). This involves specific weaknesses in WM, especially for auditory-linguistic material, and difficulties with direction and sequencing (Snowling, Duff, Petrou, Schiffeldrin, & Bailey, 2011). Children demonstrate difficulties in single word decoding, inability to read fluently, poor rapid renaming, and problems in phonological short term memory. This condition affects around 5% of the population and mainly occurs in males (Coleman, Gregg, McLain, & Bellair, 2009; Richardson & Ross, 2000). Approximately 80% of US children with learning disabilities are found to be dyslexic (Stordy, 2000). Brain imaging on dyslexic individuals has highlighted lipid abnormalities (Richardson, Cox, Sargentoni, & Puri, 1997), which may be reduced with ω-3 PUFA supplementation (Richardson, 2004a).

In a five month open-pilot Swedish study involving 19 dyslexic children (16 boys and eight girls) between the ages of nine and 17 consuming 480 mg/d DHA, 108 mg/d EPA, 96 mg/d GLA, 35 mg/d AA and vitamin E. A word-chain test and an interview were used as the main assessment tools. These detected a significant improvement in reading speed and motoric-perceptual speed (letter decoding) compared with baseline levels. They also recorded a gradual improvement in schoolwork and a perceived overall benefit (rated by child and parent) when assessed at the 6, 12, 16 and 20 week periods (Lindmark & Clough, 2007).

A 90 day Finish study involving 61 dyslexic children between the ages of seven and 13 years found no significant improvement in reading, fluency, spelling, maths, attention or

behaviour compared with the control group. It must be noted however that these children were provided with 500 mg/d EPA and 400 mg/d carnosine, but no DHA (Kairaluoma, Närhi, Ahonen, Westerholm, & Aro, 2008).

Attention Deficit Hyperactivity Disorder (ADHD) is characterised by developmentally inappropriate and disabling levels of inattention, impulsivity and/or hyperactivity (Voigt et al., 2001). These behaviours can harm school performance and family relationships (Hirayama, Hamazaki, & Terasawa, 2004). Assisting children with this condition is a common occurrence for the majority of teachers as worldwide prevalence ratings are estimated at 5.29% (Polanczyk, de Lima, Horta, Biederman, & Rohde, 2007). It is often associated with poor motor coordination, and males are affected more than females (Breen, 1989; Gershon, 2002; Richardson, 2006; Richardson & Puri, 2000; Scahill & Schwab-Stone, 2000), with male-to-female prevalence rates varying from 9:1 to 6:1 (Gaub & Carlson, 1997). Females diagnosed with ADHD have more speech/learning problems and lower verbal intelligence, although they have similar behavioural profiles (Breen, 1989; Gaub & Carlson, 1997). The ratio of ADHD females to males also correlates with the notion that males are more vulnerable than females to deficiencies in highly unsaturated fatty acids (HUFAs) (Burdge et al., 2002; Burdge & Wootton, 2002; Giltay, Gooren, Toorians, Katan, & Zock, 2004). ADHD children also have a higher likelihood of sleeping problems and, hence, wake over-tired in the morning (Kaplan, McNicol, Conte, & Moghadam, 1987; Owens, 2005; Richardson, 2006). As PUFA's may play a role in the initiation and maintenance of normal sleep (Freudigman & Thoman, 1993), it is, therefore, possible that inefficient intakes are a contributing factor to the sleeping problems associated with ADHD (Yehuda, Rabinovitz, & Mostofsk, 1998).

Children with ADHD had significantly lower concentrations of DHA, DGLA and AA than those in the control group (E. Mitchell, Aman, Turbott, & Manku, 1987). Another study involving 53 boys with ADHD found they had significantly lower concentrations of DHA and AA, as well as EPA compared with those in the control group (Stevens et al., 1995). After receiving DHA for 4 months 25 children with ADHD showed a 2.6 fold increase in plasma phospholipid DHA compared with the control group (Voigt et al., 2001).

In a study involving 6-12 year old boys with behaviour, learning and health issues a greater number of behaviour problems, temper tantrums, sleep problems, and health problems were reported in children with lower ω-3 HUFAs (Stevens, Zentall, Abate, Kuczek, & Burges, 1996). It is believed that ADHD involves difficulties in the synthesis of HUFAs rather than a lack of their EFA precursors (Richardson & Ross, 2000). Difficulties arise when undertaking research in the area of ADHD as there is a high comorbidity with dyslexia and dyspraxia, as well as the misdiagnosis of bipolar children as mentioned earlier (Kirby et al., 2009; Richardson, 2004a).

After reviewing all published literature in this field, researchers believed there was sufficient evidence to suggest that children with ADHD related symptoms might respond to PUFA supplementation, and that those with the attention subtype and/or learning difficulties might respond even better (Bloch & Qawasmi, 2011; Sinn et al., 2010). Numerous studies involving children with ADHD have been undertaken in the last decade, many of which have been outlined in Appendix D2 (Hirayama et al., 2004; Johnson, Ostlund, et al., 2009; Joshi et al., 2006; Milte et al., 2012; Richardson & Puri, 2002; Stevens et al., 2003; Vaisman et al., 2008; Voigt et al., 2001). A basic comparison has been made of all ADHD findings listed in Appendix D2 and the results outlined below. As these studies focus on children with special ADHD needs rather than mainstream children results have been used to provide background information and therefore further details have not been included.

In various international studies involving children with ADHD between the ages of six and 13, significant improvements were found in anxiety, attention and behaviour with supplementation higher than 450 mg DHA for studies lasting longer than 12 weeks (Richardson & Puri, 2002; Sinn, Bryan, & Wilson, 2008; Stevens et al., 2003). There was no treatment effect for those studies over a shorter duration or using lower DHA levels (Hirayama et al., 2004; Johnson, Östlund, Fransson, Kadesjö, & Gillberg, 2009; Milte et al., 2012; Voigt et al., 2001).

Studies using doses above 400mg EPA also found significant improvements with the active group. For example a 15 week Swedish study using 500mg/d EPA, 2.7mg/d DHA and vitamin E recorded a significant improvement in inattention (teacher rated) after supplementation compared with the control group (Gustafsson et al., 2010). Whilst a 16 week Canadian study where the dosage was dependent on the weight of the child

and varied from 200mg DHA and 500mg EPA to 400mg DHA and 1000mg EPA, parents noted a significant improvement in ADHD symptoms after supplementation compared with baseline scores (Bélanger et al., 2009). Supplementation with 400mg/d ALA was associated with improvements in attention, impulsivity, restlessness and self-control in a three month open study involving 5-12 year olds with ADHD compared to baseline scores (Joshi et al., 2006).

In summary this section has highlighted the complexity of research involving the influence of ω-3 PUFA on people with neurodevelopmental disorders. Rarely do people exhibit classical symptoms solely related to one disorder as many conditions are comorbid. Improvements in depression have been linked with an increased intake of ω-3 PUFA (Nemets et al., 2002; Peet & Horrobin, 2002b; Su et al., 2003). Doses greater than 1g /d of EPA have been shown to be less effective at reducing symptoms of depression than doses less than this (Peet & Horrobin, 2002b). Whilst supplementation of EPA and DHA may be more effectual than DHA alone (Kidd, 2007).

Findings associated with bi-polar disorders are very inconsistent (Freeman et al., 2006), whilst schizophrenia research has highlighted the importance of investigating the interaction between ω-3 and ω-6. It is believed that PLA²may be overactive in schizophrenia and small amounts of AA, EPA or EPA and DHA may improve symptoms (Hoen et al., 2012; Peet & Horrobin, 2002a). Children with autism/Asperger's syndrome have been shown to have higher concentration levels of phospholipase A² compared to controls (Bell et al., 2004). The influence of ω-6 has also been noticed in aggression research where the placebo group taking LA ω-6 PUFA demonstrated more aggression than those in the ω-3 group who took EPA and DHA (Itomura et al., 2005). Reduced ω-3 PUFA intakes has been linked with increased anti-social behaviour (Buydens-Branchey & Branchey, 2008; Gesch et al., 2002; T. Hamazaki et al., 1996; T. Hamazaki et al., 2002).

Supplementation with ω-3 and ω-6 has been linked with improvements to dyspraxic children's motor skills compared to baseline levels (Stordy, 2000) and reading, spelling and behaviour improvements compared with the control group (Richardson & Montgomery, 2005). Supplementation with ω-3 and ω-6 has also been linked with improvements to dyslexic children's reading speed, letter decoding and school work compared to baseline levels (Lindmark & Clough, 2007), however a study using

supplementation solely with 500mg/d of EPA identified no treatment effects (Kairaluoma et al., 2008). Children with ADHD have significantly lower ω-3 and ω-6 blood levels than control groups (E. Mitchell et al., 1987; Stevens et al., 1995). Supplementation studies have produced varied results although research appears to indicate that 450 mg/d DHA, 400mg/d EPA or 400mg/d ALA for at least 12 weeks is needed (as documented above).

Future research is needed investigating links between ω-3 and ω-6 and young children suffering from depression and dyspraxia. The links between ω-3 and ω-6 in schizophrenia research also highlight the need for future research to involve RBC and phospholipid biomarkers.

Until relatively recently no intervention studies had been conducted focusing on 'general' classroom children (Hughes & Bryan, 2003; NEMO Study Group, 2007; Stevens et al., 1995) using every day standard educational assessment activities. The majority of ω-3 PUFA research focusing on children has used batteries of mainly American and European devised tests, investigating IQ, verbal learning, memory, problem-solving and visual recognition (Bélanger et al., 2009; Hirayama et al., 2004; Kairaluoma et al., 2008; D. Kennedy et al., 2009; Kirby, Woodward, Jackson, Wang, & Crawford, 2010b; Muthayya et al., 2009; NEMO Study Group, 2007; Sinn et al., 2008; Voigt et al., 2001). Academic performance can be affected by many things including self-discipline and mind wandering which may be related to a person's WM (Alloway & Alloway, 2010), this in turn can influence behaviour and attitude. The following section is devoted to the associations between increased fish intake and children's cognition and the effects of ω-3 PUFA supplementation of mainstream general healthy children. Many of these studies investigated behaviour and/or cognition changes.

Cognitive Benefits of ω-3 PUFA Supplementation

As there are many aspects which come together to form the umbrella term 'cognition', the difficulty in researching changes in cognitive ability is immense (Wainwright & Colombo, 2006).

Increased fish consumption has been linked to increased cognitive performance in two Swedish studies involving a large sample size. An extensive questionnaire was completed by 10,837 15 year olds. The questionnaire was very detailed and asked about

socioeconomic conditions and dietary information. A year later school grades were accessed via the national register. Grades were significantly higher for children who ate fish more than once a week than those who ate fish less than once a week. Data was adjusted for confounding factors such as parents' education and socioeconomic status (J. Kim et al., 2010). In the other study 3 years later 4792 18 year old males from this group took part in military training where they underwent intelligence testing. Their intelligence test score was able to be obtained from the Swedish Military Conscription Register and linked with the original questionnaire data. Those males who ate fish more than once a week gained higher intelligence scores as well as performing better with verbal and visuospatial skills than those who ate fish less than once a week (Aberg et al., 2009).

The McNamara study showed that 1200 mg/d DHA supplementation for 8 weeks in 33 healthy boys (8-10 years old) increased activation of left dorsolateral prefrontal cortex during performance of a sustained attention task, using functional magnetic resonance imaging. Boys in this double blind randomised placebo controlled study received either 400 mg/d or 1200 mg/d DHA or a corn oil placebo for 8 weeks (McNamara et al., 2010). The RBC DHA composition was positively correlated with dorsolateral prefrontal cortex activation.

The majority of ω-3 PUFA intervention studies prior to 2005 have involved children with learning or behavioural needs and few have involved mainstream children. The majority of these studies have not identified any clear significant treatment effects (Baumgartner, Smuts, Malan, Kvalsvig, et al., 2012; K. Hamazaki et al., 2008; D. Kennedy et al., 2009; Kirby et al., 2010b; McNamara et al., 2010; Muthayya et al., 2009; NEMO Study Group, 2007; Richardson, Burton, Sewell, Spreckelsen, & Montgomery, 2012; Ryan & Nelson, 2008). It must be noted that some studies found significant results involving mainstream children with learning difficulties (Richardson et al., 2012), or associations between DHA RBC levels and improved listening and vocabulary scores (Ryan & Nelson, 2008). As already stated, a summary of these leading studies investigating cognition and ω-3 PUFA supplementation in mainstream children can be found in Appendix D2.

The only study involving mainstream normally achieving children which found significant cognitive improvements using ω-3 PUFA supplementation was a South

African study. For six months 183 7-9 year olds consumed 127 mg/d DHA in a fish flour spread or a control spread. After supplementation an assessment battery and a reading and spelling test identified a significant improvement in the ability to remember words (word recognition and discrimination) compared with the control group (Dalton et al., 2009). To contrast this a very recent eight-and-a-half month South African study involving iron-deficient 6-11 year olds identified no significant benefits of supplementation with 420 mg/d DHA, 80mg/d EPA and 50mg/d iron (Baumgartner, Smuts, Malan, Kvalsvig, et al., 2012). Although these studies involved slightly different methodologies comparisons can be made as both used the Hopkins Verbal Learning Test (HVLT) as one of their assessment measures. Both were double blind placebo controlled involving an even gender mix of primary school aged children. The average age in the experimental group for the Dalton et al. study was 8.2±0.7 years whilst it was 8.9±1.3 years for the Baumgartner et al. study. The Baumgartner et al. study, involved children who were anaemic and was a 2-by-2 factorial trial. For this reason only results from the 81 children in the group which received DHA and EPA and no iron will be used as a comparison with the 91 children who received DHA in Dalton et al.'s study. This latter group was not anaemic although children in both groups had low levels of ω-3 PUFA in their habitual diets. In the Dalton study the HVLT cognitive memory scores improved after supplementation in both experimental and control groups compared with baseline scores. In the Baumgartner study however scores decreased after supplementation in both experimental and control groups compared with baseline values.

These differences may reflect the influence of different placebos (medium-chain triglycerides in the Baumgartner study and an 'analogous spread' in the Dalton study). The AA level in the Baumgartner study increased after supplementation in both experimental and control groups (who both received supplementation with the placebo). These higher levels of ω-6 may have influenced the availability of DHA and EPA resulting in negative effects on cognition as found in previous studies (Agostoni et al., 1997; Neggers et al., 2009; Novak et al., 2008; Whalley et al., 2004).

Two UK studies investigated the effects of ω-3 PUFA supplementation on mainstream primary school children's behaviour and moods (D. Kennedy et al., 2009; Kirby et al., 2010b). Kennedy et al. (2009) involved 90 healthy 10-12 year olds who consumed either 400mg or 100mg of DHA for 8 weeks. This is a relatively short duration

compared to most recent studies and there was no EPA supplementation. Kirby et al. (2010b) involved 450 healthy 8-10 year olds who consumed either 400mg DHA and 56mg EPA or placebo for 16 weeks. The EPA supplementation is low compared to other recent studies. Neither study demonstrated any significant differences between ω-3 PUFA and placebo groups after supplementation.

Both studies used extensive testing which reduced the reliability of refuting the null hypothesis. One study utilised an internet battery and a tailored version of the computerised Cognitive Drug Research battery. The tests included Picture Presentation, Immediate Word Recall, Simple Reaction Time, Digit Vigilance Task, Choice Reaction Time, Spatial Memory, Numeric Working Memory, Delayed Word Recognition and Delayed Picture Recognition. They also completed a mood and Fatigue Visual Analogue scale at baseline and post-intervention (D. Kennedy et al., 2009). After all these tests were analysed there was only one significant finding. It identified a significant improvement in the speed of word recognition with a DHA supplementation level of 400mg and a lesser effect was found for those taking 1000mg compared to baseline level. Due to the extensive range of tests undertaken these results have been attributed to chance fluctuations rather than treatment effect.

The other study also used a wide range of cognitive assessment measures. These included the Kaufman Brief Intelligence Test, the Wechsler Individual Achievement Test (which tested word reading, pseudo-word reading and spelling), the Working Memory Test Battery for Children (which tested digit recall, block recall and backward digit recall) Creature Counting and the Matching Familiar Figures Task. The children's handwriting was analysed using the Computerised Penmanship Evaluation Tool whilst teachers and parents completed the Swanson, Nolan and Pelham rating scale for ADHD as well as the Strengths and Difficulties questionnaire. Using the Swanson, Nolan and Pelham rating scale, teachers rated those receiving supplementation as less inattentive and hyperactive/impulsive compared with baseline scores. The parents also rated those receiving supplementation as less hyperactive/impulsive compared with baseline scores. Using the Strengths and Difficulties questionnaire the children receiving supplementation received improved scores on total difficulties, emotional symptoms, conduct problems, hyperactivity and pro-social behaviour compared with baseline scores by their teachers. To contrast this, improvements in the total difficulty score were recorded by teachers for both groups but only the scores of the children in the placebo

group reached significant levels. Parents recorded a significant decrease in pro-social scores for the placebo group compared with baseline levels. Although this study utilised a wide range of cognitive and behavioural measures, unlike the Kennedy (2009) study it did not highlight the chance of a Type 1 error as a possible justification for its findings. It did however highlight that after all these tests were undertaken there were only 3 significant differences between the groups and one of these was in favour of the placebo (Kirby et al., 2010b).

The above paragraphs highlight the problematic nature of multiple assessment measures. Willatts and Forsyth (1981) suggested that the diversity in findings were due to the inconsistencies of cognitive performance measures. They believed that some cognitive performance measures provided relatively limited assessment across a broad range of abilities, which may or may not have been sensitive to PUFA intake. The selection of the assessment tools needs to take account of many factors which include "the purpose of testing, conditions of the testing, the tester's expertise, the availability of materials, and the cost" (Barkley, 1997b, p. 112).

In summary, the above section has identified that the majority of intervention studies have not demonstrated any significant ω-3 PUFA treatment effects (Baumgartner, Smuts, Malan, Kvalsvig, et al., 2012; K. Hamazaki et al., 2008; D. Kennedy et al., 2009; Kirby et al., 2010b; McNamara et al., 2010; Muthayya et al., 2009; NEMO Study Group, 2007; Richardson et al., 2012; Ryan & Nelson, 2008). One sole South African study which used an assessment battery and a reading and spelling test identified a significant improvement in one aspect of a test (the ability to remember words) (Dalton et al., 2009). Another study found parents of children in the ω-3 PUFA group, believed there were improvements in their children's behaviour, however their teachers recorded improved behaviour with those children in the placebo group (Kirby et al., 2010b).

Despite the lack of positive findings in most studies, the need for further research involving mainstream children is highlighted by 3 studies which identified links between increased brain activation, improved cognition and ω-3 PUFA supplementation. Two of these studies were Swedish and identified links between improved academic grades, IQ, verbal and visuospatial skills and increased fish intakes (Aberg et al., 2009; J. Kim, 2010). The other study involving mainstream boys

identified a positive correlation between RBC DHA composition and dorsolateral prefrontal cortex activation (McNamara et al., 2010).

Identifying suitable measures sensitive enough to detect changes to cognitive ability and behaviour is extremely problematic. The following section outlines many of the measures utilised in ω-3 PUFA research involving children. A more detailed explanation relating to specific tests has been provided in Appendix D3.

Cognitive performance assessment tools commonly used in ω-3 PUFA research involving children

Different tests measure different aspects of cognition or behaviour and this must be taken into account when interpreting and comparing results across different tests (Hughes & Bryan, 2003, p. 420). Table 3 outlines various measures commonly used in ω-3 research involving children.

Table 3: Children's behaviour and cognitive tests commonly used in ω-3 research to assess major domains

Targeted Domain	Measures
General intelligence/ General cognitive ability	ABC, BSID, KABC, KBIT-11, SBIS-IV, WISC, WJ-R, WPPSI-R
Attention and Concentration	CPT, CRS, CST, DS, NEPSY,SNAP-IV, ST, TEA-Ch, TMT-A&B, TOVA, WISC-III
Memory	CVLT, DS, HVLT, KABC (face recognition, hand movements, number recall, word order, spatial memory), LW, NEPSY(face, names, narrative, sentence repetition, lists), RAVLT, WISC-III, WLT, WMS, WMTB-C
Visuospatial organisation	TMT-A, WAIS-R
Executive function	GNG, NEPSY(tower, statue, knock & tap), TMT, WCST, WFT
Cognitive or psychomotor speed	MFFT, TMT-A, WFT
Motor	BSID, MABC, NEPSY (finger tap, manual motor sequences, finger discrimination), ROS
Non-verbal processing	KABC simulation scale, KBIT-11
Social/emotional adjustment	CBS, CRS (behaviour)
Language	AN, BSID, CVLT, HVLT, KBIT-11, NEPSY (language), OR, RAVLT, RW, PPVT-III,WD, WFT, WIAT-II, WISC-III & IV, WRAT-IV
Mathematics	WIAT-II, WISC-III, WRAT-IV

Abbreviations for cognitive measures: ABC: Aberrant behaviour checklist, AN: animal naming, BSID: Bayley Scales of Infant Development, CBS: child behaviour checklist (Connors rating scale), CRS: Connors Rating Scale, CPT: Connors continuous performance test, CST: concept shifting test, CVMT: California Verbal Learning Test, DS: digit spans forward and backwards of WAIS-R, GNG: Go-No-Go, HVLT: Hopkins Verbal Learning Test, KABC: Kaufman Assessment Battery for Children, KBIT-2: Kaufman Brief Intelligence test (second edition), LW: list words, MABC: Movement assessment battery for children, MFFT: Matching familiar figure test, NEPSY: Developmental Neuropsychological Assessment, OR: oral reading, PPVT-III: Peabody Picture Vocabulary Test (third edition), RAVLT: Rey Auditory Verbal Learning test, RW: Rey's words immediate and delayed recall, ROS: Rostock-Oseretzky Scale, SBIS-IV: Stanford-Binet Intelligence Scale (fourth edition), SNAP-IV: Swanson, Nolan& Pelham rating scale, ST: Stroop test, TEA-Ch: Test of everyday attention for children,TMT (A and/or B): trail making test, TOVA: Test of variables of attention, WCST: Wisconsin Card sorting test, WD: word discrimination, WFT: word fluency test, WIAT-II: Wechsler Individual Achievement Test (second edition), WISC-III: Wechsler Intelligence Scale for children (third edition), WISC-IV: Wechsler Intelligence Scale for children (fourth edition),WJ: Woodcock-Johnston Psycho-educational test Battery-Revised, WLT word learning task, WMS: Wechsler memory scale, WMTB-C: Working Memory Test Battery for Children, WRAT-IV: Wide range achievement test (fourth edition), WPPSI-R: Wechsler Preschool and Primary Test of Intelligence-Revised.

The use of a range of tests in a standard test battery provides a stable basis for comparison, however it also has serious limitations as some tests are of 'dubious value' (A. Benton, 2000) and multiple testing enhances the chance of Type 1 error. The following paragraphs highlight some test considerations.

"Many studies have continued to employ standard executive function measures developed for adults without fully considering the limitations in childhood populations" (Archibald & Kerns, 1999, p. 116). Tests which have proven successful in one field are sometimes applied to another with varying success. The following identifies various concerns related to commonly used tests.

"Cognitive tests are devised within a particular culture and therefore contain stimuli that relate to that culture, its social customs, images and vocabulary" (Grigorenko & Sternberg, 1999, p. 30). An example of this is the *Bayley Scales of Infant Development* which is based on behavioural items of a normative sample of children however the order of attainment of the milestones may differ in other cultures. American children learn to squat after they learn to crawl and stand whereas in Bali crawling is considered animal-like and is discouraged and children semi-squat, sit, squat and then stand (Super, 1981). Children may appear to be less developed using the *Bayley Scales of Infant Development* however it may simply be the tool of measurement not identifying these cultural differences. Although some tests are suitable for a wide range of ages, they are sometimes stated to be biased against ethnic minorities. Performance on the P*eabody Picture Vocabulary Test* may reflect socioeconomic and/or ethnic patterns of vocabulary usage rather than cognitive ability (Champion, Hyter, McCabe, & Bland-Stewart, 2003; Stockman, 2000).

Normed tests should only have a lifespan of approximately 15-20 years, in order to correct for the Flynn effect, which is the general trend for an increased IQ over time with each subsequent generation (Strauss et al., 2006). As countries' demographic compositions change over time additional caution is needed when using outdated normative data.

When choosing a test for research numerous issues need to be considered to ensure the selected test is the most practical whilst also being the most effective measure. Some tests take a great deal of time, for example the *Kaufman Assessment Battery for Children* (KABC) takes 75 minutes, for school-age children (Grigorenko & Sternberg, 1999) . Whilst the cost of some tests may also prove to be prohibitive, e.g. *The Wechsler Preschool and Primary Test of Intelligence* costs over US $1,120 for 25 tests

(2013 US price http://www.pearsonassessments.com/HAIWEB/Cultures/enus/Productdetail.htm?Pid=WPPSI-IV).

In a study which used a fMRI, researchers were able to identify an association between an increase in brain activation and an increase in RBC DHA levels (McNamara et al., 2010). The challenge for researchers is to investigate whether this change in brain activation can result in a measurable improvement in cognitive and academic ability. Research has investigated planning, developing strategies, testing hypotheses when problem-solving, focusing attention, inhibiting irrelevant information stimulation, collating memories and the higher order aspects of memory (Bryan et al., 2004; Hughes & Bryan, 2003; Thatcher, 1991). Few however relate specifically to academic achievement such as reading, writing, spelling and classroom and school behaviour. The following sections outline tests utilised in ω-3 research which investigate children's attention and literacy ability.

Attention Measuring attention is inherently difficult to do objectively (Kirby et al., 2010b). The ability to focus and sustain attention, to divide attention between stimuli and to inhibit distraction is essential for learning (Hughes & Bryan, 2003). Miller and Weiss (1981) believe that children become increasingly more able to selectively attend by ignoring potential distractors, somewhere between 7 ½ and 10 ¼ years of age, as selective attention is developed. Various researchers have written about tests which have been developed to assess attention including: Neuropsychological Assessment (NEPSY), Test of Everyday Attention for Children (TEA-ch), of which 'Creature Counting' is an example, and Wechsler Intelligent Scale for Children- third edition (WISC-III), with the 'Digital Span Forwards' an example (Troyer, Moscovitch, & Winocur, 1997). The Creature Counting test has been found to be "less accurate with children with attention deficit hyperactivity disorder" (Hughes & Bryan, 2003, p. 416), whilst the performance on the Digit Span Forwards is poorer in boys (but not girls) with dyslexia who were more deficient in PUFAs (Hughes & Bryan, 2003).

Executive functions, as stated above, are control processes which affect one's overall output (Hooper et al., 2002). When a person is required to consciously respond to a new situation rather than a routine or well-learned behaviour the executive functions are employed (Cheatham et al., 2006). Because executive functions draw on many cognitive

abilities, it is very difficult to identify one single reliable test (P. Burgess & Alderman, 2004). This may be one reason why little research has been undertaken to investigate links between executive functions and ω-3 PUFA supplementation (Hughes & Bryan, 2003).

A more detailed outline of these tests and others mentioned in this thesis is contained in Appendix D3.

Verbal Fluency

Verbal fluency is dependent on many executive functions (Abwender, Swan, Bowerman, & Connolly, 2001; Baldo, Schwartz, Wilkins, & Dronkers, 2006; Koren, Kofman, & Berger, 2005), but rather than investigating aspects of executive functioning it is often easier to identify dysfunction (Bryan & Luszcz, 2000; Henry & Crawford, 2004a; T. Ross, 2003). Tests of verbal fluency have been shown to detect and measure executive dysfunction, such as frontal lobe damage (Baldo et al., 2006; Bryan & Luszcz, 2000; B. Cohen & Stanczak, 2000; S. Lanting, Haugrud, & Crossley, 2009). For this reason they are widely used in cognitive psychology and neuro-psychological assessment (B. Cohen & Stanczak, 2000; Henry & Crawford, 2004a; Sauzéon, Lestage, Raboutet, N'Kaoua, & Claverie, 2004), and have been used by about 50% of neuropsychologists (Butler, Retzlaff, & Vanderploeg, 1991).

By the time children are at school the majority of the brain is fully developed with the exception of the pre-frontal lobes. These lobes are believed to be responsible for executive functioning, which includes the ability to undertake and switch between two separate tasks, remember, focus and ignore disruptions, and hold and use LTM information (Abwender et al., 2001; Baddeley, 1996; Baldo & Dronkers, 2007; Borkowski, Benton, & Spreen, 1967; Bryan & Luszcz, 2000; Crawford, Moore, & Cameron, 1992; Gaillard et al., 2003; Phillips, 1997; Rende, Ramsberger, & Miyake, 2002). Any investigation into the effects of ω-3 PUFA on executive functioning would need to utilise tests specifically designed to investigate these attributes. A wide variety of these fluency tests are available and commonly used. For this reason this section will focus on providing background information on these tests, including outlining the numerous versions available, discussing their reliability and applicability for children, explaining various analysis techniques and outlining previous findings involving interactions with age, gender, ethnicity and education levels.

Tests of verbal fluency have been used in adult and childhood research to investigate a variety of cognitive processes, including word knowledge, access to semantic memory, long-term verbal memory, attention (Ruff, Light, Parker, & Levin, 1997) executive functioning (Bégin, Langlois, Lorrain, & Cunnane, 2008; Henry & Crawford, 2004a), traumatic brain injury (Henry & Crawford, 2004b), as well as speed of information processing (Bégin et al., 2008; Kalmijn et al., 2004), vocabulary size, WM and inhibition of irrelevant words (Sergeant, Geurts, & Oosterlaan, 2002). Patients with Alzheimer's disease (Tröster et al., 1998), multiple sclerosis (Henry & Beatty, 2006; Tröster et al., 1998), Parkinson's disease (Henry & Crawford, 2004c; Tröster et al., 1998), Huntington's disease (Henry, Crawford, & Phillips, 2005) and schizophrenia (Henry & Crawford, 2005) have also been involved in research utilising fluency tests.

It is believed that fluency is a multifactorial task involving bilateral frontal and temporal brain regions (Baldo et al., 2006; Boivin, Giordani, Berent, & Amato, 1992; Henry & Crawford, 2004a; Parks, Loewenstein, Dodrill, & Barker, 1988; Robert et al., 1998). For this reason ω-3 PUFA research has often involved some form of verbal fluency test (Bélanger et al., 2009; Kairaluoma et al., 2008; Kirby et al., 2010b; Milte et al., 2012; Muthayya et al., 2009; Sinn & Bryan, 2007).

Fluency tasks require participants to generate as many words as possible within a set timeframe. These tests may be ‘phonemic’ (letter fluency) where participants generate (say or write) as many words as possible beginning with a specific letter or ‘semantic’ (category fluency) where participants generate (say or write) as many words as possible within a certain category e.g. fruit (Alvarez & Emory, 2006; Mathuranath et al., 2003; T. Ross, 2003; Spreen & Strauss, 1998).

Specific versions of the phonemic (letter) fluency test include: Controlled Oral Word Association, Word Fluency, Letter Fluency, FAS Test, Category Fluency, Phonemic Fluency, Semantic Fluency, Controlled Verbal Fluency and the Thurstone Word Fluency Test (TWFT) (Strauss et al., 2006), and all are examples of controlled association recall tasks as outlined in Appendix D3 (Bird, Papadopoulou, Ricciardelli, Rossor, & Cipolotti, 2004; Bryan & Luszcz, 2000; Bryan, Luszcz, & Crawford, 1997; Strauss et al., 2006).

Word fluency tests (WFT) have been used to investigate a diverse range of cognitive and neural dysfunctions. New Zealand researchers have used WFT in adult and

childhood research studies to investigate bacterial meningitis (Grimwood, Anderson, Anderson, Tan, & Nolan, 2000), dyslexia (Reynolds, Nicolson, & Hambly, 2003), leukaemia (Raymond-Speden, Tripp, Lawrence, & Holdaway, 2000), dementia (McGaughey, 2002), depression (Douglas & Porter, 2009), brain injury (Furlonger, Sleigh, Havill, Marsh, & Kersel, 2000), aphasia (C. McCann, Lee, Purdy, & Paulin, 2011), multiple sclerosis (Drew, Starkey, & Isler, 2009), amyotrophic lateral sclerosis (Meier, Charleston, & Tippett, 2010), Parkinson's disease (Donovan, Siegert, McDowall, & Abernethy, 1999) and links between criminal offending and learning disabilities (Rucklidge, McLean, & Bateup, 2009).

In a study investigating a variety of WFT, the delivery style (written verses oral) or cognitive modality (convergent, i.e. semantic/categorical verses divergent, i.e. phonemic/letter fluency) was believed to be irrelevant (B. Cohen & Stanczak, 2000). In a meta-analysis, phonemic fluency tests were found to be more sensitive than Wisconsin Card Sorting Tests (Henry & Crawford, 2004a), Trail Making Test-Part A, and Wechsler Adult Intelligence Scales (WAIS).

Fluency tests have been used in research for many years, with the first written WFT being used by Thurstone in 1938 (Alvarez & Emory, 2006; A. Benton, 1994). It could be administered independently to a large group of children, rather than 1-1 as required by the majority of other fluency tests. Children are given 5 minutes to recall words beginning with the letter S, after which they are then given four minutes to recall four letter words beginning with the letter C. Identifying '*S*' words is considered a high association and hence easier task whereas 'C' words is considered a low association more difficult task (Bolter, Long, & Wagner, 1983; B. Cohen & Stanczak, 2000).

Development and analysis of verbal fluency tests continued from the first Thurstone publication. Bousfield and Sedgewick, in 1944, developed an unconstrained free-recall task, during which participants produced as many examples as they could on some known category (Bousfield & Sedgewick, 1944). In doing so, it was noted that participants frequently grouped these items into clusters of associated items (Bousfield & Cohen, 1955). Miller, in 1964, applied the TWFT to patients with focal brain lesions (B. Cohen & Stanczak, 2000). This stimulated Benton who, although he found the procedure unsuitable at times, devised an oral version of the same procedure (the FAS Test) (Ruff, Light, Parker, & Levin, 1996). The term "controlled oral word association"

(COWA) was quickly coined by Benton, to avoid confusion between the terms "word fluency" and "fluency/non-fluent" as dimensions of aphasic speech (Ruff et al., 1996). In the 1960s, an international team (including Benton, Spreen, Renzi and Vignolo) worked on developing the COWA as part of an international multilingual asphasia test battery, which used new sets of letters (CFL and PRW) (A. Benton, 1969; Ruff et al., 1996). Different sets of letters were found to be appropriate for other languages e.g. Spanish (Ruff et al., 1996). It was then noted that generally words were produced in spurts during which the participants were not necessarily thinking about another word, but rather about another subcategory (Gruenewald & Lockhead, 1980). Analysis methods were refined with a method of measuring clustering being developed by Raskin, in 1992, which incorporated semantic and phonemic clustering (Raskin, Sliwinski, & Borod, 1992). As these are a key part of modern fluency analysis, they are explained in more depth in the following paragraphs.

Verbal fluency outputs increase between the ages of 5 and 11 (dramatically between 5 and 7) and by age 11-12 adult levels of verbal fluency are obtained (Sauzéon et al., 2004). Six year olds generally have a very low output (less than five words per minute) with 15-16 year olds achieving just under ten words per minute (Spreen & Strauss, 1998). The spurts of words (relating to a set topic) generated in a set time, began to be called temporal clusters (Abwender et al., 2001; T. Ross, 2003). These spurts are evidenced as a short time interval between words in a cluster and an even longer pause between clusters (Koren et al., 2005). Fluency involves the recollection of words within a subcategory (clustering) and then switching to a new one when the subcategory is exhausted (Kavé, Avraham, Kukulansky-Segal, & Herzberg, 2007; Kavé, Kigel, & Kochva, 2008; Troyer et al., 1997). "Clustering depends on processes such as verbal memory and verbal storage and is related to temporal lobe functioning" (Weiss et al., 2006, p. 502); it is thought to be a relatively automatic process (Strauss et al., 2006). Clusters include two or more consecutive words that begin with the same two letters, e.g. shine and shake, differ only by a vowel sound, e.g. sick and sack, or are homonyms, e.g. sum and some (Abwender et al., 2001; T. Ross, 2003). "Switches" are the transitions between clusters, including single words. Switching is often seen as an executive function involving cognitive flexibility in shifting from one subcategory to another, and is thought to involve a relatively effortful process, involving the frontal lobe (Kavé et al., 2007; Kavé et al., 2008; Koren et al., 2005; Strauss et al., 2006;

Troyer, Moscovitch, Winocur, Leach, & Freedman, 1998). It is switching, not clustering, that is decreased under conditions of divided attention (Troyer et al., 1997), with switching being seen as an important cognitive component on tests of attention (Meiran, 1996).

Troyer in 1997 refined the coding for 'switching' and 'clustering' techniques (Abwender et al., 2001; T. Ross, 2003; Troyer et al., 1997) as well as developing 'cluster ratio scores' which reflected the percentage of the total words generated that were clusters (Abwender et al., 2001). She also did not allow 'task-discrepant clustering', where semantic clusters were counted in phonetic fluency tests. In doing so, category confusion with such words as *slip* and *slap* was eliminated. Task-discrepant clustering is believed to be more indicative of deliberate strategy utilisation, compared with 'task-consistent clustering' (utilising only phonemic clusters in a phonemic fluency test) (Abwender et al., 2001). Troyer did, however, allow the cluster size to be variable, depending on the number of words contained within that cluster, rather than considering each pair of related consecutive words as a single cluster (Abwender et al., 2001). Troyer's analysis technique is now commonly used with neuropsychological tests, "however research on switching and clustering among children is relatively scarce" (Kavé et al., 2008, p. 350). These strategies are constantly being analysed and refined. Ross (2003) has stated that additional retrieval strategies need to be considered and has suggested these to include antonyms e.g. *love-loath* as well as words that are commonly used together e.g. *foreign-film.*

Optimal fluency requires a balance between clustering and switching (Robert et al., 1998). The number of switches and clusters reflects strategic retrieval processes associated with frontal lobe activity, whereas the size of the clusters measures the extent of lexico-semantic knowledge associated with temporal lobe activity (Abwender et al., 2001; Sauzéon et al., 2004). Troyer (2000) noted that the mean cluster sizes were larger when people were asked to identify words beginning with C compared with those who were asked for words beginning with S. Changes in cluster sizes and frequency of switching has been shown to change with age and gender. In a study by Sauzéon et al. (2004) it was found that cluster sizes decreased with age until age 11-12, whereas switching increased with age. Weiss et al. (2006) found that switching was highly correlated with the total number of words generated, and that women switched more

often between categories, while men produced larger cluster sizes and generated a lower total number of words.

The effects of gender, age and years of education on fluency ability have varied. Although little verbal fluency research has been undertaken with children (Kramer, Delis, Kaplan, O'Donnell, & Prifitera, 1997) looking at research involving adults may give us an indication of what to expect.

The influence of gender on all aspects of verbal fluency is not clear (Mathuranath et al., 2003). Numerous studies have reported that women outperform men in fluency tests (Codorniu-Raga & Vigil-Colet, 2003; Herlitz, Airaksinen, & Nordström, 1999; Herlitz, Nilsson, & Bäckman, 1997; Johnson-Selfridge, Zalewski, & Aboudarham, 1998; T. Ross, 2003; Weiss et al., 2006), although other authors have found little evidence of this (Brickman et al., 2005; Harrison, Buxton, Husain, & Wise, 2000; Kempler, Teng, Dick, Taussig, & Davis, 1998; Spreen & Strauss, 1998; Troyer et al., 1997).

One early research study stated that sex differences in verbal learning were minimal or non-existent during the primary school years, emerging only after puberty when the hormonal and psychosocial influences increased (Maccoby & Jacklin, 1978). These findings were contrasted by two studies involving children, which showed 5-16 year old girls outperform their male counterparts (Codorniu-Raga & Vigil-Colet, 2003; Kramer et al., 1997).

Explanations for these gender differences have been neuro-anatomically attributed to less lateralised hemispheric specialization, increased oestrogen levels (pre-pubertal girls have oestrogen levels eight times higher than pre-pubertal boys) and decreased testosterone levels (negatively correlated with verbal fluency in elderly men) (Koren et al., 2005). Other studies attribute girls as having better verbal skills, increased vocabularies and less developmental reading disabilities (Herlitz et al., 1999; Kramer et al., 1997; S. Lanting et al., 2009; Lezak, 1995; Weiss et al., 2006). One study found that the cerebellum was at full adult size for girls aged 7-11, but not for boys (Caviness et al., 1996). Cohen and Stanczak's (2000) results indicated that females produced significantly more *S* words but not *C* words.

Correlations between age and word fluency have been varied (Bryan & Luszcz, 2000; Bryan et al., 1997; Johnson-Selfridge et al., 1998). Herlitz et al. (1997), Pendleton,

Heaton, Lehman, and Hulihan (1982) and Schaie and Strother (1968), reported correlations between age and word fluency, whereas Cauthen (1978) as well as Mittenberg, Seidenberg, O'Leary, and DiGiulio (1989) found no significant correlation. It is important to note that all of these studies involved adult participants. Fluency performance improves during childhood "with a dramatic increase between ages five and seven years" (Strauss et al., 2006, p. 503) and stabilises around the age of 11-12 (Sauzéon et al., 2004). Sauzéon and colleagues found that age modified both the number of switches and clusters formed (2004). With a more extensive vocabulary, more words are generated before a phonemic subcategory is exhausted. Troyer (1997) on the other hand found differences in ages minimal.

As fluency tests have been shown to be effective measures of executive function the following paragraphs will outline which regions of the brain are activated when undertaking aspects of these tasks. The left prefrontal cortex has been consistently linked with verbal fluency test performance (Janowsky, Shimamura, Kritchevsky, & Squire, 1989; Parks et al., 1988) and verbal fluency activities readily activate the dorsolateral prefrontal regions involved in language processing (Gaillard et al., 2003; Kavé, Kukulansky-Segal, Avraham, Herzberg, & Landa, 2010; Pihlajamäki et al., 2000).

Generally people with frontal lobe damage demonstrate poor phonemic fluency, but 'normal' semantic fluency (Troyer, Moscovitch, Winocur, Alexander, & Stuss, 1998), possibly because words are not likely to be organised in the lexicon on the basis of the initial letter (Bryan & Luszcz, 2000). This conclusion also supports 1994 findings where a finger tapping study was shown to disrupt letter fluency, whereas an object decision task disrupted semantic fluency (Martin, Wiggs, Lalonde, & Mack, 1994). In a meta-analysis, findings showed that phonemic verbal fluency activated not only the frontal lobes but also the thalamus, parietal lobes and temporal lobes (Alvarez & Emory, 2006). An in-depth study using functional MRI scans showed that certain semantic categories engaged frontal/strategic search processes if they were broader and required frequent switching between subcategories, for example the notion of the "supermarket" required switching between subcategories such as dairy products, meats, fruit etc.(Baldo et al., 2006). When WM mechanisms were involved for example to hold in mind the task rules, maintain set and avoid repetitions, the parietal regions as well as the frontal and temporal cortex were involved (Baldo & Dronkers, 2006).

In a comprehensive compendium analysing neuropsychological tests, verbal fluency tests were seen to be well established with a "venerable past". It was noted that they were quick and simple to administer, and did not require any test material, except a timer, and, unlike many other tests, verbal fluency tests did not have low ceilings. Test-retest reliability of the total scores was also stated as high and verbal fluency tests were seen as appropriate for use when tracking changes (Strauss et al., 2006). Over a six-week period, Cohen and Stanczak (2000) reported a reliability of 0.79 for 70 adult students (mean age 20.6±4.36 years) when using written word fluency tests (Thurstone version) with no practice effects.

In a study of over 400 adults, significant effects of ethnicity on fluency scores were found even when income, education and reading scores were taken into account (Johnson-Selfridge et al., 1998). In a study involving 317 adults from five different ethnic groups, a significant effect of ethnicity independent of age and education was found (Kempler et al., 1998). Several studies have suggested that linguistic factors, rather than cognitive ability, may influence neuropsychological test performances (Kempler et al., 1998). These factors include such things as how common words beginning with S and C are in any given language and the proportion of mono-syllabic or multi-syllabic words in any language (Kempler et al., 1998). Longer words take more time to retrieve from memory and are less successfully stored and manipulated (Baddeley, 1990). As the letter C is not found in Arabic and many Pacific Island languages, including the Samoan and Maori alphabets, this could mean many non-European children have more difficulty thinking of these words and, therefore, less would be identified. An increase of one or two words, when only a few have been identified, would be more significant than with a large list generated by the letter S.

This section has highlighted the validity of verbal fluency tests to measure cognitive ability. Although numerous ω-3 PUFA studies utilising fluency tests have occurred with middle aged and elderly participants, far less have been undertaken with children and no study was found to have demonstrated any effects of ω-3 on fluency. The tests utilised included the Peabody Picture Vocabulary Test (PPVT) (Ryan & Nelson, 2008), NEPSY (F & S) (Muthayya et al., 2009), WISC-III (Kairaluoma et al., 2008) and the second edition of the Wechsler Individual Achievement Test (WIAT-II) (Kirby et al., 2010b). More detailed information of these selected studies can be found in Appendix D2.

Verbal fluency is reflected in academic achievement but is not a true measure of it. Few studies have assessed academic ability as opposed to cognitive ability (Fergusson, Beautrais, & Silva, 1982; Horwood & Fergusson, 1998; Rogan & Gladen, 1993). The following section outlines ω-3 PUFA research which has focussed on academic ability.

Academic Achievement

Spelling

Contrary to the view of spelling as a rote memorisation skill, it actually requires active consideration of the sounds, patterns and meaning of written language (Masterson & Apel, 2010). In order to support and assess spelling, traditionally teachers choose spelling lists from commercially available material, basal readers or other books read by students. Alternatively sometimes the selected wordlist represents high frequency words, words particular to a theme or focus of the week or words that can be grouped based on a specific orthographic feature (Masterson & Apel, 2010). Factors which affect spelling vary across developmental levels and "assessment instruments must contain words that tap into these factors for every developmental level it is supposed to test (Calhoon, Greenberg, & Hunter, 2010, p. 166). When selecting an appropriate test enough items to reach a basal and/or ceiling must be provided, but not so many as to cause frustration for the participant. Research has shown that spelling cannot be adequately assessed with only one standardised test. Different tests target different orthographic patterns. For this reason "results indicate that caution is needed when making diagnostic or research decisions based on scores received on an individual spelling test" (Calhoon et al., 2010, p. 169).

Children who are learning to spell rely on their mental resources to encode words. It is believed they have fewer choices of efficient spelling strategies (Steffler et al., 1998). As the children get older, they become more flexible at choosing an appropriate spelling strategy, until familiar words eventually are spelt automatically using very little cognitive energy (Steffler et al., 1998). In a Canadian study of 93 children between the ages of 7 ½ and 11 years old, 52% of spelling errors came from the 7-8 year olds with only 5% coming from the 10-11 year olds, with the younger children being slower to generate the words (Steffler et al., 1998).

Few ω-3 PUFA studies have utilised spelling tests. Two observational studies identified improvements in spelling linked with higher ω-3 PUFA intakes. One of these studies involved dyslexic adults where it was noted that spelling was correlated with total ω-3 RBC concentrations (Cyhlarova et al., 2007). The other involved 8-12 year-olds who were also dyslexic. In this study the males with higher fatty acid deficiency symptoms (FADS) showed a significantly lower spelling ability. Female levels did not show significant differences however a trend was evident for spelling but in the opposite direction to that of the boys (Richardson et al., 2000). Contrasting this, an intervention study involving dyslexic children found no significant difference in spelling after 90 days of supplementation with EPA. This spelling test required the 9-11 year olds to write 20 common words of 2-3 syllables from dictation as well as undertake a spelling subtest of *Lukilasse* (a national Finish test), without time pressure (Kairaluoma, Närhi, Ahonen, Westerholm, & Aro, 2009).

All six intervention studies involving ω-3 PUFA have involved testing children's spelling ability (Dalton, Smuts, Witthuhn, Wolmarans, & Benade, 2005; Kairaluoma et al., 2008; Kirby et al., 2010b; Milte et al., 2012; NEMO Study Group, 2007; Richardson & Montgomery, 2005).

Two of these studies found significant improvements in spelling ability of those children receiving ω-3 PUFA supplementation (Dalton et al., 2009; Richardson & Montgomery, 2005). Whilst another study found a significant association between increased RBC DHA and improved spelling but only in a small subgroup of 17 children with reading difficulties (Milte et al., 2012).

The only study to identify links between spelling ability in mainstream children and ω-3 PUFA supplementation was a six month South African study involving 7-9 year olds. This utilised a spelling test requiring a word to be read to the child, singly and within the context of a sentence. Rather than an improvement in spelling results being recorded, the control group showed a decline in scores which was not replicated in the intervention group. An alternative conclusion could be drawn from these results, that being that no improvement may have occurred with the ω-3 PUFA group and the placebo may have negatively affected the children's spelling ability. This would support the study's findings that there was a marginal inverse correlation between ω-6 and spelling (Dalton et al., 2009).

The Durham study involving 5-12 year olds with DCD utilised the Wechsler Objective Reading Dimensions to identify significant spelling improvements with ω-3 PUFA supplementation over a period of 3 months (Richardson & Montgomery, 2005). The Wechsler Individual Achievement Test- third edition (WIAT-III) was utilised with 90 7-12 year old Australians with ADHD. After four months no treatment effect was identified between groups however an association was made between DHA RBC levels and spelling improvements in a subgroup with reading difficulties as stated above (Milte et al., 2012). The WIAT-III was also utilised in another Australian study (NEMO Study Group, 2007), whilst the earlier second edition version of the WIAT-II test was used in a Welsh ω-3 PUFA study (Kirby et al., 2010b). No significant findings were detected between groups in either of these two studies.

In summary of the above studies which investigated the influence of ω-3 PUFA on children's spelling ability, significant improvements in spelling were identified in dyslexic males (Richardson et al., 2000), children with DCD (Richardson & Montgomery, 2005) and a subgroup of children with ADHD and learning difficulties (Milte et al., 2012), but not in mainstream children (Kairaluoma et al., 2008; Kirby et al., 2010b; NEMO Study Group, 2007).

(Kairaluoma et al., 2008; Kirby et al., 2010b; Milte et al., 2012; NEMO Study Group, 2007). One ω-3 PUFA study involving mainstream children did not find improvements related to increased ω-3 intake but rather found a lack of regression of spelling ability (Dalton et al., 2009). These findings highlight the need for additional research involving spelling and large sample sizes of mainstream children, where subgroup analysis can also be undertaken.

Literacy

Many of the above studies which investigated spelling used the same assessment battery and also investigated aspects of reading. Supplementation with ω-3 PUFA resulted in improvements in reading age in children with DCD (Richardson & Montgomery, 2005), dyslexic males (Richardson et al., 2000) and children with reading difficulties (Richardson et al., 2012). It also has been linked with improvements in word reading in subgroups of children with ADHD and learning difficulties (Milte et al., 2012), but not in mainstream children (Kairaluoma et al., 2008; Kirby et al., 2010b; Milte et al., 2012; NEMO Study Group, 2007; Richardson et al., 2012).

In a South African study involving mainstream children a unique test was utilised. This test involved children being shown a simple illustration and being asked to identify the words or short answers, from list of multi-choice items that pertained to the illustration. The correct answers were tallied to obtain a raw score. This new test identified a marginally significant improvement in reading with ω-3 PUFA intake (Dalton et al., 2009).

The above sections have outlined studies involving ω-3 PUFA supplementation. The majority of normative data relate to American and British samples (Halperin, Healey, Zeitchik, Ludman, & Weinstein, 1989; Kirk, 1992; Riva et al., 2000) and, therefore, may not be appropriate for other cultures. For this reason, other countries are now undertaking similar research to develop normative values (Riva et al., 2000). It is unknown whether NZ children undertaking these tests would be disadvantaged.

NZ teachers utilise a wide range of tests to investigate children's literacy learning and development. Although well-researched and validated in their ability to identify changes to literacy ability these tests have not previously been used in any NZ ω-3 PUFA studies. The following section outlines some of the tests available to NZ teachers. This is followed by a detail of one of the most commonly used NZ reading tests.

New Zealand teachers are able to select from a wide range of literacy and numeracy tests (e.g. PAT, STAR, BURT) to assess children's progress and academic achievement. Some of these are developed within NZ and others have been brought from other countries, such as the Peters (UK) and Schonell (Australian test derived from identifying adult spelling errors) spelling tests (Peters, 1975; Schonell, 2003). Many of the teachers, however, do not rely on the accuracy of these imported tests with only 22% of NZ Year 5 teachers using the Peters test and 34% using the Schonell spelling tests (Dunn & Marston, 2003). In 2005, two of the main NZ generated reading tests (STAR and asTTle) were evaluated. The results showed that STAR demonstrated unreliability of the difference score and had a ceiling effect, whilst asTTle (**AS**sessment **T**ools for **T**eaching and **LE**arning) (Ministry of Education, 2005) results exhibited neither of these (Parr, Timperley, Reddish, Jesson, & Adams, 2007, p. 63). The following sections will outline two of the NZ national tests used for assessment of academic ability by the majority of NZ primary schools.

asTTle Reading

This is a curriculum-based, teacher-managed, voluntary-use educational resource" (Hughes & Bryan, 2003, p. 420) and is designed with reference to multi-literacy and multimodality approaches (Cope & Kalantzis, 2000) consistent with the NZ curriculum (Ministry of Education, 2005a).

Literacy (reading and writing) and numeracy form the basis of asTTle tests. Responses are recorded on surface features (grammar, punctuation and spelling) and curriculum processes (processing information, exploring language and thinking critically). In order for a teacher to see change and growth within one level, sub-levels are developed that track these features. These sub-levels are labelled: basic (items that require partial mastery of knowledge and skills that are fundamental for proficient work at the level), proficient (items that demonstrate applications of the knowledge and skills of the given level) and advanced (items that are difficult applications of the knowledge and skills at this level).

Structure of Observed Learning Outcomes (SOLO) cognitive processing taxonomy (Hattie et al., 2004, p. 1 Introduction) has been utilised in asTTle to categorise student performance on the reading tasks. Students' depth of thinking (surface and deep), are compared to all students in the reference group. Each level increases the demand on the amount of WM or attention span needed (Hattie & Brown, 2004). The 'surface' category includes uni-structural and multi-structural stages where-as the 'deep' category includes relational and extended stages. Detail of these stages are outlined in Hattie and Brown (2004) and Ministry of Education (2005b).

AsTTle enables a teacher to specify curriculum content and difficulty. It provides a one-off snapshot of student achievement, and should be triangulated with other information such as observations, class work or other diagnostic tools (Biggs & Collis, 1982). The content options are identified in the asTTle curriculum maps; for reading these are *Finding Information, Knowledge, Understanding, Connections, Inference and Surface Features.* Reading curriculum achievement objectives used in asTTle have been identified in Appendix B4. The proportion of each desired content area can be adjusted (i.e. very few/none, few, some, many or most). By selecting '*a few*', around 10% or between 3 and 5 questions in the test will be placed in that content area. The level of difficulty is also able to be determined. As all asTTle tests are calibrated to curriculum

levels 2 to 6, it does not matter what content is in each test; the overall scores are on the same scale. AsTTle benchmarks the performance of the over 90,000 students asTTle has tested. This is achieved using the Item Response Theory (IRT) mathematical model (Hambleton, Swaminathan, & Rogers, 1991). “Because of the power of the IRT, the scores derived from these tests are comparable - even though students sat tests that contained different items at different times of the year” (Ministry of Education, 2005b, pp. chapter 3-25).

AsTTle scores can be used to track growth by comparing a student’s learning history with the norms (based on the results of over 100,000 children) underlying asTTle (Ministry of Education, 2008). The average growth per year is generally 25 to 50 points. The more items a student completes within the range of appropriate difficulty the more robust the score estimation for the student will be. A student must make a genuine attempt on at least 25 questions for the asTTle score estimation to be reasonably accurate. The “standard error of measurement has been calculated as 15 scale points, creating a 65% confidence interval around the reported score” (Hattie et al., 2004, pp. 25, Chapter three). “The average gain for primary school students using asTTle assessments is just over one sub-level every year... scores that are within 15 points of each other are not different by any meaningful statistical amount ” (Hattie et al., 2004, pp. 27, Chapter three).

Numeracy

As stated above, literacy and numeracy abilities or disabilities have often been shown to be linked. As both have been shown to be complex tasks involving executive functions it is a logical assumption that they could be influenced by supplementation with ω-3. Links are further emphasized as “roughly 20-25% of children with ADHD show one or more specific learning disability in Math, reading, or spelling” (Burgess, Stevens, Zhang, & Peck, 2000, p.327S). The inferior frontal cortex has been shown to be activated in arithmetic studies and may also be a region subserving WM and cognitive control functions (Baldo & Dronkers, 2007, p. 233). The majority of research studies have focused on tests devoted to cognitive ability, with only a handful of studies focussing on investigating links between ω-3 PUFA intake and mathematical ability or achievement.

Cognitive and visual differences recorded between children who have been formula fed and those who have been breastfeed are often attributed to the DHA in the mother's breast milk (C. Lanting & Boersma, 1996; Morley, 1996). In a NZ study teacher ratings (on a 1-5 scale) for 8 year olds showed a significant association between national test results for mathematical achievement and duration of breastfeeding, even when socio-economic and maternal education were considered. When these children were 11 years old a significant association was still found between mathematics scores and the duration of breastfeeding for the child, even when socio-economic factors, and maternal education were considered (Horwood & Fergusson, 1998).

Similar associations were found in a 1996 American study, which investigated links between ω-3 PUFA intake and boys' academic achievement (including maths). Amongst the numerous measures utilised in this study, teachers were required to evaluate the learning ability of each participant compared with his peers in reading, maths, handwriting and overall academic ability on a 1 to 5 scale. Those children with a lower ω-3 PUFA intake were recorded by teachers to have lower maths attainment scores compared with their peers (Stevens et al., 1996).

Only two intervention studies were found to have investigated links between children's mathematical ability and ω-3 PUFA intake. These double-blind placebo controlled studies investigated the effects of ω-3 PUFA supplementation on mathematical ability involved a RMAT screening test (Kairaluoma et al., 2008) and the WAIT screener test (NEMO Study Group, 2007). Both of these studies found no changes to children's mathematical ability linked with ω-3 PUFA intake. Additional research is needed to determine whether these results reflect that ω-3 PUFA does not influence mathematical ability or whether a test has yet to be found which is sensitive enough to detect changes attributed to the influence of ω-3 PUFA supplementation.

NZ Numeracy

In the last two decades, NZ has undertaken extensive research investigating children's numeracy, resulting in the development of the Numeracy Development Project. Children can be tested on numerous aspects of numeracy including understanding and recall of basic facts (Ministry of Education, 2008a). The Numeracy Development Project has identified there is often a mismatch between student's knowledge and the strategies they are proficient at using. They may have more knowledge than they are

able to use, or possess powerful strategies but lack the knowledge to apply them to more difficult numbers or situations. The NZ "Number Framework emphasises that the process of deriving number facts using mental strategies is important in coming to know and apply these facts. It also demands that students come to know a broader range of facts than previously, including groupings of "benchmark" numbers, and that they have knowledge of factors of numbers and decimal and fraction conversions at the higher stages" (Ministry of Education, 2008a, p. 14). The numeracy project provides specific tests and testing procedures at each level enabling teachers to compare results across classes and schools. These tests are routinely used in NZ classrooms to assess changes to a child's specific mathematical ability yet have not yet been trialled in intervention studies.

Behaviour/attitude evaluations

Behaviour is influenced by many things such as the home and school environment (B. Taylor & Wadsworth, 1984). Learning is a social act, and it is believed that learning with others is usually more effective than learning alone (Goswami, 2008). The following section outlines the wider implications of supplementation on areas such as behaviour, attitude and classroom dynamics.

Language and communication are seen as being central to this social process (Goswami, 2008). Language provides both a symbolic system for communication as well as enabling children to reflect upon and change their own cognitive functioning (by developing 'metacognitive' and 'executive functions' skills). This development requires an interaction between trustworthy and benevolent sources of shared cultural knowledge (Gergely, Egyed, & Király, 2006). With behaviour being critical to learning and the learning environment, any modification to even one child's behaviour can have a profound impact on the classroom dynamics and learning of others (Dishion & Dodge, 2005). Many famous people have talked about having expectations and meeting them, whether they are set high or low (Alexander Pope, Lord Byron, Benjamin Franklin, Earl Nightingale, Ralph Marston, Charles Kettering, John Walcot, Brian Tracy, Michael Jordan, Jack Nicklaus). The Thomas theorem relates to the notion of the self-fulfilling prophecy where if people define a situation as real, then the consequence will be that the situation becomes reality (W. Thomas & Thomas, 1928). When parents,

teachers and children alter their expectations (either raise them or lower them) these often can become reality (as explained later in this section).

In a NZ study of 4644 participants over the age of 15 years it was shown that personal perception of good mental and physical health was linked with fish consumption (Silvers & Scott, 2002). Numerous studies have researched links between ω-3 PUFA and changes in behaviour (Brue, Oakland, & Evans, 2001; Richardson & Montgomery, 2005; Richardson & Puri, 2002; Stevens et al., 1996; Stevens et al., 2003; Vaddadi, 2006; Voigt et al., 2001). These changes may be attitudinal or may be visible in changes with relationships or play and study habits. Investigating these changes is very problematic as the majority of measures are subjective (Sinn et al., 2008).

Many teachers and parents are adamant they are able to detect changes in behaviour well before they are able to be proven using standardised tests. Numerous studies have found that parents' responses can be "just as accurate as developmental-behavioral screening tests in detecting children with disabilities" (Dewey, Crawford, & Kaplan, 2003, p. 87). Parent-completed behaviour rating scales have been found to be effective when used to diagnose children's attention problems (Aman et al., 1987; Barkley, 2006), ADHD symptoms (including those undiagnosed) (Mulhern, Dworkin, & Bernstein, 1994), DCD (Richardson & Montgomery, 2005), psychosomatic problems such as being shy, anxious, restless and impulsive (Joshi et al., 2006; Richardson & Montgomery, 2005; Richardson & Puri, 2002) oppositional defiant behaviour and conduct (Richardson & Montgomery, 2005; Stevens et al., 2003) and autism (Amminger et al., 2007). Teachers have also demonstrated the ability to detect behavioural changes (Arnold et al., 1989; Hirayama et al., 2004; Kirby, Woodward, Jackson, Wang, & Crawford, 2010a; Richardson & Montgomery, 2005; Stevens et al., 2003).

Commonly used standardised tests (CPRS, CASQ, SDQ, SNAP) have been used in numerous international ω-3 PUFA supplementation studies to investigate parents' perceived changes to conduct (Kirby et al., 2010b; Stevens et al., 2003), impulsive behaviour (Gustafsson et al., 2010), oppositional behaviour, mood swings and restlessness behaviour (Bélanger et al., 2009; Joshi et al., 2006; Milte et al., 2012; Richardson et al., 2012; Richardson & Montgomery, 2005; Richardson & Puri, 2002; Sinn & Bryan, 2007; Voigt et al., 2001) and peer relationships and pro-social behaviour

(Kirby et al., 2010b). Other studies have used questionnaires to investigate parents' perceptions about changes to children's aggression (K. Hamazaki et al., 2008; Hirayama et al., 2004; Itomura et al., 2005; Joshi et al., 2006; Kairaluoma et al., 2008) linked with ω-3 PUFA supplementation.

Thirteen international studies have been undertaken to investigate parents' views of the influence of ω-3 PUFA supplementation on their child's behaviour. Ten of these involved ADHD children and one involved dyslexic children (Kairaluoma et al., 2008). Seven used Connors Parent Rating Scales (Bélanger et al., 2009; Gustafsson et al., 2010; Milte et al., 2012; Richardson et al., 2012; Richardson & Puri, 2002; Sinn, 2007; Voigt et al., 2001), one the Connors Abbreviated Symptom Questionnaire (Stevens et al., 2003) and another one using the Swanson, Nolan and Pelham rating scale for ADHD (Kirby et al., 2010b). The remaining studies utilised questionnaires (Hirayama et al., 2004; Itomura et al., 2005; Joshi et al., 2006; Kairaluoma et al., 2008; Lindmark & Clough, 2007). Parents in half of these studies identified a significant improvement in some aspect of their child's behaviour compared to those with children in the placebo groups (Itomura et al., 2005; Joshi et al., 2006; Richardson et al., 2012; Richardson & Puri, 2002; Sinn & Bryan, 2007; Stevens et al., 2003).

Only two studies have involved parents of mainstream children and both of these studies utilised tests designed to investigate ADHD symptoms (Kirby et al., 2010b; Richardson et al., 2012). The study which linked parents' detection of changes in their child's behaviour and ω-3 supplementation didn't involve a 'normally-achieving' mainstream group but rather children underperforming in reading ($\leq 33^{rd}$ centile) (Richardson et al., 2012). The risk of misinterpreting subgroup analyses due to the limited power of the interaction test in trials designed to detect overall treatment effects (Brookes et al., 2004) also needs to be considered when reviewing such findings.

Occasionally teachers have been asked if they have noticed changes in children's behaviour (Gustafsson et al., 2010; Itomura et al., 2005; Kirby et al., 2010b; Richardson et al., 2012; Richardson & Montgomery, 2005; Sinn, 2007; Stevens et al., 2003). Gustafsson et al., (2010) used a similar methodology to Richardson and Montgomery (2005) and teachers in both studies were able to detect improvements in behaviour for those children in the ω-3 group. However the children in Gustafsson et al.'s (2010) study had ADHD whilst those in Richardson and Montgomery's (2005) study were

children with DCD. Teachers but not parents were shown to detect changes in children's behaviour using Connors Rating Scales (Gustafsson et al., 2010) whilst in another study the reverse was found using the same Connors scales (Richardson et al., 2012).

One study has identified an association between children's ability to detect changes in their own behaviour and ω-3 supplementation (Lindmark & Clough, 2007) however this was not a placebo-controlled trial. On rare occasions children in ω-3 PUFA intervention studies are asked if they have noticed changes in their behaviour. Intervention studies which have investigated changes to mainstream children's behaviour have used a hostility-aggression questionnaire (K. Hamazaki et al., 2008; Itomura et al., 2005), the Barratt Impulsiveness Scale (K. Hamazaki et al., 2008) and children's mood via an internet battery (D. Kennedy et al., 2009). Studies which involved children with ADHD (Voigt et al., 2001) and dyslexia (Kairaluoma et al., 2008; Lindmark & Clough, 2007) used questionnaires or interviews. Significant changes in behaviour attributed to treatment were only found with studies involving mainstream children (Itomura et al., 2005; D. Kennedy et al., 2009). In the Itomura et al. (2005) study the girls in the placebo group felt they were more aggressive, whilst there was no change in the ω-3 group. Supplementation for this study was via consumption of bread and sausages which contained ω-3 PUFA. Although parents were asked not to heat or toast the bread, many parents did. Although the compliance was excellent the quality of the blind was not high due to the fishy smell of the heated food. In Kennedy et al.'s 2009 research the two treatment groups which received DHA (400 or 100mg/d) showed a significant increase in 'relaxation' ratings. There were however significant differences between the active and placebo groups at baseline prior to any intervention, which led these researchers to refute the validity of the significant behavioural findings.

Occasionally studies ask those involved to predict whether they (or their child or student) are in the active or control/placebo group. In a 2009 study, 6 out of 7 parent-couples and only 3 out of 7 of these children made a correct prediction (Raz, Carasso, & Yehuda, 2009). In a 2006 study "no group differences regarding participants' ability to guess their group allocation" was found (Frangou, Lewis, & McCrone, 2006, p.48). In another study when "patients were asked to guess their randomization status, 86% of the omega-3 group guessed correctly, compared with 63% of the placebo group. Although in some cases the guess was based on the presence of a fishy aftertaste, in many cases

"it was based on the patient's perceived clinical response (or lack thereof in the placebo group)" (Stoll et al.,1999, p.411). Adding flavouring agents has been suggested to improve the validity of double-blind trials (Su, Shen, Huang, Stoll, Damico, Marangell, & Severus, 2000).

These predictions/guesses are commonly used to test the notion of the study being blind to all participants. This also supports the notion that people can perceive a difference but may not be able to say specifically what it can be attributed to. They just have a 'gut feeling'. These feelings are very real and often very valid (Office on Child Abuse and Neglect, 2003). Some acknowledge these feelings as being a vital part of the job, for example journalists (Schultz, 2007), nurses (Stern, 1983) counselors (King, 1983) entrepreneurs (Cave & Minty, 2004) and police (Linfoot, 2008) whilst others such as many educators promote a more evidenced based approach (Mann, Holmes, Hayes, Burge, & Viscount, 2001; Osterman & Kottkamp, 1993; R. Smith, 2006).. Doctors and surgeons are becoming aware of the validity of intuitive knowledge (gut feeling) (Hartley & Sagar, 1994; Van den Bruel, Thompson, Buntinx, & Mant, 2012). Research has shown that at times students (Bulpitt & Martin, 2005) do have an accurate feeling of their own abilities as do their parents (Olson, Logan, & Lindsey, 1989).

It is important to consider the 'Hawthorne effect'. This is where people change their behaviour as a result knowing they are part of a study rather than as a consequence of the study. In ω-3 PUFA intervention trials it would be very easy to assume that any significant findings were the result of ω-3 PUFA. Yet an alternative influence could be the expectation of participants (children, parents or teachers), or that simply participating in the research changes the participants. Little is known about how people can influence participant's expectations about treatment (Kalauokalani, Cherkin, Sherman, Koepsell, & Deyo, 2001) however research has shown that expectation levels of the participants (Kalauokalani et al., 2001; Mondloch, Cole, & Frank, 2001) and those of their parents (Spafford, Von Baeyer, & Hicks, 2002) can influence the end result. Parents expectations have been linked with increased children's school attainment scores (Ma, 2001) and improved behaviour (Anthony et al., 2005).

The Pygmalion effect is sometimes used to refer to children showing improvement due to teachers' raised expectation levels (Rosenthal & Jacobson, 1968). This has been

shown to occur not just on the individual child but also with the whole class (Rubie-Davies, 2010; Rubie-Davies, Hattie, Townsend, & Hamilton, 2007).

People have been shown to change as a result of their belief that something could help them (whether or not their actually received it) (Beecher, 1955; Shapiro, 1960). Relying on people's feelings and beliefs has been very controversial for centuries. The term 'placebo' has been used for centuries, with the ecclesiastical term indicating a promise to please the Lord in medieval prayer. In the second half of the 18th century the word placebo became an integral part of the medical-pharmacological vocabulary (Gensini, Conti, & Conti, 2005). Gaddam in the mid 1950's referred to placebos as 'dummy pills', yet also stated that they could be effective in 40% of cases (Gaddum, 1954 as cited in Beecher, 1955). Others have written about these as effective therapeutic tools (Gensini et al., 2005). "In some circumstances a placebo is no better than nothing at all; in others it is apparently more effective than the drug under trial; sometimes it seems actually to work in the opposite direction" (Parkhouse, 1964, p. 69). The placebo effect/response directly involves the participant's expectations (Shapiro, 1960) and accounts for approximately a third of responses (Beecher, 1955; H. Cho, Hotopf, & Wessely, 2005; Walsh, Seidman, Sysko, & Gould, 2002).

Houston (1938, as cited in Shapiro, 1960, p. 114) likens the pages of medical history to the log of an old-fashioned ocean voyage, in which it was noted that on a set day a whale spouted or a flying fish was sighted but never mentioned that what was constantly seen almost exclusively was the vast expanse of water (the placebo effect). The above paragraphs highlight the need to focus on more than just linking key results to an intervention or treatment (whether or not they answer the research questions). The influence of expectations, beliefs and behaviours has been shown in many studies to influence results and these need to be taken into consideration where ever possible.

Summary of the above literature related to ω-3 PUFA

Dietary fats play an important role in the healthy functioning of our bodies. The human body is unable to produce ω-6 and ω-3 PUFA and these must be gained from the diet. EPA and DHA are mainly derived from oily fish and seafood, AA from meat and eggs and ALA and most ω-6 tend to be derived from nuts, seed and vegetable oils. LA is converted to AA and ALA converted to DHA through a cascade of metabolic processes. This conversion process involves competition for enzymes. It is believed that this

competition may limit the amount of the alternate PUFAs able to be metabolised. An increase in ω-6 PUFA consumption may alter the availability of DHA and EPA in the body. Over the centuries industrialisation has enabled people to use and consume large amounts of vegetable oils which are high in ω-6 PUFA. This has greatly increased the intake ratio of ω-6: ω-3 PUFA. Many believe this ratio is critical to neurological and cognitive development. Others believe intake levels of ω-6 or ω-3 PUFA or both are key. Considerable research has been undertaken to investigate the effects of supplementation with EPA, DHA, ALA, GLA and assorted vitamins and micronutrients on neurological and cognitive development. Associations have been found between increased ω-3 PUFA levels and improvements in behaviour and cognitive ability.

The majority of research has involved people with a neurological or behavioural impairment (brain lesions, Alzheimer's, schizophrenia, ADHD, dyslexia, autism etc.). Individuals differ in their ability to convert EFAs to LC-PUFAs and it is believed that an inefficient conversion may be partially responsible for learning and behaviour problems such as ADHD. Children with ADHD are common in NZ classrooms. Supporting these learners has proven very problematic however supplementation with at least 480mg/d DHA or 400mg/d EPA or ALA for at least three months has been linked with significant improvements in children's anxiety, attention and/or behaviour.

Selecting appropriate assessment measures is critical to the success of the research study. Culture, language, time, cost, experience, materials, conditions, and domain being measured are some of the many aspects which need to be considered before selecting an appropriate assessment tool. Research has shown that each of these variables can impact markedly on results, with some being shown to be culture specific. It is still unclear whether international tests can be successfully applied to intervention studies involving NZ multicultural children.

In the past fluency tests have been used by over 50% of neuropsychologists. They have commonly been used to investigate executive function and have successfully been used in ω-3 intervention studies with middle-aged and elderly participants. It is only in the last decade they have been used in childhood ω-3 PUFA research; however none of these studies has detected significant findings. In the publication of this research, the majority of studies have not included the detail of fluency analysis, possibly because the tests were one of many in a 'test battery' undertaken by the children. It appears

however, that all of these studies have used traditional analysis techniques rather than the more current clustering and switching. Due to the number of tests in these batteries many researchers have identified that significant findings may simply be due to Type 1 error. The inconsistencies of duration, dosage and age groups in these studies could have contributed to the inconsistent results.

Few ω-3PUFA intervention studies have focused on academic achievement. In order to evaluate literacy, tests need to be calibrated to determine the appropriate level and content material. One NZ national test (asTTle) that has been designed in the last decade provides the ability to adjust the complexity of questions and allows the proportion of questions related to surface features or deep challenging questions to be altered. Results are able to be compared against an item response bank of over 100,000. Analysis has shown asTTle to be very reliable but it is yet to be utilised in an intervention study.

Numeracy and literacy are seen to be strongly linked and generally the main focus of NZ academic learning. In the last decade NZ has developed a Numeracy Development Project which encourages teachers to test children's maths ability on a regular basis in order to inform their teaching and enhance the child's learning. These NZ maths tests have not been utilised in intervention studies and results may add to the few existing ω-3 studies which have included maths testing.

Learning is a very social act and the behaviour of one person can have an influence on the learning of others and on the classroom dynamics as a whole. Any modification to behaviour can influence not only that child's learning but also the learning and behaviour of those around them. The above section has shown that changes in behaviour, attitude, cognitive ability and academic achievement can sometimes be identifiable and measured. Usually these tests are in the form of checklists and rating scales, whilst few subjective questionnaires have been used (especially involving parents, teachers and children) to detect changes in behaviour and learning.

Summary of Literature Review Chapter

Purvis (2007) has identified a number of studies whose media reports have gained widespread public attention, because of claims linking ω-3 PUFA to improvements in concentration, reading and memory Very few of these studies have focused on

typically developing healthy school-aged children and, hence, more research is needed (Bourre, Bonneal, Clement, & Dumont, 1993; Bryan et al., 2004; Kirby et al., 2010b).The results from the few studies that have involved these children, have typically used laboratory tasks (such as tests of short-term memory) and have been published in medical and nutrition journals with "surprisingly few papers published directed towards the educational research community" (Kirby et al., 2009, p. 1). There is a need for research that links any cognitive benefits of ω-3 PUFA more directly to schools and families.

This literature review has been divided into two sections, the first section was devoted to the brain, its development and how it is used to undertake literacy, mathematics and other executive functions. The second section built on the first and provided background detail to what ω-3 is, how it affects the body and more importantly the influence it may have on the brain and the tests which are used to detect this.

The first section identified the need for research involving executive functions. Brain development, working memory and executive functions all play a major role in academic achievement. Age and gender are two of the many variables which also impact on this achievement. The brain undergoes rapid growth where nutritional requirements are increased between the ages of seven and nine and again between 14 and 16. What the optimal age is to detect the effects of ω-3 PUFA supplementation has yet to be determined. Males appear to be at a disadvantage for the ALA/EPA/DHA conversion process and are more likely to suffer from ADHD and FADS. Females on the other hand may require more DHA during puberty. Debate exists whether differences between the genders can be detected on executive tasks in children under 12 years old. Whether these discrepancies are able to be detected in an ω-3 PUFA intervention study has also yet to be determined.

Retrieving facts from long term memory is believed to require less executive functioning and therefore use less frontal lobe activity. Whether supplementation with ω-3 PUFA has a greater impact on those children who are yet to or who are still acquiring facts compared with those who have them memorised is also yet to be determined. The impact of ω-3 PUFA supplementation on those children who are at the rote learning stages involved in spelling and maths basic facts as opposed to those who have the facts memorised is still unclear.

The second section has highlighted the importance of EPA and DHA on cognitive development. EPA and DHA are derived mainly from oily fish and seafood which is consumed in varying amounts depending on ethnicity, socio-economic groups etc. In a 2002 nutritional survey for NZ it was shown that fish was consumed weekly by 37% of NZ children. As this research is now ten years old its' validity should be questioned. Many international organisations recommend a minimum of two portions of fish per week (one a fatty fish) to gain the recommended ω-3 PUFA intake of 510 mg/day for 9-13 year old boys, and 410 mg/day for 9-13 year old girls. In countries where children are poorly nourished, supplementation with ω-3 PUFA has been linked with decreased school absentee rates compared to placebo groups, however minimal research has been undertaken to investigate whether the attendance rate can be influenced by ω-3 PUFA supplementation in well-nourished children.

The influence of ω-3 PUFA supplementation on a child's behaviour, cognitive ability and academic achievement is still uncertain. Studies have identified links between increased brain activation, improved cognition with ω-3 PUFA supplementation. It is yet to be determined if differences can be detected with supplementation over 480 mg DHA or 400 mg of EPA or ALA over a period longer than 12 weeks. The majority of ω-3 PUFA intervention studies involving mainstream children have not demonstrated any significant ω-3 PUFA treatment effects. One study identified a significant improvement in the ability to remember words and one study found parents of children in the ω-3 PUFA group, believed there were improvements in their children's behaviour, however their teachers recorded improved behaviour with those children in the placebo group.

Brain development, working memory and executive functions all play a major role in academic achievement. Age, gender, and intelligence are a few of the many variables which also impact on this achievement but their involvement is not fully understood. Intervention studies frequently use a battery of tests to assess changes in cognitive development. The use of assessment batteries and the need to consider multiple variables has led to the possibility of Type 1 errors occurring in most of ω-3 PUFA studies which have identified significant findings. The development of an effective and sensitive cognitive assessment tool is yet to be determined.

Fluency tests are frequently used to measure changes in cognitive development and are often incorporated in these test assessment batteries. It is unclear whether the TWFT can be successfully applied in the NZ setting, or if differences between groups can be detected using current fluency analysis strategies.

Research has also shown that international tests cannot always be effectively used and relied upon in other cultures and countries. No educational test which has been developed in NZ for New Zealanders has ever been used in an ω-3 PUFA intervention study. The NZ asTTle reading test is now commonly used in thousands of NZ schools and provides national normative data but it has never been used in an ω-3 PUFA intervention study. The effectiveness of this tool needs to be tested.

Parents are keen to see the influence of supplementation on academic achievement rather than scores related to cognitive ability. Two recent studies have identified associations between spelling and ω-3 PUFA supplementation; however this has not been tested in the NZ setting.

Omega-3 has been linked with improvements in behaviour and attitude. Small modifications to children's behaviour can have identifiable effects on the class dynamics and learning. Sometimes a person can have a feeling that change has occurred but there is no accompanying supporting evidence. Research into whether a teacher or parent can notice a difference in general behaviour, attitude and classroom tone is rarely investigated. Minimal ω-3 research has been undertaken to investigate if a child can notice a difference in their own attitude or behaviour. Whether these differences are specific to school activities or home-life and if they are related to moods such as aggression or their ability to focus and learn has also not been investigated. Rarely are children, parents and teachers all involved in the one ω-3 study.

If ω-3 PUFAs affect the different attributes related to children's learning (behaviour, concentration, memory, attention, speed of information processing etc.), it can be hypothesised that this should affect school achievement as assessed using standardised national tests and should be able to be accurately observed by children, parents and teachers. "Studies have produced mixed and inconclusive results and, consequently, the current evidence for the effect of PUFA supplementation on learning and behaviour remains unclear. There are many differences in their outcomes, with some reporting positive effects from PUFA supplementation, and just as many observing no significant

effect" (Kirby et al., 2010b, p. 720). It is anticipated that this study will add supporting evidence to this supplementation debate.

Reseach Questions

Justification for selection of research question/s

This literature review has not only provided background ω-3 PUFA information but also identified gaps and inconsistencies in previous research findings. Many of these researchers have stated concerns that the majority of LC ω-3 PUFA intervention studies have focussed on special needs rather than mainstream children (Hughes & Bryan, 2003; NEMO Study Group, 2007; Stevens et al., 1995) and until the present date the majority of this research has only focussed on changes to cognition and literacy.

This has provided justification for the over-arching holistic research question.

Does dietary supplementation with LC ω-3 PUFAs influence academic achievement in mainstream primary school children?

Academic achievement encompasses a broad range of subjects but is frequently regarded as ability in numeracy and literacy. This achievement can be influenced by many things including the behaviour and attitudes of children, parents and teachers. Various studies have investigated the effects of ω-3 PUFA supplementation on individual aspects of academic achievement. A handful of studies have also found that the influence of ω-3 PUFA supplementation varies depending on the participant's age or gender. The following research relates to ω-3 PUFA supplementation and its influence on specific aspects of children's academic achievement.

Improvements in reading ability have been associated with ω-3 PUFA supplementation in many studies (Milte et al., 2012; Richardson et al., 2012; Richardson et al., 2000; Richardson & Montgomery, 2005) however only a couple have involved mainstream children (Dalton et al., 2009; Ryan & Nelson, 2008). Several studies have investigated changes in fluency levels after ω-3 PUFA supplementation (Kairaluoma et al., 2008; Kirby et al., 2010b; Muthayya et al., 2009; Sinn & Bryan, 2007) with significant improvements being found in one study involving children with ADHD (Milte et al., 2012) and another with mainstream children (Ryan & Nelson, 2008). Changes in spelling ability due to ω-3 PUFA supplementation have been investigated in several

studies (Dalton et al., 2009; Kairaluoma et al., 2008; Kirby et al., 2010b; NEMO Study Group, 2007) but only in three studies involving special needs children have these changes been found at significant levels (Milte et al., 2012; Richardson et al., 2000; Richardson & Montgomery, 2005).

Although a large proportion of a school day involves learning about mathematics few studies have investigated changes in numeracy ability associated with ω-3 PUFA supplementation (Kairaluoma et al., 2008; NEMO Study Group, 2007; Stevens et al., 1996).

Parents have frequently demonstrated an ability to detect changes in their child's behaviour, attitudes and mood associated with ω-3 PUFA supplementation, however only two of these studies have involved mainstream children (Itomura et al., 2005; Richardson et al., 2012). Children have been able to detect changes in their own behaviour, attitudes and mood after ω-3 PUFA supplementation(Itomura et al., 2005; D. Kennedy et al., 2009; Lindmark & Clough, 2007). Investigating teacher predictions is much rarer with only three studies showing an ability to detect changes in children's behaviour, attitudes and mood after ω-3 PUFA supplementation however all of these involved special needs children (Gustafsson et al., 2010; Richardson & Montgomery, 2005; Stevens et al., 2003).

Many of these changes have been identified with a specific age group or with a specifc gender. Stevens et al. (1996) indentifed an increase in boys maths achievement associated with ω-3 PUFA supplementation whilst Richardson et al. (2000) indentifed an increase in the reading age of boys. Differences (but in the opposite direction) were found between boys and girls aggression levels after ω-3 PUFA supplementation (Itomura et al., 2005). The small sample sizes of many previous studies has meant that investigating interactions between children's age groups has been difficult , however it has been shown to be an influencing factor in many adult ω-3 PUFA supplementation studies(Crowe, Murray Skeaff, Green, & Gray, 2008; Zhou, Kubow, & Egeland, 2011). Age is a recognised factor in most education research (Bong, Cho, Ahn, & Kim, 2012; Haines, Diekhoff, LaBeff, & Clark, 1986).

The above summary has identified inconsistencies in findings regarding the influence of ω-3 PUFA supplementation on specific aspects of academic acheivement. To

investigate each of these aspects a further six research questions were included in this study.

Specific research questions

1. **Does dietary supplementation with LC ω-3 PUFAs influence fluency ability in primary school children?**
2. **Does dietary supplementation with LC ω-3 PUFAs influence spelling ability in primary school children?**
3. **Does dietary supplementation with LC ω-3 PUFAs influence reading ability in primary school children?**
4. **Does dietary supplementation with LC ω-3 PUFAs influence retention of basic facts knowledge in primary school children?**
5. **Is the effect of LC ω-3 PUFA supplementation influenced by the age or gender of primary school children?**
6. **Does dietary supplementation with LC ω-3 PUFAs produce a change in behaviour at home, on the playground and in the classroom in primary school children, as assessed by parents, teachers and children?**

Chapter 3

Methodology

Chapter 3: Methodology

This section will provide a brief overview of the study design. This will be followed by detail and justification of all aspects of the study including; subjects/participants (their eligibility and recruitment), procedures (treatment, randomisation and allocation), tests and testing procedures (asTTle, word fluency, basic facts, questionnaires, and fish and seafood intake) followed by an explanation of analysis strategies and dissemination of results. The guiding document for reporting the methodology, results and discussion sections of this parallel groups, randomised trial study was the well-known CONSORT statement (Schulz, Altman, & Moher, 2010).

The over-arching objective of this study was to investigate the effects of dietary supplementation with LC ω-3 PUFA on the academic achievement of 8 – 13 year old 'general' classroom children.

Six research questions were developed to investigate the specific effects of LC ω-3 PUFA supplementation on these children's fluency, spelling, reading ability, basic facts recall and behaviour both at home and at school, with regards to the children's gender and age.

Study Design

This study was a double-blind, randomised, placebo-controlled trial over a period of 15 weeks. The active treatment was a dietary supplement containing LC ω-3 PUFA, whereas the placebo contained vegetable oil. All outcome measures were obtained at pre-treatment (baseline) and at the conclusion of the study. The 15 week trial period (as outlined in Figure 7) was suitable for this kind of intervention (Bourre et al., 1993; Bryan et al., 2004; Richardson & Montgomery, 2005). As shown in Figure 7 the first half of the year (terms 1 and 2) was devoted to planning the study and gaining ethics approval, whilst the second half of the year (terms 3 and 4) was devoted to data gathering and the supplementation period. The study population was stratified, by age and gender, before randomisation to the treatments. This ensured that the treatment groups were similar with regard to these potential confounding factors. All measurements and data analyses were undertaken blind, with the principal investigator and participants not cognisant of the group allocation (double-blind).

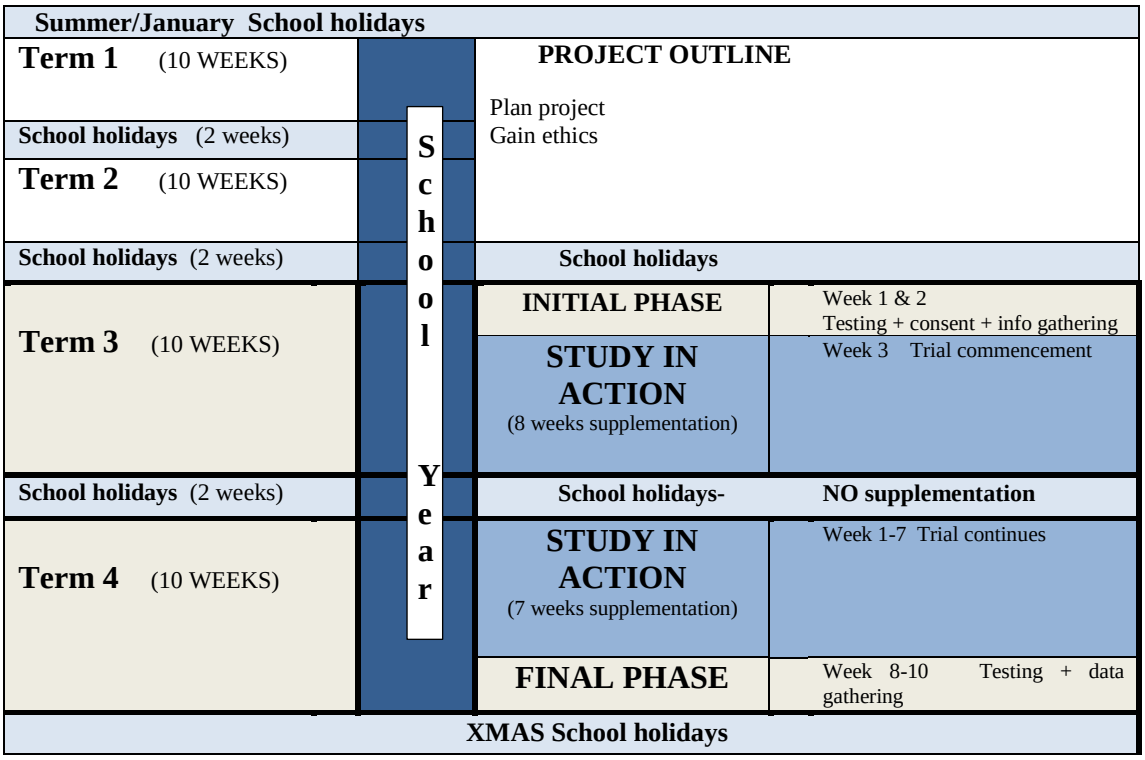


Figure 7: Brief overview of the study as related to the NZ school year

Methodology

The research was a randomised double blind placebo controlled trial. This is the most commonly used method for intervention studies as it is considered a 'gold standard methodology' (Rochon et al., 1999; Rosenzweig, Brohier, & Zipfel, 1993; Treweek et al., 2010). Of the 30 recent intervention studies undertaken to investigate the effects of ω-3PUFA on childrens' learning and behaviour 24 utilised this methodology (as shown in Appendix D2). The remaining 6 trials were open studies where all participants knew they are receiving supplementation. In these types of studies changes after supplementation may be documented but doubt can always remain as to what contributed to the observed changes.

Another methodology is the observational study, where associations are investigated between abilities or behaviours and the participants' ω-3 PUFA levels. Although valuable data can be gathered using this methodology it fails to isolate the variables which could be associated to any findings.

The double blind randomised nature of the study eliminates bias as all involved (participants and researchers) are unaware of the treatment groupings (Odgaard-Jensen

et al., 2011; Treweek et al., 2010). In this way any differences between treatment groups in academic achievement could be linked with the treatment rather than to altered expectations or behaviours of child, teacher, parent or researcher. In other studies altered expectations have been shown to influence performance. Parental expectations have been linked with children's attainment scores (Ma, 2001), their behaviour (Anthony et al., 2005) as have teacher expectations (Rosenthal & Jacobson, 1968) and participants' own expectations have also been shown to influence outcomes (Mondloch et al., 2001). For this reason blinding throughout the study was critical.

Being aware of biases is important in a pursuit of objectivity. Where a positivist approach believes the researcher and participants are independent of each other, this study utilizes a post-positivist approach. This approach accepts that theories, backgrounds, knowledge and values can influence the researcher's interpretation of results.

Currently in medical research there is a heavy emphasis on RCT's however these are rarely utilised in educational research, which prefer more qualitative methodologies (Torgerson & Torgerson, 2001). Those few studies which have utilised RCT's have rarely involved educationalists (Torgerson & Torgerson, 2001). In the last decade however there has been a call for more 'evidenced based research' both in NZ, the UK and US education systems. The USA now expects Federal education research to have a 90% empirical evidence base and states RCTs are at the top of the educational research heirachy(Benseman, Sutton, & Lander, 2005).

This study had a strong education base as the researcher was an educationalist with a science background (rather than a scientist with an interest in education). The design of this study involved the researcher working with the school management to plan what methods would be suitable for all involved. This reflected the researcher's pragmatic approach to the research. This philosophy emphasised the practical nature of what was seen as 'truth' ie. the truth was recognised to be a reflection of what was observed to be happening rather than a theory guiding the research. In this way the research was viewed as a 'pragmatic randomised controlled study'(Hotopf, 2002). As outlined in the following section the participants reflected the heterogeneity of NZ children, rather than those with existing 'conditions' such as ADHD, whilst the assessment measures were wide and varied and reflected 'real world' practice (Hotopf, 2002).

Subjects/participants

This study initially involved 213 children between the ages of 8-13 years old (with a mean age of 10.0±1.37 years). All children attended the same multicultural school. The study involved a wide range of ethnic groups as shown in Table 4. Figure 8 outlines the flow of the particpants over the 15 week study period in a format suggested in the well known and utitlised CONSORT statement (Schulz et al., 2010).

The following section outlines the eligibility criteria, recruitment procedures and demographics of the participants.

Eligibility Criteria

The study was approved by the Massey University ethics Southern A committee (09/27) and open to mainstream school children 8-13 years of age who met the inclusion criteria. The inclusion criteria consisted of being 8-13 years old, having no allergies to fish, being willing to take capsules of LC ω-3 PUFA, or vegetable oil (placebo) every day at school for 15 weeks, not taking any other form of medication, and parental and child consent.

Recruitment

In 'first world countries' research has tended to focus on those children in low socio-economic groups, as their diet often does not contain high levels of EFA (NEMO Study Group, 2007). In education in NZ the term 'decile' is used rather than socioeconomic grouping. Decile 1 schools are the 10% of schools with the highest proportion of students from low socio-economic communities, whereas decile 10 schools are the 10% of the schools with the lowest proportion of these students. It is assumed that supplementation within low decile/low socio-economic communities, may produce a more pronounced effect than with children, from high decile communities, who consume moderate to high amounts of EFA (Frensham, Bryan, & Parletta, 2012). For this reason, it was decided to base this study in an urban relatively low decile school. A principal of a decile 3 primary school was approached to ascertain the school's willingness to participate in this study. The researcher had previously taught at this school for a number of years and, therefore, had already established a strong rapport with senior management and some Board of Trustees members (school governing body). The project was approved by the principal and Board of Trustees and then

discussed with staff. A staff meeting for the teachers of the relevant classes was held and their consent to participate was sought (see Appendix A1 for a copy of the staff information sheet). Parents of children in these classrooms were sent an information letter inviting them and their children to participate (see Appendix A2 for a copy of the parent information sheet). Parents were asked to complete a screening form in order to check that children met eligibility requirements (see Appendix A4), as well as a food frequency questionnaire to determine frequency and type of seafood and ω-3 PUFA enriched food products consumed each week, as shown in Appendix A5. Finally, children who met the inclusion criteria were invited to participate in the study and their assent sought (see Appendix A3 for a copy of the child information sheet). Although this is often seen as an additional level of unnecessary complexity, (Everson-Bates, 1988; Janofsky & Starfield, 1981; Smyth & Weindling, 1999) it was seen by the researcher as an essential way of obtaining full informed consent from all participants.

Of a school roll with 259 possible candidates in the 8-13 age range 213 (82%) returned forms, consenting to participate in the study as can be seen in Figure 8. These children were located across 10 classrooms, each class with its own teacher. In a school with poor parental literacy, English being a second or third language for many and minimal involvement in school activities, this was seen as an exceptional response. A 10% drop-out rate was experienced during the study as shown in Figure 8. These results were similar to those of Dalton et al. (2009) whose 6 month South African study also had a 10% drop out rate, and Itomura et al. (2005) whose 3 month Japanese study had a 7% drop out rate. Both of these studies involved mainstream children aged between 7 and 12 years old in placebo controlled trials.

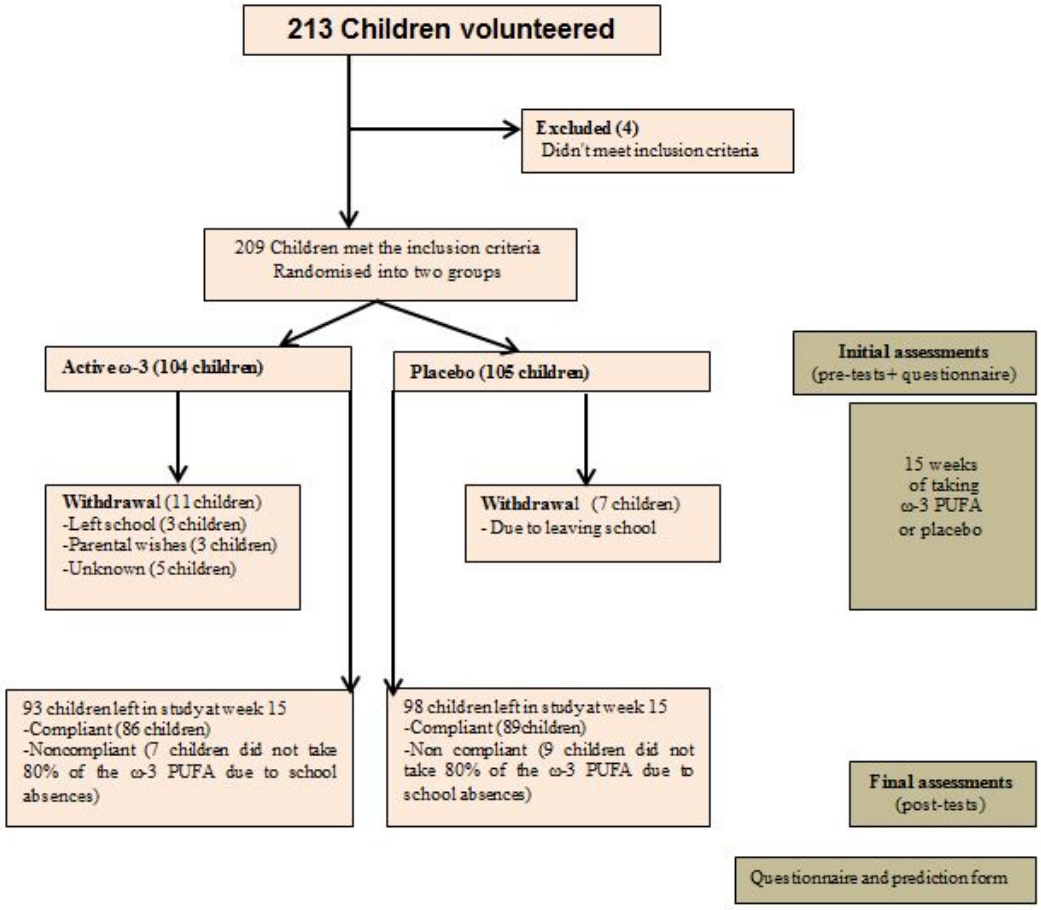


Figure 8: Flow of participants through the study

Typical of NZ urban low decile schools, the children who participated in this study represented a variety of ethnic groups, including Pacific Island, NZ European (Pakeha), indigenous Maori, Asian and Middle Eastern as shown in Table 4. When comparing the ethnic percentages in this study with the 2010 NZ national ethnic composition (72% NZ European, 24% Maori, 10% Asian, 12% Pacific Islanders and 1.2 % Middle Eastern, Latin American and African) for the age group 0-17 years old (Ministry of Social Development, 2010), the high proportion of Pacific Islanders and Maori in this sample group quickly becomes apparent. The mean age was 10.0±1.37 years. There was no difference in distribution of ethnicities between the placebo and ω-3 groups χ^2 (4) =3.1, p= 0.54.

Table 4: Ethnicity, gender across the participants' age groups

Ethnicity	Total n(%)	Total		Age group			
				8-9 years old		10+ years old	
		Male n(%)	Female n(%)	Male n(%)	Female n(%)	Male n(%)	Female n(%)
Pacific Island	60 (37%)	33(36%)	27(39%)	16(41%)	8(31%)	17(32%)	19(43%)
NZ European	42 (26%)	28(30%)	14(20%)	14(36%)	5(19%)	14(26%)	9(20%)
Maori	41 (25%)	21(23%)	20(29%)	8(20%)	10(38%)	13(25%)	10(23%)
Asian	18 (11%)	10(11%)	8(11%)	1(3%)	2(8%)	9(17%)	6(14%)
Middle Eastern	1 (0.6%)	0(0%)	1(1%)	0(0%)	1(4%)	0(0%)	0(0%)
TOTAL	162 (100%)	92 (100%)	70 (100%)	39 (100%)	26 (100%)	53 (100%)	44 (100%)

Procedures

The school database was used to provide initial information, which included children's full names, dates of birth, ethnicity and class room number. In the questionnaires sent to parents, this information was also sought and provided a double check of the data provided by the school. In the case of data inconsistency, the records were rechecked for other irregularities and the parents' response was used. Children (who assented and whose parents consented) were stratified according to age and gender and then randomly assigned to one of two groups consuming either capsules containing ω-3 PUFA or placebo capsules containing medium chain vegetable oil. Vegetable oil was selected as it contained no ω-3 PUFA and was soluble at room temperature. Palm and olive oil were rejected as possible placebos, as both these have been linked with benefits for the physical symptoms of fatty acid deficiency (Sinn, 2007). Research studies by Sinn (2007) and Stevens et al. (2003), found that both active and placebo groups showed significant improvements in these fatty acid deficiency symptoms, which they attributed to the selected placebo oil.

In NZ, the academic year follows the calendar year. As shown in Figure 7, the NZ primary school year is usually broken up into four, ten-week terms, with term one starting in January or February and term four finishing in December. As this study took half a school year (2 terms or the equivalent to one American school semester), it commenced immediately after the school holidays in the first week of term three, in order to minimise disruption to the school. This allowed time for the school governing board to consider the proposal, discussion with teachers and parents, sending and returning of consent forms and questionnaires, arranging distribution of capsules to ensure minimal school disruption as well as all baseline pre-testing. The first two weeks

of this term and the last three weeks of term four were used for data collection. In this way the 15 week study was spread across two terms (weeks 3-10 in term three and weeks 1-7 in term four), with the children receiving no supplementation during the school holidays (in order to avoid variability in compliance). Lindmark & Clough (2007) and Frensham et al. (2012) in responding to the high number of short-term interventions (8-12 weeks) suggested that such interventions be at least 12 weeks.

Treatment

The research assistant affixed children's name labels to the appropriate allocated capsule canisters. The children's names were also written on the lids of the canisters for quick retrieval in the class. These canisters were placed in colour-coded boxes and stored in a locked storeroom. Every day, monitors (trained Year 7 and 8 students, overseen by the classroom teacher) went to the classes to distribute the supplements and recorded whether the children took their supplements and if the children had consumed fish or foods high in ω-3 PUFA the previous day (as explained in Appendix A6). Every fortnight, the remaining capsules in each child's canisters, were counted and correlated with the daily logs, in order to measure the compliance ratio. The children took the intervention, at school at the same time every day, 10 minutes prior to lunchtime, whilst under the supervision of the class teacher and two trained year 7 or 8 monitors.

The daily dose of 4 capsules per day contained 540mg EPA and 360mg DHA (18:12 EPA: DHA) resulting in consumption of a total of 900mg of ω-3 PUFA per day (for 5 days a week). This dosage of ω-3 PUFA is achievable through the diet (equivalent to approximately 1-2 portions of oily fish/week). Dosages used in other studies varied markedly from 250 mg DHA and 5 mg EPA per day for a 3 month study (Meguid et al., 2008) to 700 mg DHA and 840 mg EPA per day for a 6 week study (Amminger et al., 2007). Even studies with the same duration and similar participants, e.g. children with ADHD, did not to have a commonality in dosage. See Appendix D2 for details regarding these studies.

To ensure compliance, the children were given the capsules under supervision during school hours. This meant the children received the active ingredient only during school terms, and only five days a week. As the increase in blood phospholipids due to the intake of EPA and DHA is dose and duration dependent (Arterburn et al., 2006), a higher dosage compared to other studies was chosen to achieve elevated ω-3 PUFA

status over a shorter duration of time (although still at a level achievable by un-supplemented dietary intake). Thus despite the short timeframe this should have ensured saturation of the tissues. Katan (1997) has shown that a dosage of 1g of EPA and DHA per day resulted in a rise in blood phospholipids of 1% (as measured using RBC DHA which has been shown to be the most efficient biomarker of DHA in the brain (Kuratko & Salem Jr, 2009). Although RBC ω-3 PUFA levels only reach a steady state after 4-6mo, the greatest increases are achieved within the first 4 to 8 weeks of supplementation (Katan et al., 1997). When supplementation is stopped RBC DHA takes longer than 24wks and EPA 4wks to reach baseline levels. Thus, it is expected that the levels of EPA and DHA would have remained elevated in the RBC during the 2wk holiday period (Arterburn, 2006) thus justifying the 15 week timeframe of this study.

Both placebo and active capsules were gel-coated and were able to be swallowed whole or chewed. All children were provided with an individually named drink bottle, which was filled with tap water. Children were encouraged to put the capsule in their mouth and drink some water to help swallow the capsule. The sipper-ends of the drink bottles were replaced every fortnight for hygiene reasons. The gel coating was orange coloured and fish shaped to maximise the appeal for children. Both capsules were flavoured with a natural sweetness enhancer, with a sugar free orange extract (±400mg/capsule). All the flavours had maltodextrin as carriers, which were calculated at (±400mg/capsule). This helped ensure that both placebo and active capsules looked, smelled and tasted the same. Orange, peppermint or substitute flavours have been recommended to disguise the 'fishy taste' in ω-3 PUFA trials (Stoll et al., 1999; Su et al., 2000). Both types of capsules were provided by Good Health (an Auckland based, local health food company). This 100% NZ owned company was certified, audited annually and was a Good Manufacturing Practice facility. This company solely supplied the capsules and assisted with randomisation. It had no involvement in study design, results analysis or interpretation.

Randomisation and Blind Allocation

A research assistant randomly allocated the children, by use of random numbers, to either the active or placebo group, after stratifying into gender and age groups. The randomisation scheme was generated using the Randomization.com website (http://www.randomization.com). This information was recorded and stored by this

person and was not revealed to the researchers or participants until after the trial had concluded. As is common practice in clinical trials (Margo, 1999; Vickers & de Craen, 2000), after all data gathering was completed, children were informed which group they were in and those in the placebo group were given 15 weeks supply of ω-3 supplements, to ensure they were not disadvantaged by being in the placebo group.

Children wrote their names on all tests and questionnaires, as they were not allocated ID numbers, thus reducing the likelihood of errors. Participants, those administering the interventions and those assessing the outcomes were all blinded with respect to the group assignments. At the time of data entry, names were replaced with ID numbers, to ensure confidentiality. The following section outlines the tests and testing procedures utilised.

Tests and Testing Procedures

The over-arching objective of this study was to investigate the effects of ω-3 PUFA on the academic achievement of 8–13 year old 'general' classroom children. Six research questions were developed to investigate the specific effects of LC ω-3 PUFA supplementation on these children's fluency, spelling, reading ability, basic facts recall and behaviour both at home and at school, with regards to the children's gender and age. The Thurstone Word Fluency and asTTle reading tests were undertaken at baseline and at endpoint. The researcher was also permitted access to the school database to utilise any test results from around the baseline and endpoint periods. These tests will be explained in the following sections.

High printing costs had prohibited the school from previously using asTTle and the school was therefore keen to assess its value and ease of delivery, prior to the National standardised testing which was to be introduced the following year. This enabled staff and children to become familiar with the testing processes and for this reason all middle and senior school (children 8-13 years old) children undertook the tests not solely those in the study.

It was important the children were not required to sit for an extended period of time and that conditions were as 'normal' as possible. For this reason, the tests were undertaken on the school site and took approximately an hour to deliver. Where possible the same person (researcher or deputy principal) delivered each class's tests in the same room, to

ensure consistency. The rooms were as quiet as possible, without distractions and children were provided adequate space and individual desks.

The following sections will provide further detail and justification for each specific test utilised.

Thurstone Word Fluency Test

This section focusses on the investigation as to whether dietary supplementation with LC ω-3 PUFAs influences fluency ability in primary school children (first research question).

Verbal fluency tests are quick, relatively enjoyable and non-threatening to children. For this reason they were the first test the children were required to complete. In this way the children 'warmed up' to the testing process. The following section states which fluency test was selected, why it was appropriate for this study, and how it was delivered and assessed.

As stated in the literature review, verbal fluency tasks have been widely used in cognitive psychology and neuropsychological assessment (Sauzéon et al., 2004). These tests require the participant to generate words according to a category or subcategory in a limited amount of time, and are believed to be a sensitive indicator of brain dysfunction (Spreen & Strauss, 1998). These tests are quick to administer (usually 4 to 5 minutes), do not require additional material, are cost effective, do not have low ceilings, have good test-retest reliability, and can be undertaken one-on-one or in a written form with large groups of people (Strauss et al., 2006). A written form was selected as appropriate for this study and was administered in a class setting.

Numerous varieties of verbal fluency tests exist, such as the COWA, Letter Fluency, FAS-Test and Controlled Verbal Fluency (Strauss et al., 2006). This study utilised the TWFT, where children have 5 minutes to recall words beginning with the letter S. Proper nouns, non-English words, repeats and words differing only in suffix (i.e. sing, singing, sings) are excluded. Children then have four minutes to recall four letter words beginning with the letter C.

Traditionally, scoring of results was determined by the number of words given, minus any unacceptable responses such as when words had been repeated (Troyer et al.,

1997). Misspelled words were counted as correct if the error did not affect the correctness of the word in terms of fluency (Strauss et al., 2006). Results were determined not only by the number of correct words given but also the use of strategies to generate words (Abwender et al., 2001), the switching between the strategies and the accuracy of these words, using the Troyer Strategies (2000; 1997). Troyer (1997) defined a cluster as a group of successively generated words that met one of four conditions: the words began with the same first two letters (e.g. *sn*ap, *sn*ake), differed only by a vowel sound (e.g. s*i*p, s*a*p, s*ou*p), rhymed (e.g. sand, stand) or were homonyms (e.g. sum, some). For this reason a cluster had to be one of four kinds, and must contain at least two words. A switch was a transition between these clusters.

In this study the number of words and the strategies used in their generation were identified as:

- Total 'number of correct (*S* or *C*) words' (the number of words produced, excluding errors and repetitions).
- Total 'number of (*S* or *C*) word clusters'.
- 'Number of words in the biggest cluster' (number of words listed in the largest cluster- not counting the first word).
- Average number of words written in each (*S* or *C*) word cluster.
- Total 'number of (*S* or *C*) word switches' (number of changes from one cluster to another).

In the case when smaller clusters were embedded within larger ones or two categories overlapped, but all items could correctly be assigned to a single category, only the larger common category was used, for example *sly, slipped, slim, slam* all begin with *sl* but an additional cluster was not scored for the last two words which differ only by a vowel sound.

During the initial scoring of the word fluency results using Troyer's, 1997 methods, it became clear that additional clustering strategies were being utilised by the children. These additional clustering strategies included: synonyms (e.g. shop, store), homophones (e.g. sauce, source), anagrams (e.g. silver, sliver), alliteration (e.g. *str*ide, *str*ike), assonance, (e.g. sum, sometimes), pararhymes, (e.g. ***s***ho*rt*, *s*ma*rt*), conceptual associations, (e.g. solar, system), contextual association, (e.g. spring, summer), homonyms (or partial homonyms), (e.g. sir, surge), addition or deletion neighbours, (e.g.

simile, smile), word classes, (e.g. sale, sell), as well as words which shared a letter sequence, (e.g. *sta**g**na**nt**, ser**g**ea**nt***). Ross (2003) has suggested that antonyms and words with conceptual association, e.g. foreign and film be also included in analysis.

A decision was made to re-analyse the children's fluency test results using Troyer's system but with the addition of the clustering strategies just mentioned. To acknowledge the combination of Troyer's definition, together with the elaborated clustering strategies identified in this study, the term 'Number of (*S* or *C*) word clusters & new associated clusters' is used in all tables in the results section.

The statistical methods for analysing the fluency test results are outlined in the statistical analysis section within this chapter.

Spelling

This section focusses on the investigation as to whether dietary supplementation with LC ω-3 PUFAs influences spelling ability in primary school children (second research question).

Weak links were found between ω-3 PUFA supplementation and spelling ability in research undertaken by Richardson and Montgomery (2005) and Dalton et al. (2009) but not by Kairaluoma et al. (2009), NEMO Study Group (2007) and Kirby et al. (2010). Investigating changes to NZ children's spelling ability is very difficult because, in most classes, every child has an individualised aspect to their weekly spelling list. Generally these words are derived from errors identified in classroom written work. For this reason changes in spelling ability was investigated using the words generated in the verbal fluency tests. This process had also previously been utilised to investigate accuracy of spelling (Troyer et al., 1997). Strategies for analysis will be explained in the statistical analysis section within this chapter.

Immediately after undertaking and completing the fluency test, the children were asked to undertake a 40 minute literacy test. The selection and delivery of this test will be explained and justified in the section below.

asTTle Reading Tests

This section focusses on the investigation as to whether dietary supplementation with LC ω-3 PUFAs influences reading ability in primary school children (third research question).

AsTTle is a relatively new assessment tool, which has been designed in NZ and is able to cater for a diverse ethnic mix, including Maori and Pacific Islanders (Ministry of Education, 2005b). This NZ standardised educational test formed the main assessment tool to determine the participants' reading ability prior to the commencement and at the conclusion of the study.

The asTTle tests are based on numeracy and literacy (reading and writing). Teachers are able to assess the numeracy and literacy development of students in Years 4 to 12 (8-13 year olds who are at Level 2-5) against the performance of nearly 100,000 NZ students (Ministry of Education, 2008). As stated in the literature review, teachers can individualise an asTTle test and determine what aspects are investigated, as well as the level of difficulty. This unique feature enables the personalisation of each test to meet the specific needs of the teacher or school.

Teachers can "administer a pre-and post-test version of asTTle, which could be the same or different test, to assess growth over time" (Hattie et al., 2004, pp. 4, Chapter 5); it is "appropriate to use materials in any one curriculum area up to a maximum of four times a year" (Hattie et al., 2004, pp. 19, Chapter three); it is for these reasons that asTTle was able to be used as a test/re-test in this study.

The unique ability to adjust and calibrate each test for a preferred depth of thinking and cognitive processing made asTTle a very attractive data gathering tool. After discussions with a number of classroom teachers and researchers of asTTle, it was decided to use asTTle reading tests (rather than the asTTle writing or numeracy tests) as results from these were deemed more manageable, consistent and reliable. The ability of students is reported on a transformed asTTle scale (aRs – for reading). The mean of all students in year 5 to 7, in our sample, was set to 500, with a standard deviation of 100.

Table 5 provides a summary of questions in each test which required various solution strategies. As stated in the literature review these strategies were based on the SOLO taxonomy categories, to ascertain cognitive processing. More specific detail relating to

this is provided in Appendix B4. Although the majority of questions, were at a difficulty level similar to that of the national norm, a few extra questions above and below this were also selected to ensure questions catered for gifted and remedial children (as shown in Table 5). Each test was also calibrated to ensure that the majority of questions probed for a deeper understanding (involving greater frontal lobe use), rather than surface cognitive processing.

Table 5: Calibrated settings for each asTTle test

asTTle slider settings		# of questions in Level 2 test	# of questions in Level 3 test	# of questions in Level 4 test
Cognitive processing	-Surface	12	13	11
	-Deep	22	20	23
Content	-Understanding	Many	Many	Many
	-Connections	Most	Most	Most
	-Inference	Most	Most	Most
Content	-Finding information	14	8	12
	-Understanding	20	15	16
	-Inference	20	19	19
	-Knowledge	7	2	6
	-Connections	14	16	10
	-Surface features	0	4	1
Difficulty	- Level 2	Most	Many	-
	- Level 3	Some	Most	Many
	- Level 4	-	Many	Most
	- Level 5	-		Some

To ensure a consistent and therefore comparable delivery of each asTTle test, the test conditions were highly structured (as outlined in asTTle instructions) (Ministry of Education, 2005b, pp. 26, Chapter 2). Results could then be compared against NZ national means. The following section details these conditions and the process undertaken by all children (as outlined in the asTTle manual) (Ministry of Education, 2005b).

The asTTle demographic and attitude section (as shown in Appendix B5) was completed together, with the test administrator reading the question and waiting for all children to fill in the appropriate responses, before moving to the next question. Children also had the 4 grades of ‘smiley faces’ explained for the attitude likert scale

questions. Once all the children had successfully completed this section they were given 5-10 minutes to complete the 4 practice questions. These questions are included in Appendix B6 and allowed practice of the response techniques needed in the test i.e. shading response boxes, ordering responses and filling in missing spaces. Once all children had completed this, a timeline was written on the board and they were instructed to start. Every 10 minutes a section of the timeline was crossed off, allowing the children to monitor their test progress. Early finishers were asked to check their work and then had their tests removed, whilst they read silently until the remainder of the 40 minutes had elapsed. Examples of three levels of asTTle tests, their marking guides and information about test delivery has been provided in Appendix B1, B2 and B3.

The asTTle test provides a clear marking schedule for each question. Once marks are entered for each question the asTTle computer programme provides a detailed breakdown of the child's ability. The asTTle scoring engine calculates each student's performance using the Item Response Theory techniques to determine the weighted aggregate performance across the items classified as requiring either surface or deep cognitive processes. The asTTle Consul Report displays the cohort performance of students compared to that of national groups as shown in Appendix B7. AsTTle reports the first two SOLO levels (uni-structural and multi-structural) combined as 'surface thinking' and the latter two levels (relation and extended) combined as 'deep thinking' (Hattie & Brown, 2004). It also collates all the attitude scores and provides an average mean score (4 being a score which indicated the child had a very positive attitude and 1 indicated the child had a very negative attitude).

The following section outlines additional maths tests and how their results were utilised in this study. These tests were administered by school staff as part of their regular assessment programme and access to this data was gained via ethics and Board of Trustee and principal approval. A brief overview of each test is provided below. The statistical methods for analysing these results, has been outlined in the statistical analysis section within this chapter.

Numeracy (Basic facts)

This section focusses on the investigation as to whether dietary supplementation with LC ω-3 PUFAs influences retention of basic facts knowledge in primary school children (fourth research question).

New Zealand teachers frequently assess children's mathematical ability in order to assist their progress. Classroom teachers administered standardised basic facts tests to all Year 4-8 children every term. An example of one of these tests has been provided in Appendix B8. Each test contained five columns, which the children were given one minute per column to complete.

The first column had 20 basic addition questions (using numbers between 1 and 9). The next column had 20 basic subtraction questions (using numbers between 1 and 20). The third column had 20 multiplication questions (using numbers between 2 and 9). The fourth column contained 20 basic division questions (using numbers less than 80 and a number between 2 and 9, which divided exactly into it). The last column contained 20 questions which were a mixture of addition, subtraction, multiplication and division.

Questionnaires to Investigate Accuracy of Prediction and Detection of Changes to Behaviour

This section focusses on the investigation as to whether dietary supplementation with LC ω-3 PUFAs produces a change in behaviour at home, on the playground and in the classroom in primary school children, as assessed by parents, teachers and children (sixth research question).

In order to investigate whether children, parents and teachers perceived any changes in behaviour, attitude and learning associated with the experimental treatment, questionnaires were completed at week 4 (children only) and immediately after the completion of the study (in the last 3 weeks of the school year).

Child On rare occasions children in ω-3 PUFA intervention studies have been asked if they have noticed changes in their behaviour (K. Hamazaki et al., 2008; Kairaluoma et al., 2008; D. Kennedy et al., 2009; Lindmark & Clough, 2007; Voigt et al., 2001). Intervention studies which have investigated changes to mainstream children's behaviour have used a hostility-aggression questionnaire (K. Hamazaki et al., 2008; Itomura et al., 2005), the Barratt Impulsiveness Scale (K. Hamazaki et al., 2008) and

children's mood via an internet battery (D. Kennedy et al., 2009). Studies which involved children with ADHD (Voigt et al., 2001) and dyslexia (Kairaluoma et al., 2008; Lindmark & Clough, 2007) used questionnaires.

In an open study involving an evaluation for dyslexic children an association between ω-3 PUFA intake and an overall perceived benefit and improvement in general schoolwork was demonstrated (Lindmark & Clough, 2007). Over a quarter of the children in this Swedish study perceived a benefit after the first 6 weeks of supplementation (Lindmark & Clough, 2007). This may be due to increased blood plasma phospholipid levels which rise rapidly in the first 4 weeks (Cao et al., 2006). For these reasons after four weeks of the intervention the children were presented with a questionnaire, to determine if any changes had been perceived and whether these were attributed to supplementation. Children were asked whether they noticed any changes in their behaviour, attitude and ability to remember school work (see Appendix B9).

At the conclusion of the study, questionnaires (see Appendix B10) were again distributed and collected by the principal researcher. These questionnaires investigated whether the child had noticed a change in their behaviour, attitudes towards peers and school, and their ability to learn. They were also asked to predict whether they were in the ω-3 PUFA or in the placebo group and to give reasons for this prediction. The last section questioned their thoughts on the merits of the study.

Teacher Standardised tests often do not discriminate well until considerable progress has been made. Teachers are able to fairly accurately identify young children making slow progress well before standardised tests can reliably do this (Neuman, 2003). They have also detected changes in the behaviour of special needs children's which have been associated with ω-3 supplementation (Gustafsson et al., 2010; Hirayama et al., 2004; Richardson & Montgomery, 2005; Stevens et al., 2003). A diverse range of assessment tools has been used to investigate teacher perceptions, with no one assessment measure appearing more reliable. In one study teachers but not parents were shown to detect changes in children's behaviour using the Connors Rating Scales (Gustafsson et al., 2010) whilst in another study the reverse was found using the same Connors scales (Richardson et al., 2012).

Whether supplementation can influence mainstream children's behaviour and learning and whether this can be detected by teachers is still undetermined. Only one ω-3 PUFA

study has questioned the teachers of mainstream children (Richardson et al., 2012) and although parents detected significant differences in their child's behaviour teachers did not. This highlights the need for the inclusion of a teacher questionnaire within this study design.

For this reason, teachers' views of children's behaviour, attitude and learning changes were seen as an important component of the study. This information was gathered using a questionnaire distributed at the conclusion of the study (see Appendix B12). They were also asked to predict whether each child was in the ω-3 PUFA or in the placebo group and to give any reasons for this prediction. The ten teachers involved with the children in the study were questioned. However the responses of one teacher were found to be self-contradictory and incapable of interpretation and for this reason were not included in the analysis.

Parents Fourteen international studies have been undertaken to investigate parents' views of the influence of ω-3 PUFA supplementation on their child's behaviour (Bélanger et al., 2009; Gustafsson et al., 2010; Hirayama et al., 2004; Itomura et al., 2005; Joshi et al., 2006; Kairaluoma et al., 2008; Kirby et al., 2010b; Lindmark & Clough, 2007; Milte et al., 2012; Richardson et al., 2012; Richardson & Puri, 2002; Sinn & Bryan, 2007; Stevens et al., 2003; Voigt et al., 2001). Of these, ten involved ADHD children whilst another two involved dyslexic children (Kairaluoma et al., 2008; Lindmark & Clough, 2007). Only two studies involved mainstream children and both of these studies utilised tests designed to investigate ADHD symptoms (Kirby et al., 2010b; Richardson et al., 2012). In nine of these studies parents detected changes in behaviour which were associated with ω-3 supplementation (Bélanger et al., 2009; Gustafsson et al., 2010; Hirayama et al., 2004; Itomura et al., 2005; Joshi et al., 2006; Lindmark & Clough, 2007; Richardson et al., 2012; Richardson & Puri, 2002; Stevens et al., 2003).

Questionnaires, rating scales (in a variety of forms) and symptom questionnaires formed the key assessment tools with no one tool appearing more effective. For this reason investigating parents' perceptions of behaviour and learning changes via a questionnaire was included as an assessment tool at the conclusion of the intervention (week 15).

For this reason a questionnaire completed by parents at the conclusion of the study, as shown in Appendix B11, investigated whether changes had been observed in their

child's behaviour and attitudes towards peers, home and school during the period of the intervention. They were also asked to predict whether their child was in the ω-3 PUFA or in the placebo group and to give reasons for this prediction.

Assessment of seafood and foods enriched in ω-3 PUFA

An initial food frequency questionnaire (see Appendix A5) was sent home to parents to investigate the child's intake of fish, seafood and foods enriched ω-3 PUFA, prior to the study commencing. Parents were asked how many servings of fish and seafood their child consumed each week. They were told a serving was equivalent to 80-100 g of fish/seafood (the size of an adult hand/palm) or one small tin of fish. The questionnaire listed fish and seafood commonly eaten in NZ. These were grouped together by ω-3 content e.g. albacore tuna, salmon, sardines etc. were group together as fatty fish with ω-3 greater than ~1g/100g; mackerel, snapper, barracuda and trevally were some of the examples given for the medium fatty fish group with ω-3 levels less than ~1g/100g but greater than ~0.5g/100g; Canned tuna, hoki, dory and gurnard were examples from the group of low fat fish which contain less than ~0.5g/100g of ω-3. Processed fish such as crumbed fish patties, cakes, fingers, nuggets and portions were grouped together as were shellfish (cockles, mussels, oysters, paua, scallops, shrimp/prawn and pipi).

Over the 15 weeks, monitors recorded the children's daily intake of fish, seafood and ω-3 PUFA enriched products consumed the previous day (see Appendix A6). This information provided an approximate indication of how much fish and seafood was consumed and what percentage was high in ω-3 PUFA, although it must be noted children only recorded the previous night's intake and therefore Friday and Saturday night meals were not recorded.

Power Calculations

The sample size calculation was based on the child based normed TWFT *S* word data (Kolb & Whishaw 1985, as cited in Strauss et al. 2006, p.515). It was calculated that a sample size of 128 (64 children per treatment group) would be required to be able to detect a 5 word difference (medium effect size, d= 0.5) in TWFT with 80% power and alpha level of 0.05 (2-tailed) (Erdfelder, Faul, & Buchner, 1996). For this reason, alternative fluency test data were utilised. A South African study utilised a fluency test (HVLT) and obtained a 3 word increase in the ω-3 PUFA supplemented group

(Tichelaar, Smuts, Kvalsvig, & Burgess, 2000). This data has also formed the basis for 2 other intervention studies' power calculations (Baumgartner, Smuts, Malan, Kvalsvig, et al., 2012; Dalton et al., 2009).

Statistical Analysis

SPSS (PASW Statistics 18) was utilised to analyse the data. All continuous variables were tested for normality using the Shapiro Wilk and the Kologorov-Smirnov tests, as well as detrended, Q-Q normality and box plots. Normally distributed data are expressed as mean ± standard deviation (SD) or mean (95% confidence interval (CI). Non-normally distributed data (e.g. servings of ω-3 PUFA containing foods) are expressed as median (25-75 percentile) and categorical data as frequencies and proportions.

In order to investigate whether ω-3 supplementation influenced total academic achievement a mulitvariate analysis (MANOVA) was undertaken. The total achievement was determined by combining literacy and numeracy results ie. combined maths basic facts scores (addition, subtraction, multiplication and division), asTTle scores and number of spelling errors (S and C words). Analysis utilised changes from baseline to endpoint (end minus baseline) for the above literacy measures, as dependent variables. Wilks Lambda was utilised and the partial eta squared effect size calculated with η^2 = .01 corresponding to a small effect, η^2 = .09 corresponding to a medium effect, and η^2 = .25 representing a large effect (Grimm & Yarnold, 2000).

This one over-arching research question (investigating total academic achievement) amalgamated acheivement findings, enabling the overall effects of ω-3 PUFA supplementation on 'academic achievement' to be analysed using MANOVA. Investigating what influence ω-3 PUFA supplementation had on each one of these outcomes involved specific research questions and a different analysis technique. Lack of specificity and clarity in research questions is a common design error (Wester, Borders, Boul, & Horton, 2013) and therefore six research questions were developed.

Analysis of the first five specific research questions involved calculating changes from baseline to endpoint (end minus baseline) for all main outcome variables and comparing these changes between treatment groups using analysis of covariance (ANCOVA) (controlling for baseline scores) as recommended for RCT by Fitzmaurice,

Laird and Ware (2011) and Oakes and Feldman (2001). Differences within groups between baseline and end scores were assessed using paired sample T-Tests. Effect sizes were calculated as Cohen's *d* (J. Cohen, 1988, 1992). As a guide for what constituted a small or large effect the following criteria were applied $d = 0.20$ (small effect), $d = 0.35$ (medium effect), $d = 0.80$ (large effect) (J. Cohen, 1988, 1992). These effect sizes and/or confidence intervals were reported alongside statistical information as recommended for ANCOVAs by Henson (2010).

In order to investigate whether the effect of LC ω-3 PUFA supplementation is influenced by the children's age or gender (fifth research question) the following analysis was undertaken. Interaction effects on outcome variables between treatment and gender, treatment and age (8-9 and 10-13 year olds), treatment, gender and age group and treatment and literacy ability (for basic facts only using asTTle scores below (<-44) and above (≥-44), the median of the sample), were assessed using ANCOVA (controlling for baseline values) and adding age groups, gender and literacy ability as dependent variables. A Levene's test was used to see if the variances were different in the different groups. On the few occasions when the Levene's test was found to be significant further investigation was undertaken (scatterplots and one-way ANOVA of residuals) to determine the degree to which the ratio of error variances were different. At worst the ratio was 1.6:1 (for percentage of spelling errors for C words) and for most other measures the ratio was never above 1.3:1. This is well under the median variance ratio of 2:1, and mean variance ratio of 4:1 in educational and child psychology journals (Erceg-Hurn & Mirosevich, 2008; Keselman et al., 1998).

Differences between groups for non-normally distributed and categorical data were determined using the Mann-Whitney and Pearson chi-square tests, respectively. Before the main analysis was conducted, baseline fluency data was explored for potential predictors such as age and gender using regression analysis and by determining differences between age and gender groups using analysis of variance (ANOVA). All assumptions for regression analysis were met. A *P* value of less than 0.05 was considered to be significant.

Because the total number of words differed between children generating *S* words and *C* words in the TWFT, and increased over time, the percentage of spelling errors per number of words written was calculated and utilised for all analysis.

AsTTle data were converted to standardised scores using national results as reference values (Ministry of Education, 2005a). Analysis was undertaken using all scores and then asTTle scores were divided according to the child's ability. As might be expected in a low decile school more than two-thirds of the children fell below the asTTle national mean. To gain a better understanding of whether academic ability within the low decile school was associated with the outcomes of the study the children were separated into 'high' and 'low' ability groups according to whether they scored above or below the mean (-44) for the school. AsTTle attitude Likert scale data were generated by the asTTle programme by calculating a composite score (mean) for the 6 attitude Likert-type items.

When analysing the last research question regarding perceptions of changes in behaviour a different analysis approach was used. Children and parent's responses were grouped in two categories, those who felt there had been an improvement (selecting "a lot better" or a "little bit better") and those who felt there was no change or a regression (selecting "the same as before", "a little bit worse" or "a lot worse"). Teacher's responses which recorded a change in attitude, behaviour or learning (improvement or regression) were analysed whilst responses identifying "no change" were excluded.

Parents', teachers' and children's responses were ordered according to accuracy of their prediction whether the child was in the ω-3 or placebo groups. A tally of children's responses was generated for their justification comments which included the following statements about observing: no change, an improvement in ability or memory, an improvement in behaviour, an improvement in attitude, an improvement in concentration and focus, getting on better with others, an worsening of behaviour, an worsening of results. This tally also recorded any students' comments about taste, smell and appearance.

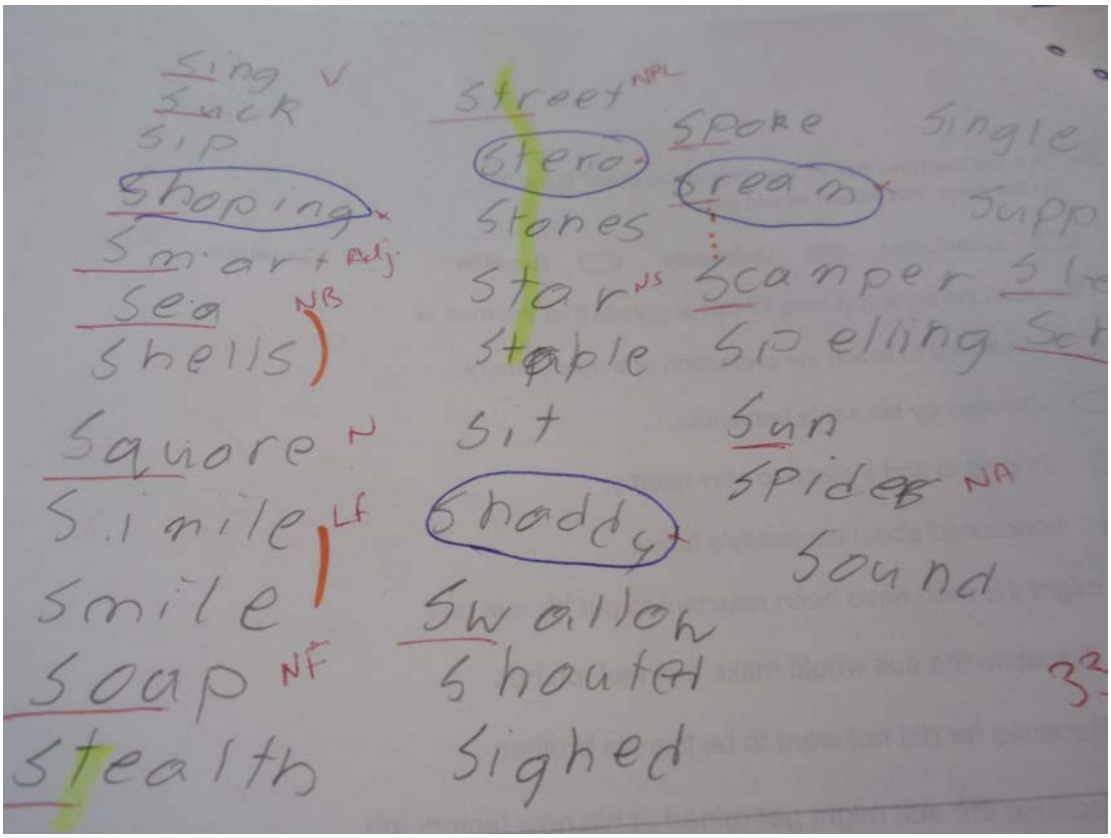
After analysis, when qualitative data were being used to support findings, each questionnaire was coded. Each respondent was given a number which was written on the top of the questionnaire preceded by a corresponding letter sequence of either CQ (for **C**hild **Q**uestionnaire), PQ (**P**arent **Q**uestionnaire), or TQ (**T**eacher **Q**uestionnaire). Any quotes which were used were always accompanied by the respondent's

corresponding code e.g. CQ54 (child number 54's questionnaire) or TQ5 (teacher number 5's questionnaire). This ensured confidentiality whilst enabling verification.

Dissemination of Results to Children, Parents and School

A verbal report was given to the school and health food company at the conclusion of the study and written reports were sent outlining progress, document analysis and findings. Summary reports were given to the school for distribution to staff, parents and children via the school newsletter.

Chapter 4



Results

Chapter 4: Results

Of the initial 209 children at the beginning of the study, there was a 91.4% retention rate with 191 children remaining in the study after 15 weeks (as noted earlier in Figure 8). Reasons for withdrawing from the study included leaving the school (7 in placebo group & 3 in the ω-3 PUFA group), parental wishes (3 in ω-3 PUFA group) and for reasons unknown (5 in the ω-3 PUFA group). Eighty-nine children (82.7%) were >80% compliant in the active group and 86 children (84.9%) in the placebo group, with the main reason for poor compliance being due to school absences (9 in placebo group and 7 in the ω-3 PUFA group). No significant differences were found between those children in the trial and those who withdrew. Results from these compliant children who remained until the conclusion of the study are outlined in this chapter. The chapter will be broken into 7 sections related to each investigation, these being; intake of food sources rich in ω-3 PUFA, word fluency tests, spelling, asTTle, basic facts, changes in children's behaviour, learning and attitude noticed by parents, children and teachers.

Consumption of LC ω-3 PUFA Food Sources

Prior to the commencement of the study parents completed a food frequency questionnaire. More than half (56%) of all parents, stated their child consumed less than 1 serving of fish or seafood per week. Table 6 shows which fish, seafood and ω-3 enriched products parents stated were consumed weekly.

A Mann-Whitney test indicated that there was no significant difference in pre-study fish and seafood consumption when utilising food frequency questionnaire responses between parents with children in the placebo group, who stated their child consumed approximately 1.14 [0.94, 1.34] servings of fish and seafood per week and parents with children in the ω-3 PUFA group who stated their child consumed 1.21 [0.99, 1.43] servings (U=3219, P=0.82 (2-tailed), r=-0.02). Nor were there any significant differences when aggregating daily child consumption responses over the 15 weeks, with children in the placebo group reporting consumption of 0.45 [0.29, 0.60] and children in the ω-3 PUFA groups reporting 0.41[0.33, 0.49] servings of fish and seafood per week (U=2836, P= 0.17 (2-tailed), r=-0.11).

Table 6: Child's weekly consumption of food sources containing LC ω-3 PUFA (as reported by parents, prior to commencement of study) (*n*=161)

Food product	Number and percentage of children in study who consumed ≥1 serving per week
Battered fish (from fish & chip shop)	35 (22%)
Fatty Fish (>1g ω-3/100g)	28 (17%)
Medium fatty fish (>0.5<1g ω-3/100g)	21 (13%)
Low fat fish (<0.5g ω-3 /100g)	30 (19%)
Processed fish e.g. fish cake, fish fingers	25 (15%)
Mussels & squid	13 (8%)
Shellfish	9 (6%)
ω-3 PUFA enriched milk	13 (8%)
ω-3 PUFA enriched bread	21 (13%)

Children were asked daily what fish they had eaten the night before (this therefore excluded Friday and Saturday nights). Over the course of the 15 week study most children (>90%) reported that they had not eaten any mussels, squid, shellfish, bread and milk enriched with ω-3 PUFA, whilst 70% stated they had not eaten any fatty fish, containing high levels of ω-3 PUFA (>1g ω-3 /100g). The most commonly eaten fish or seafood was battered fish which over a third of the children (38%) stated they had not eaten over the 15 week study period as shown in Table 7.

Table 7: Child's consumption of food sources containing LC ω-3 PUFA (reported daily by children over the course of the 15 week study)

Food product	Median number of times this food was consumed (*n*=161)		Number and percentage of children reporting no servings over the 15 weeks *n*=161
	Over the 15 weeks [25 -75 percentiles]	Each week [25-75 percentiles]	
Battered fish (from fish & chip shop)	1.0 [0.0-3.0]	0.07 [0.0-0.20]	61 (38%)
Fatty Fish (>1g ω-3/100g)	0.0 [0.0-1.0]	0.0 [0.0-0.07]	114 (70%)
Medium fatty fish (>0.5<1g ω-3/100g)	0.0 [0.0-1.0]	0.0 [0.0-0.07]	100 (62%)
Low fat fish (<0.5g ω-3 /100g)	1.0 [0.0-2.0]	0.07 [0.0-0.1]	80 (50%)
Processed fish e.g. fish cake, fish fingers	0.0 [0.0-0.0]	0.0 [0.0-0.0]	127 (78%)
Mussels & squid	0.0 [0.0-0.0)	0.0 [0.0-0.0]	152 (94%)
Shellfish	0.0 [0.0-0.0]	0.0 [0.0-0.0]	153 (94%)
ω-3 PUFA enriched milk	0.0 [0.0-0.0]	0.0 [0.0-0.0]	160 (99%)
ω-3 PUFA enriched bread	0.0 [0.0-0.0]	0.0 [0.0-0.0]	160 (99%)
TOTAL FISH & SEAFOOD CONSUMED	4.0 [2.0-8.0]	0.27 [0.13-0.53]	18 (11%)

When utilising the daily log of consumption (recorded by the monitors), over the 15 weeks the children were absent from school on average approximately 7 days, with no difference between the placebo and ω-3 PUFA groups.

In summary the above section highlights the low intake of fish, seafood and ω-3 enriched products by the children and the strong equivalence of the treatment and placebo groups in their consumption of these foods.

Changes in overall academic achievement

A MANOVA was undertaken to determine the influence of ω-3PUFA on total academic achievement. Total academic achievement was viewed as a combination of numeracy and literacy results (basic facts maths, asTTle and spelling test results). Changes between end and baseline values were utilised (as explained in the methodology section). There was no statistically significant difference in total academic achievement attributed to ω-3PUFA supplementation [Wilks' Lambda: $F(7, 67)=1.6$, $p=0.15$; Wilk's $\Lambda=0.86$, partial $\eta^2=0.14$].

Which specific aspects of the child's academic achievement were influenced by ω-3PUFA supplementation is outlined in the following sections. Whether age or gender influenced the effects of the ω-3PUFA supplementation is also reported.

Word Fluency Tests

Does dietary supplementation with LC ω-3 PUFAs influence fluency ability in primary school children and is any effect influenced by the children's age or gender?

Fluency Results at Baseline

Prior to the intervention children were asked to complete a fluency test for *S* words and *C* words (as explained in the methodology section). Analysis of this test involved identifying the number of *S* words and *C* words each child generated and the strategies they utilised (listing words related to a category or cluster, and then switching to a new category/cluster). As stated in the methodology the deputy principal assisted with initial testing, when multiple classes needed to be tested at the same time. Unfortunately 58 responses for C words for 3 classes (at baseline) were eliminated due to an instructional

error (the deputy principal incorrectly worded the test condition instructions). This resulted in *n*=162 for baseline and *n*=161 for end *S* word responses but only *n*=104 for baseline and *n*=161 for end *C* word responses. The fluency results at baseline have been explored to investigate the associations between fluency and gender and age. This has been followed with the end and change results in order to investigate any possible treatment effect.

Age was positively associated with the number of correct words generated probably because as the children got older their vocabulary increased (linear regression equation: Number of *S* words at baseline=8.89+10.1 x increased age, *n*=162, *F*=3709 (1, 161)*df*, *P*(two-tailed)<0.001, r^2= 0.19, *B*=10.08[95%CI, 6.85 -13.3]). The baseline mean, ±SD of *S* words, for 8 to 9 year olds, was 18.3±8.90 compared with 31.9±11.5 for 10 to 13 year olds (*F*= 37.9, (1, 161)*df*, *P*<0.001). The number of correct *C* words were also greater in older children compared to younger children with the baseline mean for 8 to 9 year olds being only 6.05±3.21 compared with 7.91±3.40 for 10 to 13 year olds (*F*= 6.89, (1,103)*df* , *P*=0.01), (equation: Total number of *C* words at baseline= 4.19 + 1.85 x increased age, *n*=104, *F*=6.89 (1, 103)*df*, *P*(two-tailed)=0.01, r^2= 0.05, *B*=1.86 [95%CI, 0.45 -3.26]). The lower mean number of *C* words (less than 50% of *S* words) indicated that the children found generating these much harder than the *S* words. Table 8 shows an overview of how the different age groups utilised various strategies to generate words.

Table 8: Fluency strategies used to generate words by differing age groups

Fluency Measure	Age (years)	Mean	95% CI	SD	*P* value
Number of *S* words correct	8-9 (*n=65)*	18.3	16.0-20.6	8.90	<0.001
	10-13 (*n=97)*	31.9	28.0-35.5	11.5	
Number of *C* words correct	8-9 (*n=61)*	6.05	5.23-6.87	3.21	0.01
	10-13 (*n=43)*	7.91	6.67-9.14	3.40	
Average number of *S* word clusters	8-9 (*n=65)*	2.64	2.14-3.14	1.97	0.001
	10-13 (*n=97)*	4.33	3.47-5.19	2.80	
Average number of *C* word clusters	8-9 (*n=61)*	1.54	1.27-1.81	1.06	0.11
	10-13 (*n=43)*	1.93	1.49-2.37	1.42	
Number of words in the biggest *S* word cluster	8-9 (*n=65)*	1.18	0.98-1.38	0.79	0.001
	10-13 (*n=97)*	1.67	1.38-1.97	0.94	
Number of words in the biggest *C* word cluster	8-9 (*n=61)*	1.67	1.31-2.03	1.41	0.53
	10-13 (*n=43)*	1.51	1.19-1.84	1.06	

P- Value derived from ANOVA: Comparisons between groups.

There was no significant difference between the strategies males and females utilised to generate words. Girls however generated more *S* words with an average of 28.3±11.0 words compared with boys 22.5±10.0 as shown in Table 9. Gender was positively associated with the number of correct words generated probably because girls have a larger vocabulary (linear regression equation: Number of *S* words at baseline=16.6+5.88 x gender, *n*=162, *F*=11.4 (1, 161)*df*, *P*(two-tailed)<0.001, r^2= 0.07, *B*=5.88[95%CI, 2.44 -9.32]). This however was not found to be at a level of significance when the groups were split for age and gender.

Table 9: Fluency strategies used to generate words by males and females

Fluency Measure		**Mean**	**95% CI**	**SD**	***P* value**
Number of *S* words correct	Male (*n=92)*	22.5	20.2-24.7	10.0	0.001
	Female (*n=70)*	28.3	25.7-31.0	11.0	
Number of *C* words correct	Male (*n=62)*	6.47	5.44-7.50	4.06	0.24
	Female (*n=42)*	7.33	6.42-8.25	2.90	
Average number of *S* word clusters	Male (*n=92)*	1.98	1.82-2.14	0.76	0.55
	Female (*n=70)*	2.04	1.91-2.18	0.56	
Average number of *C* word clusters	Male (*n=62)*	2.16	1.85-2.48	1.23	0.30
	Female (*n=42)*	2.41	2.09-2.72	1.01	
Number of words in the biggest *S* word cluster	Male (*n=92)*	1.59	1.35-1.82	1.13	0.65
	Female (*n=70)*	1.51	1.33-1.70	0.77	
Number of words in the biggest *C* word cluster	Male (*n=62)*	1.58	1.23-1.93	1.39	0.81
	Female (*n=42)*	1.64	1.30-1.99	1.10	

P- Value derived from ANOVA: Comparisons between groups

As children generated more words, the number of words in each cluster increased. Increasing the number of total *S* words generated, was a significant predictor of an increase in the average (mean) size of *S* word clusters and *C* word clusters generated at baseline, (size of *S* word clusters at baseline= 1.38 + 0.02 x increasing total number of *S* words, *n*=162, *F*=29.7 (1, 161)*df*, *P*(two-tailed)<0.001, r^2= 0.16, *B*=0.02 [95%CI, 0.02-0.03]) and (size of *C* word clusters at baseline= 1.5 + 0.11 x increasing total number of *C* words n=104, *F*=12.5 (1, 103)*df*, *P*(two-tailed)=0.001, r^2= 0.11, *B*=0.11 [95%CI, 0.05-0.17]. An increase in the number of words generated at baseline also significantly predicted an increase in the switching with *S* and *C* words (number of switches with *S* word at baseline= 0.38 + 0.78 x increasing total number of *S* words, *n*=162, *F*=1676 (1, 161)*df*, *P*(two-tailed)<0.001, r^2= 0.91, *B*=0.78 [95%CI, 0.74-0.81]) and (Number of

switches with *C* word at baseline= 0.49 + 0.13 x increasing total number of *C* words, *n*=104, *F*=50.8 (1, 103)*df*, *P*(two-tailed)<0.001, r^2= 0.33, *B*=0.13 [95%CI, 0.09-0.16]).

Fluency Results in response to intervention

Over the course of the 15 weeks most children significantly increased the number of words they could generate within the set time limit. Tables 10 and 11 provide a summary of the changes in strategies used by the children to generate *S* and *C* words across the 15 weeks. Although significant increases in fluency were found, the increases were not affected by whether the children received ω-3 supplementation or not. Significant interactions with a small effect size between treatment, age and gender (Cohen's *d*=0.11) were found for the *S* word clusters. These findings are investigated in more detail later in this section (see Table 13 -15). It is important to note that the high number of calculations undertaken may increase the probability of a false significant finding.

Table 10: Fluency strategies used by children to generate *S* words

Strategies used by children to generate *S* words		Placebo (*n*=80)			Fish oil (*n*=82)			*P*†	Interaction Group *Gender	Interaction Group *Age Group	Interaction Group *Gender *Age Group
		Mean	95%CI	SD	Mean	95%CI	SD				
Number of Correct *S* words	B	25.0	22.5-27.4	11.1	25.0	22.5-27.6	11.6	0.96			
	E	30.5	27.6-33.4	13.0	29.6	26.9-32.3	12.5				
	Change	5.50	3.30-7.7	9.80	4.50	2.70-6.3	8.00	0.50	0.08	0.79	0.68
	P§	*P* <0.001			*P* <0.001						
Number of *S* word clusters	B	3.5	2.9-4.0	2.6	4.0	4.0-4.5	2.6	0.21			
	E	5.1	4.3-5.9	3.5	5.0	4.3-5.6	3.1				
	Change	1.6	0.9-2.4	3.5	1.0	0.4-1.6	2.8	0.39	0.008	0.08	0.03
	P§	*P* <0.001			*P* <0.001						
Average number of *S* words written in each cluster	B	2.0	1.9-2.1	0.6	2.0	1.8-2.1	0.7	0.21			
	E	2.3	2.1-2.4	0.7	2.2	2.1-2.4	0.7				
	Change	0.2	0.1-0.4	0.9	0.2	0.0-0.4	0.9	0.68	0.87	0.30	0.74
	P§	0.014			0.04						
Number of words in the biggest cluster	B	1.5	1.2-1.6	0.9	1.6	1.3-1.9	1.1	0.39			
	E	1.7	1.5-1.9	1.0	1.7	1.5-1.9	2.0				
	Change	0.2	-0.0-0.5	1.2	0.0	-0.2-0.3	1.2	0.62	0.46	0.23	0.61
	P§	0.09			0.64						
Number of *S* word Switches	B	20.8	18.8-22.8	9.0	20.4	18.4-22.5	9.3	0.79			
	E	24.2	21.8-26.5	10.5	23.3	21.1-25.6	10.3				
	Change	3.4	1.4-5.3	8.8	2.9	1.3-4.5	7.3	0.65	0.99	0.30	0.40
	P§	0.001			0.001						

B, baseline, E, end Change: End value –Baseline value

P§-Value derived from Paired sample T-Test (2-tailed) B vs. E within groups

P†-Value derived from ANOVA: Comparisons between groups. Change value derived from ANCOVA: Comparisons between groups (using baseline as covariate)

Interaction effects were assessed using ANCOVA and adding *gender, *age group, *gender and age group as independent variables

P -at significance levels

Table 11: Fluency strategies used by children to generate *C* words

Strategies used by children to generate *C* words		Placebo (*n*=47)			Fish oil (*n*=56)			*P*†	Interaction Group *Gender	Interaction Group *Age Group	Interaction Group *Gender *Age Group
		Mean	95%CI	SD	Mean	95%CI	SD				
Number of of correct *C* words	B	6.6	5.4-7.7	3.9	7.1	6.2-8.0	3.5	0.39			
	E	9.2	7.9-10.5	4.4	9.2	8.1-10.3	4.1				
	Change	2.66	1.78-3.54	3.01	2.10	1.01-3.20	4.09	0.59	0.80	0.59	0.88
	P§	*P* <0.001			*P*<0.001						
Number of *C* word Clusters	B	1.6	1.3-2.0	1.2	1.8	1.4-2.1	1.3	0.46			
	E	2.1	1.8-2.5	1.2	2.0	1.7-2.3	1.3				
	Change	0.53	0.20-0.86	1.12	0.21	-0.20-.63	1.55	0.35	0.33	0.79	0.89
	P§	0.002			0.30						
Average number of *C* words in each cluster	B	2.2	1.8-2.6	1.3	2.3	2.0-2.6	1.0	0.69			
	E	2.6	2.3-2.9	1.0	2.5	2.1-2.8	1.3				
	Change	0.41	0.02-0.85	1.49	0.17	-0.24-.58	1.53	0.51	0.42	0.32	0.65
	P§	0.06			0.41						
Number of *C* words in biggest cluster	B	1.6	1.2-2.0	1.5	1.6	1.3-1.9	1.1	0.99			
	E	2.0	1.7-2.3	1.1	1.9	1.6-2.3	1.4				
	Change	0.40	-0.10-0.91	1.73	0.32	-0.1-0.74	1.56	0.77	0.64	0.36	0.78
	P§	0.12			0.13						
Number of *C* word switches	B	3.5	2.7-4.4	2.9	3.9	3.2-4.6	2.6	0.42			
	E	5.7	4.6-6.8	4.0	5.2	4.4-6.0	2.9				
	Change	2.08	1.20-2.97	3.01	1.30	0.42-2.19	3.30	0.28	0.96	0.95	0.84
	P§	*P* <0.001			0.01						

B, baseline, E, end Change: End value –Baseline value

P§-Value derived from Paired sample T-Test (2-tailed) B vs. E within groups
P†-Value derived from ANOVA: Comparisons between groups. Change value derived from ANCOVA: Comparisons between groups (using baseline as covariate)
Interaction effects were assessed using ANCOVA and adding *gender, *age group, *gender and age group as independent variables

Additional Analysis Building on Troyer's (1997) Scoring Methods

As detailed in the methodology chapter, Thurstone and Troyer required strict criteria for scoring these fluency tests, however on analysis it became apparent that children in this study were using a variety of additional strategies. For this reason, all tests were remarked taking into account a number of additional clustering strategies, examples of which are included in Table 12.

Table 12: Examples of linked/clustered words generated by the children during the WFT (but traditonally not allowed to be counted as a cluster- using the Thurstone or Troyer analysis strategies).

Example given by child	Explanation	Technical term and justification
simile /smile	Look almost exactly the same; only one letter different.	**Addition/deletion neighbours –** words created by adding or deleting letters.
carb/crab silver/sliver cast/cats skate/steak	Letters mixed around	**Anagrams** –word or phrase that exactly reproduces the letters in another order is an anagram.
score / skull scramble/scraps skuttle/ scatter/ scale/ scrawny scratch/secary	Start with the same sound, even though not spelt the same (or correctly).	**Alliteration** - refers to the repetition of a particular sound in the first syllables of a series of words and/or phrases.
sir/surge	Sound very similar	**Homonym** (partial example) – words that sound the same but are written differently and have different meanings.
seasons/spring/summer shampoo/shower/Sponge/scrub/soap shoe/sandals/socks/stockings science/social-studies/subjects sugar/spice/salt skateboard/scooter stew/soup sun/stars/solar/space/science swing/slide six/seven soil/spade/shovel speaking/saying/shouting/silence sweet/sour/senses square/shapes	Associated words	**Contextual association** – linked by their relevance to a shared situation or concept in the real world.
Soy/sauce sports/star solar/system see/saw sea/shore school/staff	Words often linked	**Conceptual association** – words linked by their meaning
Store/shop separate/single sink/submerge scary/spooky stupid/silly slender/skinny steaming/scalding shriek/scream/shout stinky/smelly scent/smell	Mean a similar thing	**Synonyms** – different words with identical or similar meanings.
Short/smart Chess/class slurp/sharp swap/slap	First and last few letters the same	**Pararhyme** – rhyme in which the central vowel sound of the final syllable is changed, and the consonants on either side of it are kept the same (though some of the examples from the children were only partial pararhymes)

strife/strike/stride	Same initial blends	**Alliteration** -repetition of a particular sound in the first syllables of a series of words and/or phrases.
sum/sometimes	Same initial sounds	**Assonance** – repetition of a vowel sound anywhere in the word (whereas rhyme is repetition in the last syllable).
she sells sea shells sea shore	Allusion to well-known texts, in this case a nursery rhyme or 'tongue twister'.	**Alliteration** - refers to the repetition of a particular sound in the first syllables of a series of words and/or phrases.
sale/sell	Associated words	**Word classes** – list the noun, verb etc. of the same word.
stagnant/sargent	Rhyme/ shared letters	**Shared letter sequence** – in this case s…g…nt
Stats., Carb.	Abbreviations	

After this reanalysis no antonyms were found and the overall number of clusters increased. At baseline traditional methods identified 3.71±2.61 *S* word clusters per child whilst the new method identified 4.30±3.01 clusters. A Paired-Sample T-Test indicated that there was a significant difference between the scoring methods $P<0.001$. The results suggest that the new analysis strategy is able to identify significantly more clusters compared to traditional methods of analysis.

The utilisation of these new analysis strategies (by broadening the category of words able to be included in a cluster) as described in the methodology, did not result in a significant difference between treatment and placebo groups (as shown in Table 13). Significant interactions were found between treatment and gender (as seen in Table 13). These findings are investigated in more detail in Tables 14 and 15.

Older children produced both significantly more S word clusters and more new associated clusters (number of *S* word clusters and new associated clusters = 0.43 + 1.70 x age, $n=162$, $F(2,160)= 8.21$, $P\leq0.001$, $r^2=0.09$, B=1.7[95%CI, 0.76-2.63]), whilst a similar significant finding was found for females (number of *S* word clusters and new associated clusters = 0.43 + 0.8 x gender, $n=162$, $F(1,161)= 8.21$, $P\leq0.001$, $r^2=0.09$, B=0.81[95%CI,-0.12-1.74]).

The significant interaction effects reported here and for *S* word fluency above have been elaborated upon, in the following paragraphs.

Table 13: Number of clusters generated by children when listing *S* words (using traditional and this study's newly developed method of scoring)

	Placebo (*n*=80)			Fish oil (*n*=82)			*P*†	Interaction Group *Gender	Interaction Group *Age Group	Interaction Group *Gender *Age Group
	Mean	95%CI	SD	Mean	95%CI	SD				
B	4.15	3.45-4.85	3.12	4.45	3.78-5.12	3.06	0.54			
E	6.01	5.10-6.91	4.07	6.01	5.18-6.85	3.80				
Change	1.86	1.01-2.71	3.81	1.56	0.83-2.30	3.3	0.72	0.007	0.1	0.02
P§	*P* <0.001			*P* <0.001						

B, baseline, E, end Change: End value –Baseline value

P§-Value derived from Paired sample T-Test (2-tailed) B vs. E within groups
P†-Value derived from ANOVA: Comparisons between groups. Change value derived from ANCOVA: Comparisons between groups (using baseline as covariate)
Interaction effects were assessed using ANCOVA and adding *gender, *age group, *gender and age group as independent variables
P -at significance levels

Table 14: Fluency strategies used by males and females to generate S words

		Female							Male						
		Placebo			Fish oil			*P*†	Placebo			Fish oil			*P*†
		Mean	95%CI	SD	Mean	95%CI	SD		Mean	95%CI	SD	Mean	95%CI	SD	
Number	B	3	1.97-4.03	2	4.64	3.46-5.82	2.86	0.14	2.83	2.03-3.64	2.15	2.97	2.06-3.87	2.47	0.82
S word	E	5.58	3.49-7.69	4.08	4.68	3.43-5.93	3.02		3.6	2.58-4.62	2.74	5.01	3.90-6.29	3.25	
Clusters	Change	2.59	0.54-4.64	3.99	0.04	-1.94	2.35	0.03	0.77	-1.89	2.53	2.13	1.18-3.07	2.58	0.26
	P§	0.003 (*n*=33)			0.95 (*n*=37)				0.008 (*n*=47)			*P*<0.001 (*n*=45)			
Number	B	3.41	2.23-4.59	2.29	5.28	3.71-6.84	3.79	0.33	3.23	2.37-4.10	2.31	3.49	2.45-4.52	2.82	0.82
S word	E	6.29	4.13-8.45	4.19	5.88	4.33-7.43	3.75		4.1	2.83-5.36	3.39	5.94	4.52-7.36	3.88	
Clusters	Change	2.88	0.83-4.94	4	0.6	-2.12	2.57	0.03	0.87	-2.12	2.84	2.45	1.21-3.69	3.38	0.12
& new associated	P§	*P*<0.001 (*n*= 33)			0.17 (*n*=37)				0.02 (*n*=47)			*P*<0.001 (*n*=45)			

B, baseline, E, end Change: End value –Baseline value

P§-Value derived from Paired sample T-Test (2-tailed) B vs. E within groups
P†-Value derived from ANOVA: Comparisons between groups. Change value derived from ANCOVA: Comparisons between groups (using baseline as covariate)
P -at significance levels

Significant improvements in the number of *S* word clusters produced were seen in older (10-13 year olds) females in the placebo group (Tables 14 and 15). Females in the placebo group produced significantly more clusters than those in the ω-3 PUFA group ($F(1, 67)= 5.2$, $P= 0.03$ 1.07±3.54 [95%CI, 0.22-3.26]), with a medium size effect of Cohen's d=0.56. Females in the placebo group also produced significantly more *S* word

clusters and new associated clusters than those in the ω-3 group $F(1,67)= 4.6$, $P= 0.03$, 1.77±3.88 [95%CI, 0.14-3.58]), with a medium size effect of Cohen's d=0.52.

Table 15: Fluency strategies used by females to generate S words stratified by age

		8-9 year old Females							10-13 year old Females						
		Placebo (n=11)			Fish oil (n=15)			P†	Placebo (n=22)			Fish oil (n=22)			P†
		Mean	95%CI	SD	Mean	95%CI	SD		Mean	95%CI	SD	Mean	95%CI	SD	
Number S word Clusters	B	3.18	2.00-4.38	1.78	3.4	2.36-4.44	1.88	0.77	3.95	2.53-5.38	3.21	5.5	4.35-6.65	2.6	0.09
	E	3.91	1.41-6.41	3.73	4.13	2.78-5.49	2.45		6.59	5.50-8.41	3.27	5.05	3.51-6.58	3.47	
	Change	0.76	-4.7	3.49	0.73	-2.61	2.4	0.94	3.00	1.13-4.87	4.21	-0.45	-2.39	2.7	0.01
	P§	0.5			0.26				0.003			0.44			
Number S word Clusters & new assoc.	B	3.64	2.16-5.12	2.2	3.6	2.46-4.74	2.06	0.97	4.77	3.04-6.51	3.91	6.27	4.70-7.84	3.53	0.19
	E	4.55	1.97-7.12	3.83	4.8	3.26-6.34	2.78		8.69	7.02-10.4	3.76	6.73	4.68-8.78	4.62	
	Change	0.99	-4.09	3.05	1.2	-3.18	2.88	0.81	3.91	1.94-5.89	4.46	0.45	-3.14	3.54	0.02
	P§	0.35			0.13				P<0.001			0.55			

B, baseline, E, end Change: End value –Baseline value

P§-Value derived from Paired sample T-Test (2-tailed) B vs. E within groups
P†-Value derived from ANOVA: Comparisons between groups Change value derived from ANCOVA: Comparisons between groups (using baseline as covariate)
P -at significance levels

Older females (10-13 years old) in the placebo group produced significantly more clusters than those in the ω-3 group $F(1, 41)= 6.8$, $P= 0.01$ 1.27±3.91 [95%CI, 0.58-4.58]), with a large size effect of Cohen's d=0.82. They also produced significantly more *S* word clusters and new associated clusters than those in the ω-3 group $F(1, 41)= 5.94$, $P= 0.02$, 2.18±4.34 [95%CI, 0.48-5.10]) with a large size effect of Cohen's d=0.75. It is also important to note that for the 10-13 year old females there were marked differences between placebo and ω-3 PUFA baseline levels. These were not however at a level of significance ($P= 0.09$) as shown in Table 15.

In summary, over the 15 weeks children increased the number of words they were able to generate and, consistent with previous research, generated fewer *C* words than *S* words. Analysis of the *C* word data revealed no significant effects associated with the intervention for any of the five fluency measures. A similar analysis of the *S* words found no significant main effects for the intervention, age or gender on any of the fluency measures. However, there was a significant three-way interaction effect involving the intervention, age and gender for one of the fluency measures: the number of clusters generated. Examination of the mean scores showed that the effect resulted from an increase in the number of clusters generated by older girls in the placebo group over the time of the study that was not present in the other sub-groups. Due to the number of analyses undertaken this could be attributed to a Type 1 error. An interpretation of this significant effect in support of the intervention is discussed later.

Findings indicate that dietary supplementation with LC ω-3 PUFAs does not influence fluency ability in primary school children

Spelling Errors

Does dietary supplementation with LC ω-3 PUFAs influence spelling ability in primary school children and is any effect influenced by the children's age or gender?

The self-generated words written in the word fluency tests were used to investigate changes in spelling accuracy. As the number of words the children generated increased over the 15 weeks (as shown in Table 10) one would assume the number of spelling errors would also increase over this period. This was not the case for all groups, over the 15 weeks, there was an overall 33% increase (P=0.04) in spelling errors of *S* words for the placebo group, but only a minimal 4.7% increase (P=0.66) in the ω-3 PUFA group. It must be noted however that the difference between groups was not at a level of significance (P=0.32) but had a very large effect size of Cohen's d=1.6. There was an overall 170% increase (P=0.06) in spelling errors for the *C* words in the placebo group, and again, an insignificant increase (6.6%) in the ω-3 PUFA group (P=0.88). As with the *S* words, the difference between groups was not at a level of significance (P=0.12) and had a small to moderate effect size of Cohen's d= 0.32.

When investigating the percentage of spelling errors the children made per number of words they generated, the results are more pronounced. As shown in Table 16, the

percentage of *S* word spelling errors for the placebo group increased 0.12±13.3% whilst the ω-3 PUFA groups decreased by -0.96±10.3% (F=1.00 (2,160)*df*, *P*=0.33). With the *C* words the percentage of spelling errors also increased in the placebo group by 0.52±15.8%, but decreased by -1.29±12.1% (F=1.98, (2,98)*df*, *P*=0.162) in the ω-3 PUFA group. Although neither of these were at a significant level, interaction effects between treatment and age group F=4.80, (1, 98)*df*, *P*=0.03 were found for *C* words and between treatment, age and gender, for *S* words (F=2.46, (1,161)*df*, *P*=0.05) hence, the trends were investigated further.

Table 16: Mean percentage of words generated which contained spelling errors (when listing S and C words for the fluency tests)

Number of spelling errors per words written (percentage)											
	Placebo			Fish oil			*P*†	Interaction Group* Gender	Interaction Group* Age Group	Interaction Group* Gender *Age Group
	Mean	95%CI	SD	Mean	95%CI	SD				
***S* Words** B	9.16	5.60-12.7	11.8	8.21	5.96-10.5	8.23	0.52			
E	9.28	5.82-12.7	11.5	7.25	4.95-9.55	8.42				
Change	0.12	-3.9-4.12	13.3	-0.96	-3.84-1.91	10.3	0.33	0.40	0.08	0.05
P§	*P*= 0.43 *n*=(80)			*P*=0.38 *n*=(82)						
***C* Words** B	5.79	0.86-10.7	16.4	4.55	1.72-7.39	10.4	0.68			
E	6.31	2.39-10.2	13.0	3.26	1.34-5.14	6.86				
Change	0.52	-4.24-5.28	15.8	-1.29	-4.58-2.00	12.1	0.16	0.31	0.03	0.11
P§	*P*= 0.83 *n*=(45)			*P*= 0.44 *n*=(54)						

B, baseline, E, end Change: End value –Baseline value
Percentage of spelling errors was determined by the number of words spelt incorrectly as a percentage of the total number of words written.
P§-Value derived from Paired sample T-Test (2-tailed) B vs. E within groups
P†-Value derived from ANOVA: Comparisons between groups. Change value derived from ANCOVA: Comparisons between groups (using baseline as covariate)
Interaction effects were assessed using ANCOVA and adding *gender, *age group, *gender and age group as independent variables
P -at significance levels

On further analysis when the group was split for age and gender, no significant differences were found for the percentage of spelling errors with *S* words (data not shown). Whilst a significant decrease in *C* word errors was identified in the 8-9 year olds ω-3 PUFA group (-3.75±14.1) compared to the placebo group (1.17±19.9) as shown in Table 17, F=4.5(1,56), *P*= 0.04 6.18±2.9 [95%CI, -4.42-1.69], with a medium effect size of Cohen's *d*=0.56.

Table 17: Mean percentage of words generated which contained spelling errors, when listing *C* words for the fluency tests

	8-9 year olds							10-13 year olds						
	Placebo			Fish oil			*P*†	Placebo			Fish oil			*P*†
	Mean	95%CI	SD	Mean	95%CI	SD		Mean	95%CI	SD	Mean	95%CI	SD	
B	8.38	0.22-16.5	20.6	6.68	2.10-11.27	12.7	0.70	1.91	-0.43-4.26	4.71	1.46	-0.35 -3.27	4.09	0.80
E	9.54	3.30-15.8	15.8	2.98	0.70-5.25	6.31		1.46	-0.58-3.50	4.10	3.68	0.26-7.11	7.73	
Change	1.17	-6.70-9.04	19.9	-3.70	-8.80-1.39	14.1	0.04	-0.46	-3.69-2.77	6.50	2.22	-0.93 -5.38	7.12	0.24
P§	*P*=0.76 (*n*=27)			*P*=0.15 (*n*=32)				*P*=0.77 (*n*=18)			*P*=0.15 (*n*=22)			

B, baseline, E, end Change: End value –Baseline value

Percentage of spelling errors was determined by the number of words spelt incorrectly as a percentage of the total number of words written.

P§-Value derived from Paired sample T-Test (2-tailed) B vs. E within groups

P†-Value derived from ANOVA: Comparisons between groups. Change value derived from ANCOVA: Comparisons between groups (using baseline as covariate)

P -at significance levels

In summary the above spelling section highlights that over the 15 weeks as the children generated more words in the fluency tests there was a trend for the children in the placebo group to make more spelling errors than those in the ω-3PUFA groups. When considering the number of spelling errors made per number of words generated there was a trend for the placebo group to increase in spelling errors whilst the ω-3PUFA groups decreased in spelling errors. Due to interactions being found between treatment and age group further analysis was undertaken which identified a significant reduction in spelling errors in 8 to 9 year olds in the ω-3 PUFA group when generating the difficult *C* words. Again it is important to note that analysis involved a number of calculations and the significant findings only related to one-subgroup. Therefore the reliability of these findings is in question due to a possible loss in power and the increased chance of a Type 1 error. However it is believed that due to the trend of an increase in spelling errors in the placebo group and decrease in errors in the ω-3 PUFA group combined with the significant findings within this subgroup that dietary supplementation with LC ω-3 PUFAs may influence spelling ability in primary school children but only in a small subgroup of children (8-9 year olds)

asTTle Reading

Does dietary supplementation with LC ω-3 PUFAs influence reading ability in primary school children and is any effect influenced by the children's age or gender?

Data were gathered from direct testing. AsTTle was the second test the children completed with the researcher. This test took over 40 minutes. Once data were entered into the asTTle computer programme, detailed analysis was provided on every student (as shown in Appendix B7). The student's asTTle score was measured against the national mean, as was their score on utilising surface and deep thinking strategies. Significant improvements in all these areas were found however no significant differences were found between placebo and ω-3 PUFA groups, as can be seen in Table 18. As stated in the literature review the average growth per year is generally 25-50 points, so the increase made over the course of this study demonstrated excellent academic improvement. No significant interactions between treatment and gender, treatment and age group, and treatment gender and age group were found.

Computer analysis also provided cognitive processing scores measured against the national mean, in the areas of finding information, knowledge, understanding connections and inference. As there was no significant differences between treatment and placebo groups using these two main strategies (surface and deep thinking) an ANCOVA analysis was undertaken which also identified no significance differences for the processing scores for; finding information (P=0.69), knowledge (P=0.16), understanding (P=0.73), connections (P=0.28), and inference (P=0.83). Had results for the two main strategies (surface and deep thinking) identified significant differences these processing scores would have been investigated in depth to see where changes had specifically occurred. The asTTle attitude and behaviour scores (scores derived from the first few questions asked of the children when they started the asTTle test) have been outlined in the behaviour and attitude section later in this chapter.

Table 18: Variation of asTTle reading scores from the national mean

	Placebo (*n*=80)			Fish oil (*n*=82)			*P*†	Interaction Group* Gender	Interaction Group * Age Group	Interaction Group* Gender *Age Group
	Mean	95% CI	SD	Mean	95% CI	SD				
Surface Features B	-26.6	-46.0- -7.11	87.5	-52.5	-72.9- -32.2	92.6	0.07			
E	-6.84	-27.4 - 13.7	92.3	-10.4	-29.5- 8.77	87.1				
Change	19.7	4.51- 34.9	68.3	42.1	27.9- 56.4	65.0	0.12	0.72	0.92	0.51
P§	0.01			*P*<0.001						
Deep B	-48.7	-64.7- -32.7	71.9	-44.0	-59.2- 28.7	69.5	0.67			
E	-19.0	-35.4- -2.66	73.5	-26.0	-41.3- -10.8	70.0				
Change	29.7	19.2- 40.2	47.2	17.9	6.23- 29.6	53.2	0.16	0.13	0.34	0.20
P§	*P*<0.001			*P*<0.001						
asTTle score B	-47.9	-64.5- -1.4	74.3	-51.9	-68.3- -35.4	75.0	0.74			
E	-6.8	-27.4- 13.7	92.3	-10.4	-29.0 - 8.77	87.1				
Change	41.1	27.0- 55.2	63.5	41.5	28.1- 54.9	60.9	0.99	0.59	0.33	0.36
P§	*P*<0.001			*P*<0.001						

B, baseline, E, end Change: End value –Baseline value

P§ -Value**:** Paired sample T-Test Sig. (2-tailed) B vs. E within groups

P† -Value derived from ANOVA: Comparisons between groups. Change ANCOVA significance: Comparisons between groups (using baseline as covariate)

Interaction effects were assessed using ANCOVA and adding *gender, *age group, *gender and age group as independent variables

In summary the above results indicates a significant improvement in asTTle reading strategies over the course of the 15 weeks. However, this improvement was not affected by the intervention of a dietary supplement of ω-3 PUFA. Results from this study indicate that dietary supplementation with LC ω-3 PUFAs does not influence the reading ability (as assessed by the asTTle reading test) in primary school children.

Numeracy (Basic Facts)

Does dietary supplementation with LC ω-3 PUFAs influence retention of basic facts knowledge in primary school children and is any effect influenced by the children's age or gender?

Access to the school data base provided basic facts data. These common classroom tests required the children to complete 20 addition basic facts questions, followed by 20 subtraction, 20 multiplication and then 20 division basic facts questions. The majority of children found the addition basic facts relatively easy with the mean score being

14.1±6.2. Forty-two children (42%) scored at least 70% of the correct answers (14/20) at baseline for their addition basic facts scores. With 43 (26%) of the children scoring 20 out of 20 at baseline for addition and a third (56) of the children scoring 20/20 at the conclusion, a ceiling effect was evident thus preventing children showing further gains. Subtraction had a mean score of 10.2±6.64, whilst children found multiplication and division much harder with mean scores of 7.47±5.85 and 4.64±5.47 respectively. Over the 15 weeks, all groups showed significant improvements in basic facts mean scores. Children made the greatest improvement in division with children almost doubling their initial score from 4.64±5.47 to 8.17±6.56 (t=10 (2,126)*df*, P<0.001).

Females performed significantly better than their male counterparts with addition facts, with females at baseline scoring 15.5±5.2 compared with the males 13.08±6.73 (F=4.97, (1, 127)*df*, *P*=0.03), whilst at the end of the 15 weeks there was no significant difference between the genders, even when controlling for baseline (*P*=0.14). There was no significant difference between the genders for subtraction basic facts at baseline (*P*=0.77) or end (*P*=0.91), nor for multiplication basic facts at baseline (*P*=0.19) or end (*P*=0.35); there was also no significant difference between the genders for division at baseline (*P*=0.79), but there was at the end for division when controlling for baseline, with females scoring 9.02±6.1 compared with males 7.61±6.80 (*F*=6.13, (1, 125)*df*, *P*=0.02).

At baseline older children (over 10 years old) performed significantly better than their younger counterparts (8-9 year olds) with all basic facts scoring (as shown in Table 19). At the end of the 15 weeks there was still a significant difference between the age groups for multiplication (*P*=0.04) and division (*P*=0.05) even when controlling for baseline (as shown in Table 19).

Table 19: Maths basic facts scores across the age groups

	8-9 Year Olds (*n*=56)			10-13 Year Olds (*n*=73- baseline) (*n*=71- end)			*P*†
	Mean	**95%CI**	**SD**	**Mean**	**95%CI**	**SD**	
Addition - B	10.58	8.94-12.2	6.14	16.7	15.6-17.9	4.84	*P*<0.001 F= 40.8 *df*=128
- E	14.7	13.5-15.8	4.52	18.2	17.1-18.6	52.84	*P*=0.36 F= 0.84 *df*=126
Subtraction-B	7.05	5.47-8.63	5.89	12.6	11.1-14.0	6.19	*P*<0.001 F= 26.4 *df*=128
- E	11.2	9.68-12.3	5.42	15.2	13.5-16.1	5.33	*P*=0.86 F= 0.03 *df*=126
Multiplication B	4.27	3.01-5.53	4.70	9.92	8.64-11.2	5.46	*P*<0.001 F= 38.2 *df*=128
- E	6.63	5.25-7.54	4.61	12.4	10.6-13.4	5.82	*P*=0.04 F= 4.42 *df*=126
Division-B	1.80	0.95-2.67	3.19	6.82	5.45-8.19	5.87	*P*<0.001 F= 33.4 *df*=128
-E	4.82	3.29-5.69	4.90	10.8	9.08-12.1	6.60	*P*=0.05 F= 3.97 *df*=126

B, baseline, E, end Change: End value –Baseline value

P† Baseline value derived from ANOVA: Comparisons between groups (Baseline)
P† End value derived from ANCOVA significance: Comparisons between groups (using baseline as covariate)
P-at significance levels.

No significant differences were found in basic facts tests between children in the placebo and ω-3 PUFA groups when considering the total group (as shown in Table 20). The responses to the treatments were not modulated by gender or age group.

Table 20: Maths basic facts scores

Basic facts tests undertaken by children (20 questions per test)		**Placebo** (*n*=67)			**Fish oil** (*n*=60)			*P*†
		Mean	**95%CI**	**SD**	**Mean**	**95%CI**	**SD**	
Addition	B	14.7	13.2-16.2	6.26	13.3	11.7-14.9	6.24	0.22
	E	17.6	16.4-18.3	3.72	15.9	14.7-17.0	4.27	
	Change	2.64	1.76-3.53	3.63	2.57	1.41-3.73	4.49	0.08
	P§	*P*<0.001			*P*<0.001			
Subtraction	B	11.19	9.62-12.8	6.45	9.23	7.48-11.0	6.79	0.10
	E	14.01	12.6-15.4	5.78	12.8	11.4-14.2	5.59	
	Change	2.62	1.90-3.74	3.76	3.57	2.41-4.73	4.48	0.86
	P§	*P*<0.001			*P*<0.001			
Multiplication	*B*	7.97	6.53-9.41	5.91	6.78	5.29-8.27	5.77	0.32
	E	10.3	8.80-11.7	5.97	9.39	7.80-11.0	6.11	
	Change	2.28	1.31-3.25	3.97	2.60	1.59-3.61	3.90	0.90
	P§	*P*<0.001			*P*<0.001			
Division	B	5.09	3.72-6.46	6.23	4.05	2.68-5.42	5.29	0.37
	E	8.39	6.75-10.0	6.71	7.93	6.27-9.59	6.43	
	Change	3.30	2.24-4.36	4.35	3.88	2.94-4.83	3.66	0.46
	P§	*P*<0.001			*P*<0.001			

B, baseline, E, end Change: End value –Baseline value

P§ -Value**:** Paired sample T-Test Sig. (2-tailed) B verse E within groups
P†- Value derived from ANOVA: Comparisons between groups. Change ANCOVA significance: Comparisons between groups (using baseline as covariate)

As stated in the literature review, links have been identified between numeracy and literacy (Baldo & Dronkers, 2007; Bull et al., 1999; Geary et al., 1999; Gullick et al., 2011; Räsänen & Ahonen, 1995). For this reason the interaction effects between treatment and literacy ability (as determined by asTTle scores) on basic facts scores were investigated. Significant interactions were shown for division between treatment and literacy ability (P=0.03) (as shown in Table 21).

Table 21: Interactions with maths basic facts scores

Basic facts tests undertaken by children	**Interaction Group* Gender**	**Interaction Group* Age Group**	**Interaction Group* Gender *Age Group**	**Interaction Group* asTTle Ability#**	**Interaction Group* asTTle Ability# Gender**	**Interaction Group* asTTle Ability# Age Group**
Addition	0.07	0.52	0.09	0.96	0.48	0.32
Subtraction	0.98	0.21	0.75	0.47	0.70	0.53
Multiplication	0.30	0.12	0.25	0.97	0.73	0.99
Division	0.55	0.39	0.74	0.03	0.16	0.10

#Children were categorised into low (<-44) and high (≥-44) ability using asTTle reading scores (aRs) minus the national mean at baseline
Interaction effects were assessed using ANCOVA and adding *gender, *age group, *gender and age group, *literacy, *literacy and gender, *literacy and age group, as independent variables
P -at significance levels

It is important to note that due to the high number of calculations there is a significant chance of Type 1 error.

Those children in the top half of the school for asTTle reading scores (asTTle reading aRs score minus the national mean) and in the ω-3 PUFA group showed the greatest improvement in division facts, with a mean increase of 5.37±3.78, compared with 3.20±4.22 for the placebo group (F=5.43, (2, 65)df, P=0.02), with a medium effect size (Cohen's d= 0.58), as shown in Table 22. This indicates that more able readers who received 15 weeks of ω-3 supplementation were able to recall more division facts.

Table 22: Division basic facts scores from children with high or low asTTle reading (aRs) baseline scores

Division Scores	**asTTle score (minus the national mean) <-44**							**asTTle score (minus the national mean)≥-44**						
	Placebo (*n*=28)			**Fish oil** (*n*=33)			***P*†**	**Placebo** (*n*=39)			**Fish oil** (*n*=27)			***P*†**
	Mean	**95%CI**	**SD**	**Mean**	**95%CI**	**SD**		**Mean**	**95%CI**	**SD**	**Mean**	**95%CI**	**SD**	
B	3.14	1.45-4.84	4.36	1.79	0.79-2.79	2.81	0.15	6.49	4.52-8.45	6.07	6.81	4.33-9.30	6.28	0.69
E	6.57	3.91-9.24	6.88	4.45	2.79-6.11	4.68		9.70	7.63-11.8	6.36	12.2	9.93-14.4	5.71	
Change	3.42	1.65-5.21	4.59	2.67	1.57-3.77	3.10	0.64	3.20	1.84-4.57	4.22	5.37	3.87-6.87	3.78	0.02
P§	*P*<0.001			*P*<0.001				*P*<0.001			*P*<0.001			

B, baseline, E, end Change: End value –Baseline value
Children were categorised into low (<-44) and high (≥-44) ability using asTTle reading (aRs) scores (minus the national mean) at baseline
P§-Value derived from Paired sample T-Test (2-tailed) B vs. E within groups
P†-Value derived from ANOVA: Comparisons between groups. Change value derived from ANCOVA: Comparisons between groups (using baseline as covariate) *P* -at significance levels

All age groups significantly increased their ability in division over the 15 weeks. The improvement seen in division in children with high literacy ability was particularly evident in the 8-9 year old ω-3 PUFA group (5.63±3.93) compared with the placebo group (2.57±2.59), (F=4.90, (2, 20)*df*, *P*=0.04), with a large effect size (Cohen's *d*= 1.03) as shown in Table 23.

Table 23: Division basic facts scores of children in each age group with high (≥-44) or low (<-44) asTTle reading (aRs) baseline scores

Division Score for children with high or low literacy scores		8-9 Years old							10-13 Years old						
		Placebo			Fish oil			P†	Placebo			Fish oil			P†
		Mean	95%CI	SD	Mean	95%CI	SD		Mean	95%CI	SD	Mean	95%CI	SD	
<-44	B	1.21	-3.11	2.69	0.95	-2.14	2.28	0.76	5.07	2.23-7.91	4.92	3.08	1.17-4.98	3.15	0.23
	E	3.29	0.44-6.13	4.92	3.9	1.92-5.89	4.24		9.86	5.74-14.0	7.13	5.31	2.07-8.55	5.36	
	Change	2.07	0.10-4.04	3.41	2.95	1.60-4.30	2.87	0.35	4.79	1.72-7.85	5.3	2.23	0.12-4.34	3.49	0.2
	P§	*P*=0.04 (*n*=14)			*P*<0.001 (*n*=20)				*P*=0.005 (*n*=14)			*P*=0.04 (*n*=13)			
≥-44	B	3.86	1.49-6.22	4.09	1.38	-5.29	3.16	0.16	7.96	5.26-10.7	6.54	9.11	6.28-11.9	5.86	0.42
	E	6.43	3.30-9.56	5.42	7	3.05-10.9	4.72		11.5	8.96-14.1	6.2	14.4	12.1-16.6	4.63	
	Change	2.57	1.07-4.07	2.59	5.63	2.34-8.90	3.93	0.04	3.56	1.53-5.59	4.92	5.26	3.42-7.11	3.83	0.09
	P§	*P*=0.003 (*n*=14)			*P*=0.005 (*n*=8)				*P*=0.001 (*n*=25)			*P*<0.001 (*n*=19)			

B, baseline, E, end Change: End value –Baseline value
Children were categorised into low (<-44) and high (≥-44) ability using asTTle reading (aRs) scores (minus the national mean) at baseline
P§-Value derived from Paired sample T-Test (2-tailed) B vs. E within groups
P†-Value derived from ANOVA: Comparisons between groups. Change value derived from ANCOVA: Comparisons between groups (using baseline as covariate)
P -at significance levels

Both males and females significantly increased their ability in division over the 15 weeks. Males with high literacy (above the school asTTle mean score of -44), who were in the ω-3 PUFA group showed greater improvement in division over the 15 weeks (4.86±3.86), than those in the placebo group (2.05±3.56), (F=4.60, (2, 33)*df*, *P*=0.04), with a large effect size (Cohen's *d*= 0.75), as shown in Table 24.

Table 24: Division basic facts scores of males and females with high (≥-44) or low (<-44) asTTle reading (aRs) baseline scores.

Division Score for children with high or low literacy scores		Females							Males						
		Placebo			Fish oil			P†	Placebo			Fish oil			P†
		Mean	95%CI	SD	Mean	95%CI	SD		Mean	95%CI	SD	Mean	95%CI	SD	
<-44	B	4.33	-9.17	4.37	2.69	0.37-5.01	3.84	0.42	2.82	0.87-4.77	4.40	1.20	0.37-2.03	1.77	0.13
	E	9.83	2.05-17.6	7.41	6.00	2.39-9.61	5.97		5.68	2.74-8.62	6.63	3.45	1.85-5.05	3.43	
	Change	5.50	0.73-10.3	4.55	3.31	1.47-5.14	3.04	0.36	2.86	0.85-4.88	4.54	2.25	0.78-3.72	3.14	0.69
	P§	*P*=0.03 (*n*=6)			*P*=0.002 (*n*=13)				*P*=0.007 (*n*=22)			*P*=0.005 (*n*=20)			
≥-44	B	4.63	1.76-7.51	5.96	5.69	1.93-9.54	6.22	0.45	8.25	5.56-10.9	5.75	7.86	4.18-11.5	6.37	0.85
	E	9.05	6.20-11.9	5.92	11.60	8.37-14.9	5.36		0.30	7.10-13.5	6.84	12.70	9.15-16.3	6.17	
	Change	4.42	2.20-6.64	4.60	5.92	3.64-8.20	3.77	0.21	2.05	0.38-3.72	3.56	4.86	2.63-7.09	3.86	0.04
	P§	*P*=0.001 (*n*=19)			*P*<0.001 (*n*=13)				*P*=0.02 (*n*=20)			*P*<0.001 (*n*=14)			

B, baseline, E, end Change: End value –Baseline value

Children were categorised into low (<-44) and high (≥-44) ability using asTTle reading (aRs) scores (minus the national mean) at baseline

P§-Value derived from Paired sample T-Test (2-tailed) B vs. E within groups

P†-Value derived from ANOVA: Comparisons between groups. Change value derived from ANCOVA: Comparisons between groups (using baseline as covariate)

P -at significance levels

A reanalysis was undertaken to investigate whether there was any difference in division basic fact recall in children with high or low literacy and numeracy ability after ω-3 PUFA supplementation. Significant improvements in division scores were found with children, who demonstrated a high ability in both division and literacy. Children in the ω-3 PUFA group, with a high baseline asTTle score (≥-44) and a high baseline division score (>4) showed significant improvements in their division ability. A significant difference between the placebo (with a mean score change of 2.14±3.5) and the ω-3 PUFA groups (with a mean score change of 4.38±3.14) was found (F=4.30, (2, 36)*df*, *P*=0.05, with a medium Cohen's effect size *d*= 0.68), as shown in Table 25.

Table 25: Division basic facts scores for children with varying ability in division and literacy (using asTTle aRs reading scores)

Division scores considering those above and below asTTle score national mean		Below the school mean at baseline for division (<4)							Above the school mean at baseline for division (>4)						
		Placebo			Fish oil			P†	Placebo			Fish oil			P†
		Mean	95%CI	SD	Mean	95%CI	SD		Mean	95%CI	SD	Mean	95%CI	SD	
Division for asTTle score <-44	B	0.90	0.26-1.54	1.37	0.82	0.35-1.29	1.22	0.63	8.75	5.21-12.29	4.23	7.20	3.23-11.1	3.19	0.50
	E	3.95	1.32-6.58	5.61	3.00	2.04-3.96	2.46		13.10	8.67-17.6	5.33	12.60	5.07-20.1	6.07	
	Change	3.05	0.74-5.36	4.94	2.18	1.07-3.29	2.87	0.45	4.38	1.28-7.47	3.70	5.40	1.42-9.38	3.21	0.58
	P§	*P*=0.01 (*n*=20)			*P*<0.001 (*n*=28)				*P*=0.01 (*n*=8)			*P*=0.02 (*n*=5)			
Division for asTTle score ≥-44	B	1.28	0.58-1.98	1.41	0.82	-1.98	1.47	0.22	10.90	8.78-13.1	4.77	10.90	8.42-13.4	4.73	0.98
	E	5.72	3.27-8.18	4.93	7.64	4.54-10.7	4.61		13.10	10.6-15.6	5.46	15.30	13.1-17.5	4.09	
	Change	4.44	2.09-6.80	4.73	6.82	3.92-9.71	4.31	0.20	2.14	0.55-3.73	3.50	4.38	2.71-6.05	3.14	0.05
	P§	*P*=0.001 (*n*=18)			*P*<0.001 (*n*=11)				*P*=0.01 (*n*=21)			*P*<0.001 (*n*=16)			

B, baseline, E, end Change: End value –Baseline value

Children were categorised into low (<-44) and high (≥--44) ability using asTTle reading (aRs) scores (minus the national mean) at baseline

Children were categorised into low (<4) and high (>4) division ability using school division basic facts scores (either below school mean or above the school mean of 4) at baseline

P§-Value derived from Paired sample T-Test (2-tailed) B vs. E within groups

P†-Value derived from ANOVA: Comparisons between groups. Change value derived from ANCOVA: Comparisons between groups (using baseline as covariate)

P -at significance levels

In summary results from the analyses just reported indicate that the intake of ω-3 PUFA increased the basic facts scores for the youngest and more literate and numerate boys in this trial.

Apart from the 26% of the children who scored 20/20 at baseline for addition, and therefore could not show improvement, significant improvements were made in all basic facts scores over the 15 weeks. Most children doubled their score for division over this period. No treatment effect was evident between placebo and ω-3 PUFA groups when the total group was considered. However an interaction effect was evident between treatment and literacy (asTTle ability) and on division scores. Young males (8-9 year olds) in the top half of the school for asTTle scores and who were in the ω-3 PUFA group correctly scored significantly more division basic facts after 15 weeks than those children in the placebo group. Children in the ω-3 PUFA group with above

average scores for numeracy (division) and literacy (asTTle scores), also achieved significantly better in division basic facts after 15 weeks than those children in the placebo group.

As stated previously numerous calculations and analysis using only subgroups has reduced the power and significantly increased the chance of a Type 1 error. For this reason, there is no supporting evidence that dietary supplementation with LC ω-3 PUFAs influences retention of addition, subtraction and multiplication basic facts knowledge in primary school children. There is limited support to the notion that dietary supplementation with LC ω-3 PUFAs influences retention of division basic facts knowledge in primary school children but only in a small subgroup of children (those with high literacy and numeracy ability).

In this study changes were investigated objectively via tests and subjectively by asking those involved if they have noticed or felt there has been a change in specified aspects such as learning behaviour and attitude. The following section investigates children's, parents' and teachers' opinions about any perceived differences in memory, learning, behaviour and attitudes after 4 and 15 weeks of supplementation.

Behaviour/attitude

Does dietary supplementation with LC ω-3 PUFAs produce a change in behaviour at home, on the playground and in the classroom in primary school children, as assessed by parents, teachers and children?

Children, parents and teachers completed a questionnaire at the conclusion of the study to investigate if they perceived any changes in children's behaviour, attitude and ability to remember things associated with the experimental treatment. These questionnaires were also completed by the children after four weeks. During the initial four week period of supplementation the majority of the increase in blood plasma phospholipid DHA levels occurs (Cao et al., 2006) and any changes might be more noticeable as they would not yet be 'normalised' or easily attributed to other things.

Children's responses were grouped into whether they reported an improvement (improved a 'bit' or 'a lot') or not ('stayed the same' or 'worsened'). After the first 4 weeks more children (69%) in the ω-3 PUFA group thought that they got on better with

others, compared with those in the placebo group (46%). This was shown to be at a significant level using a Chi squared test χ^2 (1, N=134)=7.15, *P*= 0.007 and was still significant after 15 weeks χ^2 (1, N=148)=4.08,*P*= 0.04, as shown in Table 26.

There was no significant difference between ω-3 PUFA and placebo groups for the remaining three questions (behaviour towards schoolwork, behaviour at home and ability to remember things). After four weeks more children in the ω-3 PUFA group (71%) thought their behaviour towards school work had improved, compared with those in the placebo group (55%) although this difference was not at a level of significance χ^2 (1, N=150)=0.25,*P*= 0.06. These findings suggest that after 4 weeks of dietary supplementation with LC ω-3 PUFAs an improvement in behaviour towards others in the classroom can be perceived by those children in the ω-3 PUFA group

Table 26: The number of children who noticed changes in their attitude or behaviour after 4 weeks and 15 weeks of intervention

Specific questions in the children's questionnaire, (completed after 4 weeks and 15 weeks of the intervention)		Number of children in placebo group who ….		Number of children in ω-3 PUFA group who…		*P* *
		Felt "a little bit better" or "a lot better" *n*(%)	Felt "same", "a little bit worse" or "a lot worse" *n*(%)	Felt "a little bit better" or "a lot better" *n*(%)	Felt "same", "a little bit worse" or "a lot worse" *n*(%)	
After taking the capsules your behaviour towards classroom schoolwork is…	After 4 weeks	38 (55)	31(45)	46(71)	19(29)	0.06
	After 15 weeks	48(64)	27(36)	50(68)	24(32)	0.65
After taking the capsules the way you get along with other children in the classroom is…	After 4 weeks	32(46)	37(54)	45(69)	20(31)	0.007
	After 15 weeks	37(49)	38(51)	48(66)	25(34)	0.04
After taking the capsules your behaviour at home is….	After 4 weeks	42(61)	27(39)	40(63)	24(38)	0.85
	After 15 weeks	40(53)	35(47)	46(61)	29(39)	0.32
After taking the capsules your ability to remember things you have learnt at school is…	After 4 weeks	31(46)	37(54)	35(54)	30(46)	0.34
	After 15 weeks	45(60)	30(40)	48(64)	27(36)	0.61

*P * Value* derived from Chi Square test. Comparison between groups
P -at significance levels

Note: These figures do not represent all children. Those children who did not notice a difference in their attitude or behaviour (ie selected the 'I feel the same' category) were excluded in order to focus only on those who believed a change had occurred.

At the conclusion of the study (after 15 weeks) the parents were asked to complete a questionnaire to investigate if they noticed any changes in their child's attitude, behaviour or ability to remember things they had learnt at school. The majority of parents felt their child's attitude and behaviour as well as their ability to remember had improved, irrespective of which group they were in. When this was analysed using a Chi squared test, findings indicated that over the 15 weeks the parents did not notice any significant changes in their child's behaviour or attitude due to supplementation, as shown in Table 27. More parents of children in the placebo group than those of the ω-3 PUFA group believed their child's attitude towards homework had improved over the

15 weeks (*P*= 0.06). Although this wasn't at a level of significance it is important to note as it counters the notion that dietary supplementation with LC ω-3 PUFAs produces a noticeable improvement in behaviour at home in primary school children. Children were not asked their views on their attitude towards homework and therefore a comparison cannot be made as to whether their views support those of their parents. These findings suggest that dietary supplementation with LC ω-3 PUFAs does not produce an improvement in children's behaviour at home which is noticeable by parents.

Table 27: The number of parents who noticed changes in their child's attitude or behaviour after 15 weeks of intervention

Specific questions in the parent's questionnaire (completed after 15 weeks of the intervention)	Number of children in placebo group who ….		Number of children in ω-3 PUFA group who…		*P* *
	Felt "a little bit better" or "a lot better" *n*(%)	Felt "same", "a little bit worse" or "a lot worse" *n*(%)	Felt "a little bit better" or "a lot better" *n*(%)	Felt "same", "a little bit worse" or "a lot worse" *n*(%)	
Your child's attitude towards homework is…	31(94%)	2(6%)	27(77%)	8(23%)	0.06
Your child's behaviour at home is…	30(77%)	9(23%)	28(70%)	12(30%)	0.49
The way your child gets along with other children is…	32(87%)	5(14%)	33(89%)	4(11%)	0.72
The way your child gets along with adults is…	31(91%)	3(9%)	27(87%)	4(13%)	0.60
Your child's ability to remember things learnt at school is…	38(95%)	2(5%)	39(89%)	5(11%)	0.30

P * *Value* derived from Chi Square test. Comparison between groups

P -at significance levels

Note: These figures do not represent all children. Those parents who did not notice a difference in their child's attitude or behaviour (ie selected the ' are the same' category) were excluded in order to focus only on those who believed a change had occurred.

All teachers believed there were differences in children's attitudes, behaviours and learning over the 15 weeks. After analysing the teachers' concluding questionnaire it was determined that teachers did not detect any statistically significant differences in children's behaviour or attitude over the 15 weeks due to supplementation, as shown in Table 28. It was believed one teacher did not fully understand the questionnaire or did not engage in the process of completing it and for this reason this one teacher's

responses to the questionnaire were withdrawn from the study. When responding to the question “Did you notice a change in the child’s attitude to school?” children in the ω-3 PUFA group showed better behaviour with results almost reaching significant level (*P*= 0.054). When all data were reanalysed including the previously withdrawn class, little difference was noticed in scores for significance levels, with the level for attitude to school reaching (*P*= 0.07). As shown in Table 28 a trend was evident as a large number of teachers’ responses relating to behaviour and attitude (but not learning) showed children in the ω-3 PUFA group gained higher ‘improved’ responses whilst the placebo group received more ‘regressed’ responses.

Two teachers felt sure they were able to distinguish between children in the ω-3 PUFA and placebo groups. These teachers generally based their surety on the changes in children's behaviour. Some teachers commented on the difficulty of trying to remember initial behaviour. Teachers were also asked if they had noticed changes to the dynamics of the class/school since the beginning of the study and to elaborate on these. The eight out of ten teachers, who stated the dynamics had changed, attributed this to changes in behaviour, atmosphere and tone of the class/school.

In summary, a trend of teachers recording a greater improvement in children’s attitude towards school for those children in the ω-3 PUFA group than the placebo group was identified; however this was not at a significant level. There was no difference in teachers’ views regarding changes to memory or learning between those children in the placebo or ω-3 PUFA group. These findings suggest that dietary supplementation with LC ω-3 PUFAs does not produce an improvement in children’s behaviour at school which is noticeable by teachers.

Table 28: The number (%) of children which teachers (*n*=9) had noticed changes in child's attitude or behaviour after 15 weeks of intervention

Specific questions in the teacher's questionnaire(completed after 15 weeks of the intervention)	Number of children in placebo group who teachers had noticed a distinct change…		Number of children in ω-3 PUFA group who teachers had noticed a distinct change …		*P* *
	"Improved" *n*(%)	"Regressed " *n*(%)	"Improved" *n*(%)	"Regressed " *n*(%)	
Changes in general behaviour	18(62%)	11(38%)	22(73%)	8(27%)	0.35
Changes in attitude to others	20(69%)	9(31%)	22(85%)	4(15%)	0.17
Changes in attitude to school	22(69%)	10(31%)	25(89%)	3(11%)	0.054
Changes in learning	38(95%)	2(5%)	35(88%)	5(12%)	0.24

*P * Value* derived from Chi Square test. Comparison between groups

NOTE responses from one classroom teacher excluded (as explained earlier)

Note: These figures do not represent all children. Children whom teachers did not notice an attitudinal or behavioural change (ie identified as 'are the same') were excluded in order to focus only on those who believed a change had occurred.

The asTTle demographic questionnaire, as outlined in the methodology chapter and shown in Appendix B5 included six attitudinal questions. Children used a likert scale of smiley faces to indicate if they really disliked (grumpy face) through to they really loved (smiling face) doing a task e.g. reading at school. The asTTle demographic and attitudinal questionnaire was completed at baseline and again at the end of the study. The responses were loaded into the computer and the asTTle computer programme provided an average mean score for each child. Comparisons were made to investigate whether changes in children's attitude with regards to reading and using the library had changed over the course of the study. After 15 weeks of supplementation there was no significant difference with regards to reading and using the library between placebo (*n*=80) and ω-3 PUFA groups (*n*=80) with regards to asTTle attitude scores (F=0.35, (1, 159)*df*, P=0.50), as can be seen in Table 29. The asTTle attitudinal questions did not identify a noticeable difference in attitude towards reading and use of the library between primary school children receiving and not receiving dietary supplementation with LC ω-3 PUFAs.

Table 29: Children's changes in attitude over the 15 weeks, as measured by the six asTTle demographic and attitude questions in the asTTle reading test.

asTTle attitude score	Placebo		Fish oil		*P*†
	Mean±SD	Median (25 percentile-75 percentile)	Mean±SD	Median (25 percentile-75 percentile	
B	3.16±0.57	3.30 (2.80-3.50)	3.15±0.56	3.30 (2.70-3.70)	0.82
E	3.19±0.58	3.20 (2.80-3.70)	3.14±0.54	3.20 (2.80-3.50)	
Change	0.03±0.49	0.00 (-0.20-0.30)	-0.004±0.51	0.00 (-0.30-0.30)	0.50
*P**	0.54	(*n*=80)	0.95	(*n*=80)	

B, baseline, E, end Change: End value –Baseline value

*P**-Value derived from Paired sample T-Test (2-tailed) B vs. E within groups
P†- Value derived from Mann-Whitney (2-tailed)

In summary after 4 weeks of ω-3 PUFA supplementation significantly more children in the ω-3 group perceived an improvement in their ability to get along with others in the classroom. After 15 weeks these significant findings were still evident. Associations were made between ω-3 PUFA supplementation and an improvement in children's views of their attitudes towards their schoolwork. Although this trend was identified it was just short of being statistically significant as was the teachers' evaluations of the children's attitude towards school. No differences in children, parents' and teachers' views regarding changes to memory or learning, was identified between those children in the placebo or ω-3 PUFA group. This supports the idea that dietary supplementation with LC ω-3 PUFAs may produce a noticeable improvement in primary school children's behaviour as assessed by teachers and children but these behavioural changes are not noticeable at home by parents.

Predictions of treatment grouping

Children, parents and teachers were asked to predict whether they were in the ω-3 PUFA or placebo group and what basis they used for this prediction. The accuracy of these predictions is shown in Table 30. This section provides examples of the justification parents provided for their prediction. In order to ensure an accurate representation of these views both accurate and inaccurate responses have been provided. This has resulted in this section being longer than the previous objective section, but is indicative of the difference between reporting quantitative and qualitative results.

Of the 149 children, who completed this question, 84 (56%) accurately predicted which group they were in, as can be seen in Table 30. Many children predicted they were in the ω-3 PUFA group, with 95 (59%) predicting they were in this group and only 54 (33%) predicting they were in the placebo group (as shown in Table 30). A Chi squared analysis on the accuracy of this prediction indicates that children in the ω-3 PUFA group were more likely to correctly identify their group than those in the placebo group χ^2 (1)= 11.5, P<0.001. Unfortunately it is not possible to determine whether this reflects real self-observed change resulting from the ω-3 supplementation or 'constructed' change resulting from an expectation of change.

Table 30: Accuracy of participants' predictions

Predictions						*P**
Participant	**Predications**		**Accuracy of prediction**			
	Placebo	**ω-3**	**Overall**	**Placebo group**	**ω-3 PUFA group**	
Children	54/149 (33%)	95/149 (59%)	84/149 (56%)	32/75 (40%)	52/74 (63%)	0.001
Parent	61/133 (46%)	72/133 (54%)	72/133 (54%)	33/66 (50%)	39/67 (58%)	0.13
Teacher	78/158 (48%)	80/158 (49%)	89/158 (56%)	43/77 (54%)	46/81 (56%)	0.86

*P** value derived from Chi Square test: comparison between groups with regard to accuracy of predictions

The main reason given by children who predicted they were in the ω-3 PUFA group (correctly or incorrectly) was improved academic ability (Table 31). Those 15 children (38%) who thought they were in this group but were actually in the placebo group stated this prediction was based on an improvement in ability, whilst the 20 (38%) children who were actually in the ω-3 PUFA group and had predicted accurately also gave the same reason. This indicates that a large proportion of children who thought they were in the ω-3 PUFA group believed their work had improved. One child in the placebo group who thought they were in the ω-3 PUFA group wrote "because it made me feel good and smart, it is a good feeling" (Child number 234's Questionnaire-CQ234).

An improvement in maths ability was also a justification for predicting the ω-3 PUFA group (correctly or incorrectly), with those who thought they were in the placebo group not mentioning this subject. Spelling was also mentioned by a few children as shown in

Table 31. Some of these children's comments included "I am better at my spelling and maths and I am much smarter"(CQ 259) and "I now have the ability to concentrate for longer. I got a bit better spelling by getting more tens (probably referring to 10/10 in spelling tests). I have gotten better at behaving. I learnt better division"(CQ19). If a child stated which aspect of maths had improved, it was always timetables and more specifically division, with comments such as "because my divide-byes (sic) have gotten better" (CQ61)

The main reason given by children who predicted they were in the placebo group (correctly or incorrectly) was a lack of change. Of the 32 children who correctly predicted they were in the placebo group 12 (38%) gave a lack of change as the reason, whilst 9 (41%) of those in ω-3 PUFA group but thinking they were in the placebo group also mentioned this. These children wrote comments such as "because my memory is still the same. Because I don't feel different. My behaviour is the same. Because my concentration is the same" (CQ91). Behaviour was mentioned more by those in the placebo group. Six (19%) of the children who accurately predicted they were in the placebo group mentioned no change or a deterioration in their behaviour e.g. Child number 211 stated the reason was "because my behaviour is the same" (CQ211).

Changes to memory were mentioned more by those children in the placebo group with 3 children (9%) who correctly predicted this group and 6 (14%) who were in the placebo group but incorrectly predicted the ω-3 PUFA group, mentioning no change or a deterioration in their memory. Memory was barely mentioned by those children in the ω-3 PUFA group (with accurate or inaccurate prediction) with only 1 child (2%) using it as a justification for their group selection. Children's justification comments included "because I usually forget things, but now I haven't" (CQ14) and "because I've noticed a better memory"(CQ67).

Table 31: Children's main reasons for their treatment group prediction

Main prediction comments related to…	Children in placebo group *n*=75		Children in ω-3 group *n*=74	
	Correct (predicted placebo) *n*=32 (percentage of placebo group)	**Incorrect (predicted ω-3)** *n*=43 (percentage of placebo group)	**Correct (predicted ω-3)** *n*=52 (percentage of ω-3 group)	**Incorrect (predicted placebo)** *n*=22 (percentage of ω-3 group)
No change	12(16%)	0	0	9(12%)
Abilities	2(3%)	15(20%)	20(26%)	3(4%)
Behaviour	6(8%)	3(4%)	5(7%)	1(1%)
Attitude	1(1%)	0	3(4%)	1(1%)
Memory	3(4%)	6(8%)	1(1%)	0
Maths	0	5(6%)	7(9%)	0
Spelling	0	1(1%)	2(3%)	1(1%)
Taste	14(19%)	4(5%)	4(5%)	3(4%)

When asked to provide reasons for their prediction few children mentioned taste. From all responses only 18 (12%) accurately stated taste as one of the justifications for their prediction. Five children referred to the colour of the capsule and/or liquid in the capsule as being a reason for their prediction. Of these 5 children only 2 were able to accurately predict which group they were in. This further supports the notion that the treatments were blinded to participants. Fishy-burps is one of the main side effects of fish oil interventions (Lee, O'Keefe, Lavie, Marchioli, & Harris, 2008). However, no children mentioned fishy burps as reason for their prediction.

Parents (*n*=133) were also blinded to the treatment as there was no significant difference in responses between parents with children in the ω-3 PUFA or placebo groups χ^2 (1)= 0.9, *P*=0.34. Parents were unable to accurately predict whether their child was in the placebo or ω-3 PUFA group, with only 72 (54%) being able to accurately predict their child's grouping (as shown in Table 32). Slightly more parents thought their child was in the ω-3 PUFA group 72 (54%) than the placebo group 61 (46%).

The majority of the parents who felt their child had not changed over the 15 weeks predicted their child to be in the placebo group (as shown in Table 32). These parents made comments such as "nothing has changed" (PQ26), and "there were no changes in behaviour or learning that I noticed" (PQ5).

Those 39/67 (58%) parents who accurately predicted their child to be in the ω-3 PUFA group focussed on improved attitude, behaviour and memory. Those 33/66 (50%) parents who incorrectly predicted their child to be in the ω-3 PUFA group when they were actually in the placebo group also justified their choice with comments related to improved attitude, behaviour and memory. These parents who believed their children were in the ω-3 PUFA group stated such things as "because he's improved in his behaviour" (PQ37), "Does better at spelling. More alert. Shows kindness to younger children. Showed an improvement in general well-being and attitude" (PQ19).

A few parents were extremely positive about the perceived improvements in their child such as "Homework: Wants to do his homework, as before he hated to do it. Behaviour: Not so jumping around all of the time before. Gets along with other children: Talks to other children easier than shying away. Gets along with adults: Has listened a lot better to adults. Remembering things learnt at school: Has a good memory of what happens at school. As his change in his eating habits, sleeping a lot better and wanting to do homework as before he really didn't do any of this much at all"(PQ219).

A few parents mentioned their child had commented on the capsule taste and this had also influenced their prediction of treatment group (see Table 32).

Table 32: Parents' reasons for treatment group prediction

Parents main prediction comments	**Parents with children in placebo group** (*n*=66)		**Parents with children in ω-3 group** (*n*=67)	
	Correct (predicted placebo) *n*=33 (percentage of this correctly predicted group)	**Incorrect (predicted ω-3)** *n*=33 (percentage of this incorrectly predicted group)	**Correct (predicted ω-3)** *n*=39 (percentage of this correctly predicted group)	**Incorrect (predicted placebo)** *n*=28 (percentage of this incorrectly predicted group)
No change	15(45%)	1(3%)	0	19(68%)
Behaviour	1 (3%)	8(24%)	11(28%)	0
Attitude	0	13(39%)	13(33%)	0
Memory	1(3%)	7(21%)	12(30%)	0
Taste	3(9%)	4(12%)	1(3%)	1(4%)

Teachers were also blinded to the treatment as there was no significant difference in teachers' responses between ω-3 PUFA and placebo groups (χ^2 (1)= 0.00, *P*=0.91)

Teachers did not make comments or predictions for every child in the study. With the 158 responses an overall accuracy score of 56% was calculated indicating that the teachers were unable to accurately predict which group the children were in. They attributed children relatively evenly to groups with approximately 48% being predicted to be in the placebo group and 49% in the ω-3 PUFA group, as can be seen in the Table 33. Teachers accurately predicted 43/77 (54%) of the children in the placebo group. Their judgement appeared to be based on a lack of perceived change or poor or deteriorating behaviour. Teachers' comments included "remained pretty constant" (TQ93), "no change" (TQ220), "Regression in behaviour" (TQ166) and "No real change in behaviour/attitude evident" (TQ105).

Teachers' justified their predictions of those children in the ω-3 PUFA group (correctly or incorrectly) based on improved concentration, attitude, behaviour and ability. The teachers accurately predicted just over half the children in the ω-3 PUFA group (56% or 46/81). Comments related to these children included "Concentrates longer, Huge improvements in writing, more confident, making new friends"(TQ172), "Shows enthusiasm towards learning, and has been very positive" (TQ13), "Huge improvement in all areas" (TQ117), "Major Changes"(TQ48) and "Has shown more focus with her learning & has made good improvements in most subjects" (TQ57) .

Similar to the reasons given by children and parents when predicting those children in the placebo group (correctly or incorrectly) teachers also focussed mainly on the lack of perceived change. The teachers also commented 10 times on the deterioration in behaviour as a justification for those they thought were in the placebo group whereas they mentioned improved behaviour 14 times for those they thought were in the ω-3 PUFA group.

Comments relating to those children in the ω-3 PUFA group (predicted correctly or incorrectly) also included improvements in spelling and maths. These comments did not occur for those children teachers thought were in the ω-3 PUFA group but were actually in the placebo group. Positive comments relating to reading and writing were made by teachers when they thought the child was in the ω-3 PUFA group (correctly or incorrectly). Comments included " increased academic results in reading, increased focus, seems less flighty"(TQ131), "improvements shown in all areas especially

spelling" (TQ141), "improved score in basic facts, spelling and also rdg (reading) level" (TQ23).

Table 33: Teachers' reasons for treatment group prediction

Teachers' main prediction comments	**Teachers' prediction of children** (*n*=77) **in placebo group**		**Teachers' prediction of children** (*n*=81) **in ω-3 group**	
	Correct (predicted placebo) *n*=43 (percentage of this correctly predicted group)	**Incorrect (predicted ω-3)** *n*=34 (percentage of this incorrectly predicted group)	**Correct (predicted ω-3)** *n*=46 (percentage of this correctly predicted group)	**Incorrect (predicted placebo)** *n*=35 (percentage of this incorrectly predicted group)
No change	14(32%)	3(9%)	1(2%)	6(17%)
Behaviour	9(21%)	8(24%)	8(17%)	6(17%)
Attitude	2(5%)	8(24%)	16(35%)	5(14%)
Concentration	1(2%)	5(15%)	15(33%)	3(9%)
Abilities	0	6(18%)	14(30%)	9(26%)
Maths	0	0	2(4%)	1(3%)
Spelling	0	0	3(7%)	1(3%)
Reading	0	4(12%)	3(7%)	1(3%)
Writing	0	4(12%)	4(9%)	0

In summary the above section relating to the accuracy of predictions supports the notion that the children, parents and teachers were all blinded as to which children were in the ω-3 PUFA and placebo groups. Children were unable to accurately predict whether they were in the ω-3 PUFA or placebo groups, with the majority believing they were in the ω-3 PUFA groups and only a third (33%) of the children predicting they were in the placebo group. Those children who thought they were in the ω-3 PUFA group mentioned improved abilities (including maths and spelling). Those children who correctly predicted they were in the placebo group generally commented on the lack of change. For this reason one could assume that they believed the ω-3 PUFA could influence their learning and behaviour.

Parents were unable to accurately predict whether their child was in the ω-3 PUFA or placebo group. The parents who predicted their child was in the ω-3 PUFA group (correctly or incorrectly) generally commented on perceived improvements in memory, behaviour and attitude. Those parents who predicted their child was in the placebo

group (correctly or incorrectly) focused on the lack of change or improvement. Teachers also were unable to accurately predict whether the children were in the ω-3 PUFA or placebo groups. Teacher's comments referred to perceived improvements in academic ability, attitude, concentration and memory when referring to those children in the ω-3 PUFA group. The differences in teachers' and parents' comments relating their predictions of supplementation groupings (correctly or incorrectly) indicates they believed that the ω-3 PUFA could influence a child's attitudes, behaviour, ability to concentrate, focus and learn. The main reasons given by children, parents and teachers for their prediction did not involve taste and therefore this supports the notion that they were blind to the treatment.

Children's views of the merits of this research

The questionnaire, given to the children after 15 weeks, also contained additional questions to ascertain whether the children felt the study was worthwhile (see an example of the questionnaire in Appendix B10). Although this information is not relevant to the research questions a discussion is included here because the researcher believed investigating the benefits for the main stakeholders (in this case the children) was seen as a key part of the research ethics. Although the research had gained ethics approval it must still be acknowledged that the children gave up 10-15 minutes each day to support this research. This may equate to more time than is often able to be devoted to some areas of the curriculum. Teachers and the principal of this study had stated they were happy to support the research if there would be a benefit to the children. Irrespective of the findings related to the ω-3 PUFA, questions were included to investigate whether the children felt there had been an overall benefit. Overall the children did feel people benefited from being in the study, with 125/148 (84%) stating they agreed or really agreed, that they had, with 94 (64%) stating they agreed or really agreed that some people benefited.

Children indicated they had been fully informed and not pressured into undertaking the study. When asked why they wanted to be part of the study 104/148 (70%) of the children strongly agreed with the statement 'I wanted to be part of the study because it might help me learn more'. This figure rose to 127 (86%) if responses were included from those who agreed with the statement. The focus was on learning rather than getting the free ω-3, because when they were asked if they wanted to be part of the study to get

free ω-3 only 64 (42%) agreed or really agreed with the statement. Over 111/149 (74%) agreed or strongly agreed that the study initially sounded exciting. Children did not acknowledge peer pressure as a reason for joining the study with only 34 (23%) stating that they agreed or really agreed with the statement 'I wanted to be part of the study because my friend was going to as well'. 83/148 (56%) thought that the study had helped them understand science better. This is a lower figure than expected but may be attributed to the fact that many teachers did not emphasise the links between the previous term's science focus and this scientific study.

Initially children had been reluctant to swallow the capsules, yet after 15 weeks of swallowing 4 capsules everyday 91/112 (62%) of the children, strongly agreed with the statement that 'Swallowing the Omega-3 capsules was easy', with 112/147 (76%) agreeing or strongly agreeing, about the ease of swallowing the capsules.

As a way of investigating the children's belief in the impact of the ω-3 PUFA children were asked if when they grew up would they give their children ω-3 capsules, 57 (38%) strongly agreed whilst another 52 (35%) were unsure. In order to eliminate the financial consideration (as these were low SES children), they were asked whether they would take them every day if the ω-3 tablets were free and 107 (71%) agreed or strongly agreed that they would. If however they had to pay for the ω-3 but it was cheap the ratios altered, with only 59 (40%) agreeing or strongly agreeing they would take them daily. Overall the majority of the children enjoyed being part of the study and 117/148 (79%) agreed or strongly agreed that they 'would be part of a study like this again'. This positive holistic view is indicative of the sound methodology utilised by this study which is discussed in the following chapter.

Chapter 5

Discussion

Chapter 5: Discussion

This research study investigated the effects of ω-3 PUFA supplementation on the academic achievement, behaviour and attitudes of primary school children. This investigation focussed specifically on changes in fluency, spelling, reading and maths basic fact knowledge and ability after 15 weeks of supplementation. Whether any concomitant changes due to the intervention could be detected by the child and his or her parents and teachers was also investigated as was the children's consumption of ω-3 PUFA rich food sources. Analysis for each of these investigations has been provided in the chapter above. The literature review chapter has provided background information and international findings relating to each of these investigations. Rather than having separate chapters devoted to discussion, strengths and limitations and suggestions for future research, this chapter is slightly longer than might be expected but will encompass all three aspects as it is believed they are inextricably linked.

The chapter is broken into two sections. The first being a discussion of the findings related to literacy, numeracy, behaviour and predictions of children, parents, and teachers. This is followed by a discussion of the methodology as it is seen as the main strength of the study. Limitations and recommendations for future research are woven throughout this chapter where applicable and remaining key ideas are presented in the concluding sections of this chapter.

Discussion of ω-3 PUFA related findings

The following section provides a discussion of all major findings in this NZ ω-3 PUFA intervention study. Where possible, interactions were identified, whilst findings and suggested trends have been linked with other international studies.

Consumption

Prior to the commencement of the study parents completed a food frequency questionnaire, whilst a log of children's fish and seafood intake was recorded every school day. There was no significant difference in pre-study fish and seafood consumption when utilising food frequency questionnaire responses between those children in the placebo and ω-3 PUFA group, nor were there any significant differences when utilising daily child consumption responses. The children's daily responses and the parents' food frequency questionnaires showed a very similar low fish and seafood

consumption rate of approximately 4 servings of fish and seafood over the entire 15 week period (Tables 6 and 7). These findings vary from a 2002 nutrition survey for NZ children aged 5 to 14, which reported that fish and seafood was consumed weekly by 37% of NZ children (Parnell et al., 2003). This data however supported Australian findings which indicated that almost 8-9 children out of 10 did not eat fish or seafood (National Health Medical Research Council, 2006a). The median (interquartile range) LC ω-3 PUFA intakes were 88mg (46-159) for the 9-13 year olds in this Australian study, indicating that only 6% of children met the aSDTs for LC ω-3 PUFA per day (Meyer & Kolanu, 2011).

This data supports evidence that NZ like many other Western Countries has a relatively low ω-3 PUFA intake level (Hibbeln et al., 2006; Stonehouse et al., 2011). Numerous international studies have showed aspects of mental health being inversely proportional to countries' fish consumption (Sanchez-Villegas et al., 2007; Silvers & Scott, 2002; Tanskanen, Hibbeln, Tuomilehto, et al., 2001). Other studies have reported improvements in learning and behaviour linked with increased ω-3 PUFA intake (Amminger et al., 2007; D. Benton, 2007; Gesch et al., 2002; Itomura et al., 2005; J. McCann & Ames, 2005; Richardson, 2004b; Richardson & Montgomery, 2005; Stevens et al., 1996; Stevens et al., 2003). The following section focusses on the influence of ω-3 supplementation on fluency.

Fluency

Does dietary supplementation with LC ω-3 PUFAs influence fluency ability in primary school children and is any effect influenced by the children's age or gender?

When focusing solely on ω-3 PUFA studies with school age participants, only one previous study was found to include a WFT, and this was a NEPSY Test utilising F and S, which identified no significant differences between the ω-3 PUFA and placebo groups (Muthayya et al., 2009). This current NZ study is believed to be the first to use the TWFT to investigate the influence of increased ω-3 PUFA intake on children's word fluency levels. It too did not show significant effects with ω-3 PUFA compared to placebo for word fluency. The following paragraphs discuss these findings.

At the conclusion of this NZ study a retrospective G* Power calculation (Erdfelder et al., 1996) was undertaken using the (*S* words) TWFT data. This demonstrated that for the 15 weeks a sample size of 154 (77 per group) provided 89% power, for a medium effect size (d=0.5) and alpha level of 0.05 (2-tailed), thus indicating the study was adequately powered.

The baseline findings in this study support those of others who have found that younger children generate less words than older children (Sauzéon et al., 2004; Spreen & Strauss, 1998), and more high association (*S*) words are generated than low association (*C*) words (B. Cohen & Stanczak, 2000). Females produced more high association *S* words than males however this was not the case with low association *C* words, where there was no difference between the genders. These findings reflect those of Cohen and Stanczak (2000) who also used TWFT. Means for both genders across all age groups fitted well with the table of norms presented in the '*Compendium of neuropsychological tests'* by Strauss, Sherman and Spreen (2006).

This study stratified for gender and also investigated group and gender-related interaction effects. Future studies are recommended to also consider gender in this way due to the following findings. Girls out-performed boys, as was found in other studies involving verbal fluency tests and children under the age of 16 (Berninger & Fuller, 1992; Codorniu-Raga & Vigil-Colet, 2003; Kramer et al., 1997; Kramer, Yaffe, Lengenfelder, & Delis, 2003). Studies however which involved adults over 18 years old (often including elderly participants) found no gender differences (Brickman et al., 2005; Kempler et al., 1998; Maccoby & Jacklin, 1978; Tombaugh, Kozak, & Rees, 1999; Troyer et al., 1997). These gender differences have been attributed to females' better verbal skills, increased vocabularies and lower level of developmental reading disabilities (Herlitz et al., 1999; Kramer et al., 1997; S. Lanting et al., 2009; Lezak, 1995; Weiss et al., 2006), reduced lateralised hemispheric specialization, increased oestrogen levels, decreased testosterone levels (Koren et al., 2005) and an adult sized cerebellum at a younger age (Caviness et al., 1996). Boys have been also shown to be less able and more reluctant readers (R. Mitchell et al., 2008; M. Smith & Wilhelm, 2009; Watson et al., 2010; Wheldall & Limbrick, 2010) which needs to be taken into consideration in test conditions as they may misread instructions and require a longer time period.

Children generated more words as they got older and the size of clusters increased as well as the frequency of their switching supporting the findings of Roberts (1997), Weiss (2006) and Sauzeon (2004). This again highlights the need for future studies to also stratify for age and investigate possible age-related interaction effects.

When comparing which strategies the children used to generate words, the findings from this study reflect those of others (Robert et al., 1997; Sauzéon et al., 2004; Troyer, 2000; Weiss et al., 2006). The children in this study found generating low association words much harder, as evidenced by the low number of words produced. For this reason the number of words in each cluster (the mean cluster size) was generally larger for *C* words than for *S* words as also found by Troyer (2000). These findings were consistent across baseline and end groups in both placebo and ω-3 PUFA groups.

The letter *C* is not found in Maori, Arabic and many Pacific Island languages. Children from these backgrounds may have had more difficulty thinking of words beginning with *C* than European children. This would mean that to successfully undertake this task these children would heavily rely on their frontal lobe (which is influenced by ω-3 PUFA levels) (McNamara, 2013). When investigating the mean number of *C* words generated by these different ethnic groups there was no significant difference. These ethnic groups were not determined by the child's first language (often the predominate language spoken at home) but rather the ethnic mix the parents wished to identify with at time of enrolment at the school. In future research questionnaires a question should be included which determines the language most frequently spoken at home to increase the reliability and usefulness of this data. As test-retest reliability of the TWFT total scores have been stated as high and are seen as appropriate for use when tracking changes (Strauss et al., 2006), further research comparing large numbers (to give adequate power) of first language speakers of Maori, Arabic or the Pacific Islands with native English speakers is suggested.

At the time of marking, numerous additional clusters were identified (these were unable to be included if using Troyer's strict criteria) and the data re-evaluated, broadening the permissible cluster categories (as outlined in the methodology chapter). All the children in this study had completed a 10 week project on astronomy earlier in the year. It was therefore of no surprise words such as space, solar, system, stars, sun were listed (clustered) when they were asked to list all the words they could think of beginning

with the letter S. This study identified new clustering strategies not previously utilised in other TWFTs. No current NZ normative TWFT data was available and for this reason these baseline findings and trends may prove valuable for future studies. It is recommended that further research be undertaken to investigate the validity of these additional clusters. On analysis when these new cluster categories were permitted, the number of clusters was significantly higher but it must be noted however that in this intervention analysis their inclusion did not affect the results.

After 15 weeks of ω-3 PUFA supplementation no difference was found between ω-3 PUFA and placebo groups using the TWFT. Significant interactions were found in clustering strategies between group, gender and age. On further analysis this significance was only evident in the 10-13 year old girls and favoured the placebo group. However due to the numerous calculations undertaken and the resulting loss of power this result could be attributed to a Type 1 error. These findings indicate that dietary supplementation with LC ω-3 PUFA does not influence word fluency, in primary school children. These findings are consistent with all other childhood fluency studies which have not detected differences between treatment groups (Kairaluoma et al., 2008; Kirby et al., 2010b; Muthayya et al., 2009; Ryan & Nelson, 2008).

The above section explained the word fluency findings using the TWFT whilst the following section discusses the spelling errors generated when children underwent this fluency test.

Spelling

Does dietary supplementation with LC ω-3 PUFAs influence spelling ability in primary school children and is any effect influenced by the children's age or gender?

Verbal fluency is reflected in academic achievement but is not a true measure of it. Few studies have assessed academic ability as opposed to cognitive ability (Fergusson et al., 1982; Horwood & Fergusson, 1998; Rogan & Gladen, 1993). The following section outlines how this NZ ω-3 PUFA study has investigated changes to academic ability. In the first instance focusing on spelling followed by aspects of reading and then knowledge of basic maths facts.

Previous international findings related to spelling have been discussed in the literature review. These findings have highlighted the possibility that ω-3 PUFA supplementation may influence children's spelling ability. Results however are very inconclusive and tend to suggest improvements occurring only in children with special learning needs such as DCD, dyslexia and those with other learning difficulties. Over the last eight years not one of the six ω-3 PUFA intervention studies undertaken has identified spelling improvements in mainstream children attributed to ω-3 PUFA supplementation (Dalton et al., 2009; Kairaluoma et al., 2008; Kirby et al., 2010b; Milte et al., 2012; NEMO Study Group, 2007; Richardson & Montgomery, 2005). Dalton et al. (2009) however did identify a lack of regression of spelling ability in the group receiving ω-3 PUFA compared with the placebo group.

Testing children's spelling can be very problematic, because spelling strategies and errors are very individualised (Calhoon et al., 2010; Neilson, Waugh, & Konza, 2011). Rather than preselecting and reading aloud spelling words presumed to be at the appropriate level for the child, the TWFT utilised in this study required each child to rapidly list words. In doing so words were written to the complexity of each child's ability and spelling errors automatically occurred during this process. The TWFT had previously been utilised to investigate accuracy of spelling (Troyer et al., 1997), however it had never been applied to ω-3 PUFA intervention research.

At baseline the younger children made less spelling errors than the older children (10-13 year olds), however the older children generated considerably more words. After 15 weeks of schooling, as would be expected all children were able to list significantly more words in the same given amount of time. Because of this variability in the number of words generated, the percentage of spelling errors generated per the number of words written was used in analysis rather than the mean number of errors generated per child. After 15 weeks of ω-3 PUFA supplementation no significant differences were found between the percentage of spelling errors written by the placebo and ω-3 PUFA groups.

As shown in Table 16, the percentage of *S* and *C* word spelling errors for the placebo groups increased whilst errors decreased in the ω-3 PUFA groups. Although neither of these was at a significant level a consistent trend was believed to be evident. The children in the placebo group showed an increase in the number of spelling errors, both in this NZ study and also that of Dalton et al., (2009). In contrast, those in the ω-3

PUFA group in Dalton's study did not alter their spelling accuracy whereas an improvement in spelling ability was evident in the children in this NZ study. This decrease in the percentage of spelling errors in the ω-3 PUFA group compared with those in the placebo group was consistent when generating both *S* and *C* words. Interaction effects were subsequently investigated between group, age and gender. A significant interactionwas identified for *C* words between treatment group and age.

Amongst the 8-9 year olds a significant difference was evident between the treatment and placebo groups. The 8-9 year olds in the ω-3 group decreased in *C* word spelling errors compared with those children in the placebo group. This is the first time spelling improvements have been associated with ω-3 supplementation in mainstream children. One other study has also identified improvements in spelling associated with ω-3 supplementation however this study involved children with DCD (Richardson & Montgomery, 2005).

The large standard deviation of spelling word errors may have influenced the amount of power needed to detect differences between treatment groups. For this reason a retrospective power calculation was undertaken. A G* Power calculation (Erdfelder et al., 1996) identified that only 24% power was present when trying to detect a medium effect size (d=0.5) in *S* word spelling errors with an 0.05 alpha level (2-tailed) and was based on a SD of 10 and a meaningful difference of 2%. To achieve sufficient power a sample size of 788 children would be required to detect any significant difference.

For C word spelling errors a retrospective G* Power calculation (Erdfelder et al., 1996) identified that only 17% power was present when trying to detect a medium effect size (d=0.5) with an 0.05 alpha level (2-tailed) and was based on a SD of 11 (mean of all 4 SD- baseline and ends in the two groups) and a meaningful difference of 2%. The mean difference however was much bigger for the 8-9 year olds (5%), and 47% power was provided when trying to detect a medium effect size (d=0.5) with a 0.05 alpha level (2-tailed). Although this was still underpowered it may provide some reason as to why significant findings were only found with this age group.

An alternative or possibly additional reason why these changes were only evident in the younger children and not older children may be the dramatic increase in verbal fluency which occurs between the ages of 5 and 7 (Sauzéon et al., 2004). It is at this young age where children are making the most spelling mistakes, with very few errors being made

by 10-11 year olds (Steffler et al., 1998). These older children would have less opportunity to demonstrate improvement due to the lower proportion of errors which were generated. Children over the age of 10 years old have been shown to use very little cognitive energy to generate familiar words which generally are spelt automatically (Steffler et al., 1998). This lack of complexity of the task would reduce the cognitive load and amount of executive functioning. In this way the task could become a less effective measure to identify any differences which may have occurred due to supplementation. This may also be why the results were found when children generated *C* words but not *S* words. The fluency results in this study demonstrated that all children found generating *C* words much harder than *S* words. A change in spelling errors of one or two words would be more significant when only a few words had been listed, than with a large list generated by the high association letter S. The task was more challenging and it would not have been able to be completed automatically. The 63% of the children undertaking the TWFT who did not have the letter C in their indigenous alphabet would have found it an even more demanding task probably requiring frontal lobe activation. No interaction between total number of words generated and ethnicity was found within this small sample. As already suggested utilising a large sample size of participants from one of these ethnic groups is a recommendation for further research.

Analysis involved a number of calculations and the significant findings were only related to one-subgroup. Therefore the reliability of these findings is in question due to the increased chance of a Type 1 error. On the other hand, due to a lack of statistical power and consequently increased risk of a Type 2 error (concluding that there is no effect when in fact there is), the significant findings within 8-9 year olds combined with the clear trend of an increase in spelling errors in the placebo group and decrease in errors in the ω-3 PUFA group cannot be ignored and warrants further investigation with larger samples. For this reason it is believed that dietary supplementation with LC ω-3 PUFAs may influence spelling ability in primary school children, with a greater potential of improvements in subgroups of children (8-9 year olds).

This research has shown that word fluency tests can be used as an effective measure to detect changes in spelling abilities as suggested by Troyer et al (1997). Further research, with much larger sample sizes to ensure sufficient statistical power, needs to be

undertaken to determine whether ω-3 PUFA supplementation influences spelling ability and whether the TWFT is a suitable tool for this measurement.

asTTle Reading

Does dietary supplementation with LC ω-3 PUFAs influence reading ability in primary school children and is any effect influenced by the children's age or gender?

As stated above asTTle was one of the two main assessment measures in this study. It is a NZ based test and therefore was seen as very appropriate for these multicultural children. Each test had been calibrated to investigate the child's use of strategies (surface and deep thinking) and level of cognitive processing (finding information, knowledge, understanding connections and inference). Computer analysis enabled these scores to be measured against the national mean. Findings indicated there were no significant differences between treatment and placebo groups using the two main strategies (surface and deep thinking). Nor were significance differences identified for processing scores (finding information, knowledge, understanding connections and inference) using an ANCOVA analysis.

As asTTle has never been used in this manner a retrospective G* Power calculation (Erdfelder et al., 1996) was undertaken utilising data gathered in this study. This calculation indicated a sample size of 506 was needed to detect a significant difference of 20 between groups over a period of 15 weeks and using a SD of 80 (small effect size, (d=0.25) at 80% power and an alpha level of 0.05 (2-tailed). The large variation as indicated by the large SD highlighted the need for a much greater sample size. The lack of significant findings using this test could also be attributed to a short supplementation period not allowing enough time between pre-and post-test to detect differences in learning, possible lack of sensitivity of the tests and/or the level of calibration of the tests (tests were calibrated for national norms rather than this low socio-economic school with norms well below national levels).

Several studies have investigated the effects of ω-3 PUFA supplementation on aspects of literacy (Dalton et al., 2009; Kairaluoma et al., 2008; Kirby et al., 2010b; Lindmark & Clough, 2007; Milte et al., 2012; NEMO Study Group, 2007; Richardson et al., 2012; Richardson & Montgomery, 2005). However improvements in reading age or word reading have only been identified in children with special needs. These have included

DCD (Richardson & Montgomery, 2005), males with dyslexia (Richardson et al., 2000), children with reading difficulties (Richardson et al., 2012) and children with ADHD and learning difficulties (Milte et al., 2012). Whilst no improvements in reading age or word reading have been identified in mainstream children (Kairaluoma et al., 2008; Kirby et al., 2010b; Milte et al., 2012; NEMO Study Group, 2007; Richardson et al., 2012).

In this NZ study, although there was a significant improvement in asTTle reading strategies over the course of the 15 weeks, this improvement was not affected by the intervention of a dietary supplement of ω-3 PUFA. This indicates that dietary supplementation with LC ω-3 PUFAs does not influence reading ability (as assessed by the asTTle reading test) in primary school children. This lack of effect could be attributed to a lack of power and needs to be confirmed in a larger study.

Although the influence of ω-3 PUFA supplementation on literacy is commonly researched numeracy is rarely investigated (Kairaluoma et al., 2008; NEMO Study Group, 2007), as shown in Appendix D2. One aspect of this study included the investigation of the effects of ω-3 PUFA supplementation on children's basic facts recall. International ω-3 PUFA studies which have investigated links with numeracy have been outlined in the literature review whilst the findings related to this study have been detailed in the above results chapter. The following paragraphs will discuss and interpret the key significant numeracy basic facts findings and use these as a basis to provide recommendations for future research.

Numeracy (basic facts)

Does dietary supplementation with LC ω-3 PUFAs influence retention of basic facts knowledge in primary school children and is any effect influenced by the children's age or gender?

The numeracy tests required the children to complete a series of 20 question basic facts tests, over a one minute time period. The tests were undertaken on a regular basis and always in the same order, that being addition, subtraction, multiplication and lastly division.

Children in NZ learn their basic facts in stages. Children in each year level focus on different basic facts operations. Initially it is addition and subtraction and then the

emphasis moves onto multiplication and finally division. After two years at school, children (7 year olds) should be able to instantly recall all addition and subtraction basic facts to 10. At around 7-8 years old children should have mastered all addition facts to 20, subtraction facts to 10, and the multiplication facts for the 2, 5 and 10 times tables and their corresponding division facts. It is not until children are 9-10 years old when they will have instant recall of all addition and subtraction facts to 20, and have mastered all the multiplication and division facts up to the 10 times table. Children who are 10-13 years old are expected to be able to identify factors and common multiples of numbers (Ministry of Education, 2008a).

The majority of children in this study therefore would be expected to have mastered their addition basic facts to 20 (especially those who were 9-13 years old). As addition was the first test they completed one might presume the children would have been more focussed and less pressured by time. In the total group analysis children found addition the easiest of the four basic facts, with 37% of the children gaining near full marks at baseline testing. For this third of the children, baseline addition basic fact responses were probably automatic, making little use of their pre-frontal lobes to complete this task as it was not reliant on executive processes (Bull & Scerif, 2001). For children with high maths ability retrieving their basic addition facts would have come directly from long-term memory, and executive processes would not have had an important role as the skill had become more automatic (Bull & Scerif, 2001). When only considering those children who scored poorly in addition (less than 15/20), an increase was seen in the ratio of boys, to girls. This imbalance was even more pronounced in the 10 to 13 year olds where 70% of the children who scored less than 15/20 in addition were boys. As 7-8 year olds have generally mastered addition basic facts any future research wishing to utilise these tests will need to involve children under this age to ensure it is cognitively challenging. Again this highlights the need to stratify and check for interactions between treatment, gender and age.

The accuracy of basic fact subtraction recall was lower than addition, with 15% of the children making less than 1 error at baseline. This figure could be attributed to the 8 year olds only mastering their basic subtraction facts up to 10 (unlike the 20 expected with addition). The accuracy of basic fact multiplication and division recall was poor with only 3% of the children gaining near full marks at baseline (less than 1 error). Normally achieving children at 9-10 years old should have mastered their basic facts but

only their addition and subtraction facts would be in instant recall. A simple single digit basic facts calculation can be solved either through a direct route using operands (e.g. 5+6), transcoded into verbal code (five plus six), with rote memory producing the answer, or the answer can be generated through an indirect semantic route in which the operands represent quantities on which semantically meaningful calculations need to be undertaken. This indirect route is utilised when rote memory cannot be undertaken (Arsalidou & Taylor, 2011). Future research involving basic facts scores should therefore determine which methods children utilised.

Of the four arithmetic operations taught at school, division is the last to be introduced and is often considered the most difficult. It typically has the largest number of prerequisite skills (Foley & Cawley, 2003; Hatfield, Edwards, Bitter, & Morrow, 2000) and requires activation of the inferior frontal cortex (Baldo & Dronkers, 2007). This could also explain why the baseline mean for division, (the fourth test undertaken) was less than 4/20 however it could also be attributed to lack of time. Children were given one minute to complete each column and were then instructed to move on to the next column. Those children who ignored this instruction and continued to focus on completing the previous columns may have run out of time to attempt the last few columns. This could be especially true for those children who did not have instant recall of basic facts but were still at the early counting stages. Placing the division questions first would be a valuable strategy in any future investigations.

Whilst concentrating on division children would have been required to utilise executive processes unlike those who would be retrieving facts from long-term memory (Bull & Scerif, 2001). It is at times of new learning when maximum changes would occur between baseline and endpoint values. With these greater improvements an increased opportunity would be provided to demonstrate differences between placebo and active (assuming the active has influenced the learning). This was evidenced by the children making the greatest improvement in division basic facts over the 15 weeks, as the majority of the children almost doubled their initial score. Whilst considerable research is needed into the effects of ω-3 supplementation on the mathematical ability of school age children, emphasis may be best placed to initially focus on division for those children who may have the ‘easier’ basic facts in instant recall.

No significant findings associated with ω-3 supplementation were found for addition. Due to the large ceiling effect for addition the chance of detecting differences between treatment groups had been greatly reduced. Again no significant treatment effects were found for multiplication. This may be due to multiplication table knowledge mainly activating the left peri-sylvian regions, which is often linked with language (Dehaene et al., 2003). Addition and multiplication rely mostly on rote verbal memory, and over time this memorisation can reduce the WM load of prefrontal lobes (Ischebeck et al., 2006).

No significant effects between treatment groups were found for subtraction. Baldo and Dronkers (2007) identified subtraction involved activation of the inferior frontal gyrus. Subtraction also requires qualitative manipulations in both main cortical networks, with the prefrontal cortex controlling strategy choice, planning and hierarchical involvement (Arsalidou & Taylor, 2011). Those children who achieve below national expected levels have been shown to have poor WM function (S. Gathercole et al., 2004) and may have difficulty "inhibiting a learned strategy and switching to a new strategy" (Bull & Scerif, p.281). Further ω-3 PUFA research specifically targeting these participants undertaking a variety of both simple and complex subtraction equations is warranted. No significant effects between treatment groups were found for division. Using a retrospective G* Power calculation (Erdfelder et al., 1996) only 46% power was available to detect a small effect size (d=0.33) at 0.05 alpha level (2-tailed). This calculation was based on a SD of 6 and a mean difference between the groups of 2. This lack of power suggests the need for further research using larger sample sizes.

Total group analysis identified no significant effects between treatment groups and basic facts scores. The gender balance was relatively static for subtraction, multiplication and division unlike for addition as stated above. Only two ω-3 PUFA intervention studies were found which investigated maths reasoning and ω-3 PUFA supplementation (Kairaluoma et al., 2008; NEMO Study Group, 2007). Like this study, both of these studies found no significant effects with ω-3 PUFA supplementation.

Children's attainment in both literacy and numeracy has been shown to be significantly associated with WM (S. E. Gathercole, S. J. Pickering, C. Knight, & Z. Stegmann, 2004). For this reason literacy ability was investigated for interaction effects with treatment.

AsTTle scores were used as a method to differentiate children with high and low literacy ability. Interaction effects were evident for division. The more able readers who received 15 weeks of ω-3 supplementation were able to recall more division facts (a challenging mathematical task requiring utilisation of their frontal lobe). Subsequent analysis, showed significant differences in division basic facts scores between the ω-3 PUFA and placebo groups, for 4 sub-groups of participants in this study (those with above average literacy, 8-9 year olds with above average literacy, males with above average literacy, and children above average at both literacy and numeracy).

Due to the large number of calculations involved in these subgroup analyses the findings could be attributed to Type 1 error. Thus, moderate support is given to the notion that dietary supplementation with LC ω-3 PUFAs influences retention of basic facts knowledge in primary school children but only in a small subgroup of children (those with high literacy and numeracy ability). For this reason a discussion of relevant literature and research associated with these findings has been provided in order to justify new research foci.

Division is the hardest of the four arithmetic operations and requires a higher cognitive load (Foley & Cawley, 2003) and inferior frontal cortex activity (Baldo & Dronkers, 2007). The largest improvement in learning (a large difference between pre and post-test scores) in this NZ study was demonstrated using these division tests. This growth in ability and knowledge may enable differences to be observed between children receiving and not receiving supplementation. As this is the first research to identify this trend further investigation is suggested.

Using the asTTle scores as a method to differentiate between children with high and low literacy ability enabled significant findings to be identified. These 30% significant improvements were evident in high ability children in the ω-3 PUFA supplementation group undertaking division compared to those in the placebo group (see Tables 22-24). This partially supports the findings of Bull, Johnston and Roy (1999) who showed a significant association between maths and reading ability. It is believed this is the first time standardised school testing results have been used to identify literacy ability when investigating interaction effects in ω-3 PUFA studies. AsTTle certainly has never been used in this manner. It is suggested that future research ensure enough power is obtained to investigate the modulating effects of literacy ability.

Analysis was repeated considering the ages (8-9 year olds and 10-13 year olds) of the high and low ability children. A significant difference was found between ω-3 PUFA and placebo groups in the high ability 8-9 year old children when undertaking division. The children in the ω-3 PUFA group showed a greater improvement compared with those in the placebo group, as shown in Table 23. There was no significant difference however for 10-13 year olds or for this 8-9 year old group when they undertook any other basic facts test. A possible reason why significant findings were restricted only to the younger 8-9 year old children may due to the educational focus for these children. In NZ division is being introduced at this age, whilst addition and subtraction would be at the maintenance stage (Ministry of Education, 2008a). It was believed that the children in this study were working at or below their age appropriate stage on the NZ Number Framework, and as one would expect they did not initially have these division facts in instant recall. For this reason over the 15 weeks these younger children had more opportunity to show growth. These children would also find the task more difficult and therefore be utilising their frontal lobe to a greater extent. Future research should identify what level of maths basic facts the children are being taught and what facts are expected to be at maintenance level.

It is believed that adults rely more on the parietal cortex when solving arithmetic problems (Dehaene et al., 2003), whereas children appear to rely on the hippocampus and prefrontal cortex (Rivera et al., 2005). Further research is needed as to whether this change is due to brain maturation (age) or with the ability required to complete the task. Research using fMRI is needed to determine whether these children proficient with their basic facts (as in the above sub-group), activate their prefrontal cortex more and their intra-parietal sulcus less.

Males in the ω-3 supplementation group who also had high literacy ability demonstrated a 37% significant improvement in their recall of division basic facts compared to placebo (as shown in Table 24). This could be because males develop the ability to retrieve maths facts faster than females (A. Rocha et al., 2005). High ability males have been shown to be faster than their female counterparts to rapidly and automatically retrieve correct answers (Royer et al., 1999). These small differences in speed can prove to be significant with multiple questions when students do not complete the test due to time constraints. These males may have had more time to complete the division questions (which were the last test to be undertaken).

Those children in the ω-3 PUFA group who were 'bright' with regards to both numeracy and literacy (those with scores above the school mean for asTTle and also above the school mean for division basic facts scores) showed a 21% greater improvement compared with those in the placebo group (as shown in Table 25). One would also expect a significant difference to be found with those children with above average basic division facts scores and those children with asTTle scores higher than the national mean (as opposed to the school mean). Although results supported this trend, findings were only close to levels of significance.

The children showing these significant results were not only good at division they were also good at comprehension. Children who are good comprehenders have been shown to have greater listening spans and are believed to be not only quicker to process linguistic material but also make greater use of strategies. These children increase their memory load, unlike those participants with a low listening span, who continue to utilise a strategy even when it is not efficient (DeBeni et al., 1998). These children therefore have the ability to select the most appropriate strategy for the task at hand. In this instance the children in the ω-3 PUFA group appear to have been more focussed and able to select the most appropriate strategy in order to gain a higher division score than those in the placebo group. Few studies have investigated the relationship between performance in mathematics and levels of phonological ability (Holmes & Adams, 2006; Passolunghi et al., 2007). Performance levels in maths have been linked with executive function skills even when statistically controlling for reading ability, IQ scores and short term memory (Bull, Johnston and Roy, 1999). This is the first study to investigate links with numeracy and literacy ability. More research investigating these subgroups (high and low ability) and links between numeracy, literacy and ω-3 PUFA is needed.

Research has identified that when undertaking arithmetic operations children rely on different brain processes and use different strategies compared with adults (Dowker, 2006; Geary & Brown, 1991; Rivera et al., 2005). For this reason researchers need to take care when they generalise results and a greater emphasis needs to be placed on childhood rather than adult research.

This study has not clearly demonstrated that ω-3 supplementation improves the recall of maths basic facts with primary school aged children. However it has clearly

demonstrated the need for further research in this area, as a trend has been shown and there has been minimal research investigating the effects of ω-3 PUFA supplementation on mathematical ability. Where possible research should not solely focus on children with learning or behavioural needs, but should also concentrate on high achieving children. Emphasis placed on division may provide a better opportunity for detection of any effects of ω-3 supplementation. Ideally the sample size should be large enough to undertake subgroup analysis for age, gender and literacy ability without loss of power.

Antisocial behaviour has been linked with lowered ω-3 PUFA dietary intake levels (D. Benton, 2007; D. Benton & Gesch, 2003; Bourre, 2005; Dani et al., 2005; Gesch et al., 2002; K. Hamazaki et al., 2008; Iribarren et al., 2004; Vaddadi, 2006; Zanarini & Frankenburg, 2003). Changes to behaviour and attitude can impact upon all aspects of everyday life including academic achievement. Identifying these changes in attitudes and behaviour however can be problematic as detection measures usually require subjective assessment (Sinn et al., 2008). The next two subsections (Behaviour and attitude and Predictions) are devoted to discussing the findings gathered by these measures.

Behaviour and attitude

Does dietary supplementation with LC ω-3 PUFAs produce a change in behaviour at home, on the playground and in the classroom in primary school children, as assessed by parents, teachers and children?

In this NZ study changes to children's attitude and behaviour were investigated in multiple ways (a variety of questionnaires and an asTTle test) and involved all participants (children, parents and teachers).

Children's attitudes towards reading and their perception of others were investigated at the beginning of every asTTle test. After 15 weeks of supplementation no significant differences were found in these attitude scores between placebo and ω-3 PUFA groups. This test contained 6 questions which required the children to rate their opinion using a scale of 4 faces (smiley to sad). Although children's opinions of themselves and others' perceptions is very interesting for the classroom teacher it is believed this aspect of the asTTle test may be insensitive as a tool for intervention studies. In a Swedish open study involving 19 dyslexic 9-17 year olds the children were also asked about their enthusiasm to read. These children believed their desire, speed of reading and general

schoolwork had improved over the 20 week period of the intervention . It must be noted that this was an open study where the children self-reported their views knowing they were receiving ω-3 PUFA supplementation (Lindmark & Clough, 2007).

Very few ω-3 PUFA intervention studies have questioned mainstream children about their views of changes to their attitude, behaviour and ability to remember things they have learnt at school. Only three intervention studies involving mainstream children have investigated changes in behaviour or mood associated with ω-3 supplementation (K. Hamazaki et al., 2008; Itomura et al., 2005; D. Kennedy et al., 2009). These studies have produced inconsistent findings, as detailed in the literature review. Another three studies which did not identify any significant improvements in behaviour and attitude attributable to ω-3 supplementation, have involved children with ADHD (Voigt et al., 2001) and dyslexia (Kairaluoma et al., 2008; Lindmark & Clough, 2007).

When children in this study were asked after four weeks of ω-3 PUFA supplementation, if they had noticed any changes in their behaviour or ability to remember things significantly more children in the ω-3 PUFA group believed they got on better with others in the classroom compared with those in the placebo group . After 15 weeks this was still at a level of significance. This perceived improvement in behaviour towards others contrasts with findings from a three month Japanese study which involved similar aged children (9-12 years old) (Itomura et al., 2005). In this Japanese study children in the ω-3 PUFA group exhibited more extraggression than those in the placebo group. The females however were less impulsive, whilst the females in the placebo group were more physically aggressive. Significant findings were not found when the study was repeated in Indonesia using the same measures (K. Hamazaki et al., 2008). These results may be due to the children in the Japanese study being aware of their treatment grouping (as explained earlier) unlike those in the Indonesian study.

Although this NZ study showed no significant difference between treatment groups with regard to 'behaviour towards classroom schoolwork' it was however very close to significant and therefore a trend was believed to be evident, particularly when coupled with the significant results reported above. After 4 weeks more children in the ω-3 PUFA group felt their behaviour towards their schoolwork had improved compared with those in the placebo group.

This improvement in behaviour may be a direct result of the ω-3 PUFA or indirectly as a result of improved attention or an improved WM which may have improved self-discipline and mind wandering (Alloway & Alloway, 2010), which can then affect behaviour. In the Swedish open study (mentioned in the first paragraph of this section) children reported improvements in their general school work. It is important to note the question is specifically related to whether their schoolwork had generally improved rather than their attitude to school work, as in the current study (Lindmark & Clough, 2007).

This perceived improvement in behaviour in the first month may be due to increased DHA and EPA blood levels. The majority of the increase in plasma phospholipid DHA levels occurs in the first 4 weeks (Cao et al., 2006) and saturation can occur within a month with high doses (Arterburn et al., 2006; Katan et al., 1997). After six weeks more than a quarter (26%) of the children in a Swedish study believed they noticed some benefit attributed to supplementation (Lindmark & Clough, 2007). In this NZ study a clear trend towards an improvement in behaviour in the ω-3 PUFA group was evident after one month, but became less pronounced after 15 weeks as shown in Table 26. This may be due to the fact that after 3 months the children had different behaviour expectations (they were in the habit of being better behaved and didn't notice a behavioural change but rather saw it as "the way they were") or attributed the changes to peers, teacher etc., not something which had occurred over the 15 week supplementation period.

These findings indicate that dietary supplementation with LC ω-3 PUFAs produces a noticeable improvement in behaviour on the playground and in the classroom in primary school children as reported by children. This is the first time mainstream children in an intervention study have demonstrated an ability to detect improvements in their own behaviour after receiving ω-3 PUFA supplementation.

As this aspect of data gathering was not onerous or time consuming it should be seen as a standard addition to future research. This is especially applicable for studies involving mainstream children, as it has been with these children where significant differences have been demonstrated. This data gathering should occur after 4 weeks of supplementation and again at the completion of the study. Studies investigating teachers' and parents' views on perceived changes to children's behaviour whilst not

asking the children themselves, appears to be not only a lost opportunity for triangulation of data but also demeaning to the child.

Children develop within a socio-cultural environment and are influenced by knowledge and behaviour in the school and family settings (Dickenson, McCabe, & Anastasopoulos, 2003; Korat, 2011; Teale, 2003). Their behaviour and that of their peers can greatly influence attitudes and attainment. "Even a relatively small effect, when it affects all children in a classroom and when it is magnified by repeated exposures across years of elementary school, may be a substantial effect" (D. Thomas et al., 2011, p. 755). Children at risk of developing behaviour problems, are vulnerable to the influences of others in the classroom setting (D. Thomas et al., 2011). If one or two children were able to be calmed or more focussed in their studies this would influence others in the class and could alter the whole tone of the class and school.

After 15 weeks teachers were asked to identify changes in each child's 'general behaviour', 'attitude to others', 'attitude to school' and 'learning'. No significant differences were identified between treatment groups for any of these four criteria. A consistent pattern however was identified by teachers' regarding changes to the children's general behaviour and their attitude to others and to school. More children in the placebo group were believed to have regressed over the 15 weeks whereas more children in the ω-3 PUFA group were believed to have improved. Although the value for 'changes in attitude to school' was close to significance, it is important to note that these three values did not reach a level of significance but rather appeared as a trend. These findings are similar to those of Richardson et al. (2012) whose teachers rated children in the ω-3 PUFA group higher than those in the placebo group in 9 out of the 13 sub-scale scores. Again no significant findings were identified but a common trend evident. These findings do not indicate that dietary supplementation with LC ω-3 PUFAs produces an improvement in behaviour on the playground and in the classroom in primary school children which is noticeable by teachers. The observed trend in improvement in behaviour however warrants further research.

Teachers have also demonstrated the ability to detect behavioural changes attributed to ω-3 PUFA supplementation, however these studies have usually involved children with special needs (Gustafsson et al., 2010; Hirayama et al., 2004; Richardson & Montgomery, 2005; Stevens et al., 2003). Often special-needs teachers have worked

with children over a number of years. This enables them to have a sound understanding of initial behaviours and attitudes prior to the commencement of any intervention study.

This NZ study and that of Richardson et al. (2012) were undertaken midway through the academic school year. It is important to stress the relevance of the time of the school year when studies were undertaken and data gathered. In order for a teacher to identify changes in children's behaviour they would have to have known the children for an extensive period of time. For this reason any future studies involving teacher evaluation should ensure that at least six months is allowed for this process prior to the commencement of the study. It is therefore important for all publications to include this information in order for other researchers to make comparisons with findings.

Teachers were also asked if the dynamics of the class/school had changed since the commencement of the study. The majority of teachers (n=7, 70%) in this study commented on these changes and attributed them to improvements in behaviour, atmosphere and tone.

If the ω-3 PUFA had been able to positively influence behavioural changes in even a few children, this may have had a pronounced influence on class and school tone and atmosphere. Teachers are often able to detect these changes but may not necessarily be able to attribute them to one specific child, or if every child is just a little calmer and a little more focussed the whole dynamics of the class may alter without the change being able to be attributed to any one person. Many teachers acknowledge the enormous influence of the behaviour of one child on the dynamics of the whole class. If the behaviour of one disruptive child is modified the effect on the class can be enormous (Dishion & Patterson, 2006; Mrug & Windle, 2009; D. Thomas et al., 2011; D. Thomas, Bierman, Thompson, Powers, & Group, 2008). For this reason although the teachers may not have been able to identify specific changes to each child, the overall effect to the classroom dynamics was perceived to be improved by 70% of the teachers. Further research into this area may also support findings related to specific individuals. It must be noted however that this perception of change could be due to the increased teacher expectation of change after the ω-3 PUFA supplementation and/or their teaching.

Parents are in daily contact with their children and therefore are extremely familiar with their behaviours and attitude. For this reason it is not surprising that they are regularly involved in the assessment of their children's behaviour and learning. As stated in the

literature review 14 international studies have investigated parents' views of the influence of ω-3PUFA supplementation on the child's behaviour. Only two of these studies have involved mainstream children (Kirby et al., 2010b; Richardson et al., 2012). Although questionnaires have been utilised with parents of dyslexic children (Kairaluoma et al., 2008; Lindmark & Clough, 2007) this is the first study to utilise this form of data gathering with parents of main stream children.

Parents in this study were unable to detect significant changes to their child's behaviour, attitude or memory. This contrasts with other international studies where parents have identified improvements in attention and behaviour of those children in the ω-3 PUFA supplementation group (Hirayama et al., 2004; Joshi et al., 2006; Richardson & Montgomery, 2005; Richardson & Puri, 2002; Stevens et al., 2003). It must be noted however that not one of these significant findings involved 'normally-achieving' mainstream children, but rather involved children with ADHD (Hirayama et al., 2004; Joshi et al., 2006; Richardson & Puri, 2002; Stevens et al., 2003), DCD (Richardson & Montgomery, 2005) or children with learning difficulties (Richardson et al., 2012). These findings suggest that dietary supplementation with LC ω-3 PUFAs does not produce an improvement in behaviour at home in primary school children which is noticeable by parents.

Significant findings have been identified using a variety of measurement tools without one measure appearing more effective than another. It is therefore recommended that future studies include some form of investigation into the parents' perceptions of changes to their children's behaviours and attitudes. Research should also be undertaken in order to evaluate which assessment tool is the most effective.

After four weeks children receiving ω-3 PUFA supplementation were able to identify significant changes in their school behaviour, however these differences were less pronounced at the conclusion of the study. After 15 weeks, teachers and parents were not able to detect any significant differences in children's behaviour or attitude which could be attributed to treatment (as shown in Tables 27 and 28). Teachers however were able to identify an overall improvement in behaviour across the senior school. To avoid disruption and increased workload to the teaching staff, the parents and teachers were not asked for their opinions after 4 weeks but solely at the completion of the study. After 3 months, the parents and teachers may have had different behaviour expectations

as they were in the habit of seeing the children being better behaved and therefore didn't notice a behavioural change but rather saw it as "the way they were". They may have attributed the changes to peers, teacher etc., and not something which had occurred over the 15 week supplementation period. Some teachers commented that after 15 weeks they had difficulty remembering the child's initial behaviour. This was seen as a limitation and for this reason questioning parents, teachers and children after one month of supplementation would be recommended.

As stated this is the first time that normally achieving mainstream children and their teachers' and parents' have used a questionnaire to measure attitudinal and behavioural changes attributed to ω-3 supplementation. The results from this study indicate that children were able to detect changes in their behaviour towards others and possibly also towards their school work. Whilst teachers demonstrated a trend in their ability to observe these improvements in children's behaviour on the playground and in the classroom, parents however were not able to detect any changes in behaviour at home which could be attributed to ω-3 supplementation.

Predictions of treatment grouping

Attitude, behaviour and self-belief however can have a very influential role in day-to-day classroom achievement or success. Child, parent and teacher expectations have been shown to have a great impact on learning and achievement (Bishop, Berryman, Cavanagh, & Teddy, 2009). If a teacher or parent has high or low expectations these are often met (for a variety of reasons as explained in the literature review). Many parents and teachers are adamant they are able to detect changes well before they are able to be proven using standardised tests. Their estimations have been considered good predictors of children's development and achievement (Entwisle & Baker, 1983; Korat, 2011). If parents or teachers have predicted or perceived a child is receiving/or not receiving supplementation they may themselves act or behave differently which may produce a difference in the child's attainment or behaviour.

Only two other mainstream ω-3 PUFA intervention studies were found which also investigated 'parents' predictions (Itomura et al., 2005; Richardson et al., 2012). These studies used this prediction data to support the investigation of blinding and did not refer to the influence of changed perceptions. The Japanese Itomura et al. (2005) findings suggest that participants were aware of their treatment grouping. The notion

that participants were not blinded was clearly stated in the publication of these findings. The implication of participants possibly knowing the treatment group and the influence this may have had on the study as a whole was not however addressed. This Japanese study identified unique significant behavioural findings in extraggression (as detailed in the literature review). As participants were no longer blind to treatment groupings all results needed to be critically evaluated because changed participant expectations may have also affected the children's learning and achievement. As the findings of this study were unable to be replicated in Indonesia (Hamazaki et al. 2008) the implications of the un-blinding of participants need to be highlighted. This subsequent Indonesian study utilised capsules rather than food as the method of supplementation (and therefore reduced the possibility of treatment detection) which may be one of the reasons the Japanese results were unable to be replicated. This highlights the possible influence of participant expectations on results.

To check whether participants in this NZ study remained blind and to investigate the possible influence of their expectations, parents, teachers and children were asked to predict treatment grouping.

When investigating responses from the parents' questionnaire half of the parents (54%) accurately predicted the group of their child. These results were not at a level of significance. The reason for their prediction was not based on the child's reference to the capsule taste, smell or appearance but on behaviour (improvements or lack-of). Teachers also were unable to accurately predict which treatment group the children were in. Like the parents their reasons were based on a lack of perceived change or poor or deteriorating behaviour (placebo group) and improved academic ability, concentration, and memory (ω-3 PUFA group). These comments by the teachers' and parents' regarding their predictions of supplementation groupings (correctly or incorrectly) indicated they believed that the ω-3 PUFA could influence a child's attitudes, behaviour, ability to concentrate, focus and learn. This change in expectation could have influenced results, had participants in the study not remained blinded throughout.

More children (n=95, 59%) thought they were in the ω-3 PUFA group than the placebo group (n=54, 33%) and frequently justified this prediction by referring to improved abilities (including maths and spelling). This placebo effect has been shown to influence

results, and has been termed ‘halo errors’ or ‘source problems’ (Aman et al., 1987; Margo, 1999). Results can be influenced when people believe they are in the active group and therefore can respond differently especially with respect to emotions such as self-belief and emotional self-regulation (Moscucci, Byrne, Weintraub, & Cox, 1987). In contrast those children who correctly predicted they were in the placebo group generally commented on the lack of change. For this reason one could assume that like the parents and teachers, the children in this study believed that the ω-3 PUFA could influence their learning and behaviour.

The influence of these expectations on behaviour and learning can be very pronounced. If a teacher or parent has high or low expectations these are often met (for a variety of reasons as explained in the literature review). If parents or teachers had predicted or perceived a child was receiving/or not receiving supplementation they may themselves have acted or behaved differently which may have produced a difference in the child’s attainment or behaviour. The same would be true of the child themselves. For this reason identifying children, teachers’ and parents’ ω-3 PUFA and placebo group prediction is recommended as it may enable a better explanation for results.

The children in the ω-3 PUFA group were significantly more accurate with their prediction than those in the placebo group. This may indicate that children were able to identify a change in themselves or their ability which they attributed to ω-3 PUFA supplementation. An alternative argument could be that these children are un-blinded to treatment. This was not believed to be the case as the children were unable to predict the treatment group (56% accuracy as shown in Table 30) and their justification for their prediction focused more on behaviour and academic ability rather than taste, smell and appearance. Fishy-burps are commonly identified as one of the main side effects of ω-3 PUFA studies (Lee et al., 2008), however no child in this study mentioned fishy burps as a justification for their prediction. In this way the trial was believed to be blinded throughout the whole course of the study, similar to results by Frangou et al., (2006) Kairaluoma, et al.,(2008) and Raz et al., (2009) and unlike other studies where the treatment (or lack of) had a tendency to un-blind participants (Moscucci et al., 1987; Stoll et al., 1999).

The two most common reasons given, by children who correctly predicted they were in the ω-3 PUFA group, were improved academic ability and/or improved memory. When they gave more detail they generally mentioned spelling and maths, more specifically times tables and/or division, for example a child said "I now have the ability to concentrate for longer. I got a bit better spelling by getting more tens [10/10]. I have gotten better at behaving. I learnt better division"(CQ19). These were also the key areas where improvements in learning could have been attributed to ω-3 supplementation (either via a trend or significant results within a sub-group).

Children are often given daily basic facts recall tests and weekly spelling tests this may enable them to have a better understanding of their learning compared with other learning areas (subjects). However it should be noted that the two areas shown by testing to have improved in the supplementation group (spelling and division) were also the two areas mostly identified by the children as justification for their ω-3 PUFA group prediction. This highlights the possibility that at times a participants 'gut feeling' may detect change prior to subjective measures. These innate or intuitive views have been demonstrated at times to be accurate although rarely acknowledged or validated (as stated in the literature review). The influence of these predications can also affect findings and for these reasons the inclusion of an open ended question investigating participants' predictions is recommended in future intervention studies.

Rarely do studies investigate children's perception of change in their behaviour or attainment over the period of supplementation. It is equally rare for studies to investigate the child's justification for their prediction of treatment grouping. By providing the opportunity for an open ended response children are able to identify any change they feel applicable. In this way not only is the researcher broadening the field of data collection, they are also ensuring that they are not limiting and/or preventing the detection of possible significant findings. When interviewing children in a Finnish study children were questioned about whether the treatment had affected their reading skills (Kairaluoma et al., 2008). This research study however also investigated changes in spelling, attention, arithmetic and memory but these were not discussed with the child. In this way potential significant findings may not have been detected. Future studies are advised to provide children the opportunity to identify any changes they have observed over the supplementation period, either via an interview or an open ended question. This may enable potential significant findings to be identified.

The results from this NZ study were not only used to discuss blinding but also possible influences on attitude, behaviour and learning changes. When contact was made with parents to gather data this was seen as an opportunity to maximise data collection. Information on parents' perceived changes to children's behaviour and attitude as well as their prediction of treatment grouping was recorded. Only one other study has undertaken this strategy (Richardson et al. 2012). Another study asked parents if they had noticed changes to their child's behaviour but did not record their prediction (Kirby et al. 2010). Whilst another study did the reverse and asked parents about their predictions but not ask whether they believed their child's behaviour had changed (Itomura et al. 2005). This demonstrates this study's ability to utilise the opportunity to maximise the data gathered and was seen as a strength but also a recommendation for inclusion in future research.

This section was devoted to participants' predictions of treatment groupings. It demonstrated that parents, teachers and children were unable to accurately predict their treatment group.

This whole section focussed on a discussion of the findings, whilst the following focusses on the strengths of this study as well as the limitations and recommendations for future research.

Discussion of the methodology of this study

For several reasons the methodology was considered to be a major strength of this study. This multifaceted methodology was original in many ways. The research-informed design, assessment measures and subsequent analysis resulted in an innovative and unique approach. These elements will be elaborated upon in the following section and will be linked with material presented in the literature review and results sections.

Design Strengths

This study was unique in that it identified a school which was supportive of the study and developed an appropriate research methodology which was relatively non-intrusive to the participants involved. Rather than utilising and implementing the commonly used, well-controlled, laboratory setting for running cognitive studies, this study was conducted in the school setting. Consultation regarding the research design was

regularly undertaken with the school and enabled total 'buy-in' and minor modifications to be made throughout the process. This resulted in minimal disruption to children, classes and the teaching programme ensuring that time was not taken away from learning. Teachers were also able to use the study as a teaching opportunity and link to the previous term's school-wide science focus. This design however was also a limitation of the study as the outcomes explored (academic progress) were subject to naturally occurring forces (what teachers did, children's motivation for school, etc.) and were not under the researcher's control. All participants came from the same region and school with similar socio-economic backgrounds and therefore many of the additional confounding variables identified by Kirby (2010a) were reduced or eliminated. International studies have involved participants from a variety of schools, with only one other study being found where all the mainstream participants came from the same school (Dalton et al., 2009).

Utilising a double blind placebo controlled methodology was seen as a major strength of this study as it ensured unbiased results that were not influenced by any pre-conceived expectations (of participant or researcher) and allowed investigation of the casual relationship between ω-3 and academic achievement. Some children in this study accurately predicted their grouping and the possible implications for this is discussed in the findings section below. In at least one other study the children and/or parents were un-blinded by being able to smell or taste the fish oil (Itomura, 2005). It is important to note however the reasons the children in this NZ study gave for their predictions rarely involved taste and generally involved perceived change or lack of change in their learning and behaviour. These results are similar to those found by Milte and colleagues (2012) who were also confident that children were blinded throughout the study period.

Another strength of the design was that prior to randomisation children were stratified by age and gender to ensure equal numbers in treatment groups. This was important because although participants may be treated equally in an ω-3 PUFA trial, males and females have been shown to react differently to ω-3 PUFA supplementation (Baumgartner, Smuts, Malan, Kvalsvig, et al., 2012; Itomura et al., 2005; Vyncke et al., 2013; Welch et al., 2006). A review of international literature suggested that the DHA in the blood lipids of women was generally higher than in men (Decsi & Kennedy, 2011). These gender differences however have mainly involved adults but it is assumed that they could also exist in children. Gender-differences can also be found in

children's cognitive abilities (M. Casey et al., 1992; J. Fletcher, 1985; Geary, 1996; F. Rocha et al., 2005; Royer et al., 1999). These differences include such things as speed of basic facts recall (Royer et al., 1999), strategy flexibility (A. Rocha et al., 2005), verbal processing (Royer et al., 1999), vocabulary size (S. Lanting et al., 2009), fluency (Codorniu-Raga & Vigil-Colet, 2003) and reading (R. Mitchell et al., 2008).

Due to these differences in gender and the influence of age the potential modulating effects of age and gender was investigated. This was also strength, as very few studies have looked at interaction effects. Stratification controlled the potential confounding effects of these variables whilst investigating the interaction effects considered if different gender and age groups responded to the treatment in the same manner.

No ω-3 PUFA research could be found which identified age-related differences in school-aged children, unlike numerous adult ω-3 PUFA studies (Crowe et al., 2008; Dewailly, Blanchet, Gingras, Lemieux, & Holub, 2002; Dewailly et al., 2001; Tavendale, Lee, Smith, & Tunstall-Pedoe, 1992; Zhou et al., 2011). Age is a recognised factor in education (Haines et al., 1986). It has been correlated with children's self-efficacy and achievement (Bong et al., 2012) as well as activation of various areas of the brain such as frontal lobes (Gaillard et al., 2000). Differences in ability have been demonstrated in differing age groups in maths (Ministry of Education, 2008a) and fluency (Sauzéon et al., 2004). Rather than stratifying for age many ω-3 PUFA studies have stratified for grade or class distribution (Baumgartner, Smuts, Malan, Kvalsvig, et al., 2012; Dalton et al., 2009; Kairaluoma et al., 2008). Unfortunately children in each class can have not only a wide ability range but also a wide age range. This can result from children being promoted to a class ahead or held back in a more junior class for any number of reasons (including height, size, social skills, academic ability, class sizes, parental wishes, teacher's strengths etc.)(Montgomery & Hirth, 2011). For this reason the children in this study were stratified for age rather than grade or class level. Only two other ω-3 PUFA studies (Joshi et al., 2006; Milte et al., 2012) have included both age and gender as stratification measures.

Other aspects which contributed to the quality of this study's design included; the children had low habitual intakes of ω-3 PUFA rich foods, the duration of the study was 15 weeks and the high compliance and retention rates. These factors (as outlined in the

following paragraphs) although not individually seen as major influences when combined demonstrated the robust nature of the selected methods.

Previous studies involving animals suggest that only 8 weeks supplementation with DHA is needed to replete brain levels in rats deprived of ω-3 PUFA (Moriguchi, Loewke, Garrison, Catalan, & Salem, 2001), and 6-12 weeks are required for rhesus monkeys (Connor, Neuringer, & Lin, 1990). In humans it is believed that 8 weeks is sufficient time to saturate plasma phospholipids (Arterburn et al., 2006). This study's trial period of 15 weeks was therefore seen as adequate and was longer than the commonly used 8-12 week period (Amminger et al., 2007; Beblo, Reinhardt, Demmelmair, Muntau, & Koletzko, 2007; Bent, Bertoglio, Ashwood, Bostrom, & Hendren, 2011; K. Hamazaki et al., 2008; Hirayama et al., 2004; Itomura et al., 2005; Joshi et al., 2006; Kairaluoma et al., 2008; D. Kennedy et al., 2009; McNamara et al., 2010; Richardson & Puri, 2002; Vaisman et al., 2008).

This study involved children from a low socioeconomic area with diets containing low levels of food rich in ω-3 PUFA. The UK Scientific Advisory Committee on Nutrition (2004) recommends a minimum of 2 portions of fish (one oily) per week (Harris et al., 2008), whilst the World Health Organization (2003) recommends up to 2 fish meals per week (Garg et al., 2006; Yashodhara et al., 2009). However only 5% of the parents in this study stated their child ate one portion of fish high in ω-3 PUFA (> 1g/100g) more than once a week. It has been suggested that studies involving children from low-socioeconomic communities with low ω-3 PUFA intakes may produce a more pronounced effect than those consuming high amounts of ω-3 PUFA (Frensham et al., 2012). For this reason this one large school from a low socioeconomic area was therefore seen as ideal.

Compliance at home could not be assured and therefore the distribution of the supplements only occurred at school and did not occur during the weekends or during the 2 week school holiday (in the middle of the study). This method of supplementation had also been utilised by Dalton et al. (2009) and Baumgartner et al. (2012). Although it could have been seen as a limitation of the study it was not believed to have had a major influence on its findings as blood biomarker levels would have remained relatively static over this time period (as argued in the literature review). The strict and rigorous way in which the supplements were distributed at school ensured high

compliance rates (83%>80% compliant). The questionnaire return rate was also very high (82%) (Herber, Schnepp, & Rieger, 2009; Treweek et al., 2010) as were retention rates (91.4%) and withdrawal rates were low (18 children) compared with many studies involving 'minority' groups (B. Chang, Hendricks, Slawsky, & Locastro, 2004; Hatchett, Holmes, Duran, & Davis, 2000). This may have been due to the involvement of the whole school community.

The number of participants was relatively large compared to other studies (D. Kennedy et al., 2009; McNamara et al., 2010) however a larger sample size may have provided the statistical power needed to detect any minor treatment effects in the total group and when investigating subgroup effects as age, gender and ability. More detail of this is provided in the discussion of limitation below.

The above has discussed the suitability of the design whilst the following section will briefly outline the merits of the selected assessment measures.

Assessment Measures

This current NZ study is believed to be the first to use the TWFT to investigate the influence of increased ω-3 PUFA intake on children's word fluency and spelling ability. It enabled a new classifaction system to be developed. This was also the first time that the NZ designed asTTle test was used in an intervention study as well as enabling subgroup analysis by distinguishing high and low literacy ability. Yet another first was the investigation of the influence on children's basic facts recall by an increased ω-3 PUFA intake. Basic facts testing is a regular occurrence in the majority of NZ primary schools and access to the school data base enabled this data to be collected without intruding on valuable learning time. Investigating teachers', parents' and children's perceptions of changes to the children's attitude, behaviour and learning during and at the conclusion of the study was unique and yet another key addition. This selection of assessment measures demonstrates this study's ability to maximise the data gathered with minimal disruption to those involved.

The remaining key limitations and future recommendations for further research will be outlined in the following section followed by a brief summary of the chapter as a whole.

Limitations and future research recommendations

More ω-3 PUFA research has been identified as needed with mainstream school-age children (Bourre et al., 1993; Bryan et al., 2004) to add to the extensive adult and children with special needs (e.g. ADHD) findings. The school in this study used the children's age and school syndicate (class grouping) to form year groups, which prevented the involvement of children younger than 8 years old. It is known however, that children 7-8 years old would be undergoing a growth spurt, as well as being in a sensitive period, where particular skills were more readily acquired (Anderson et al., 1998; D. Benton, 2008a; Hughes & Bryan, 2003). It has been suggested that during growth spurts, nutrition interventions have the greatest impact on cognition (D. Benton, 2008a; Cheatham et al., 2006; Scott et al., 1974). The lack of involvement of these younger children is seen as a limitation of this study and although a trial including these younger children with their limited written literacy ability would prove challenging, if achieved it could show more pronounced results.

As stated earlier a limitation of this study was the lack of blood biomarkers which could have been used to investigate associations with changes to attitude, behaviour and academic achievement as also suggested by Schuchardt et al. (2010).

Retrospective G* Power calculations (Erdfelder et al., 1996) identified that this study was frequently underpowered. Data suggested there was an underlying pattern of non-significant numerical improvement in scores (spelling, division and child-rated behaviour). These are the areas where other researchers have found significant findings again indicating that this study may have been underpowered (similar conclusions were drawn by Kennedy et al., 2009). When undertaking sub-group analysis participant numbers were lessened often reducing the power even further. Restricting the study to only one school had many positive effects (as stated in the section above). This did however limit the sample size and therefore reduced the power to detect differences which was a limitation. As suggested by Schuchardt and colleagues (2010) larger sample sizes and longer study durations provide greater power to enable the detection of any differences attributed to treatment. This larger sample size would also enable investigation into interaction effects that cannot be carried out effectively with smaller numbers.

Although asTTle's strength was its ability for questions to be calibrated to measure specific depths of knowledge and learning, it was found to lack the sensitivity for a study of this duration. For this reason it was seen as a limitation of this study. A study involving a larger sample size over the entire school year is recommended to investigate the efficacy of this assessment tool for intervention research. If asTTle is to be used in further studies the schools involved should already be using this assessment tool. This would allow initial testing to be targeted at the child's established reading ability which would reduce the variance amongst the year groups, increasing power and making improvements more apparent.

This study has highlighted the need for additional double blind placebo controlled studies that are longer with larger sample sizes, investigating the optimum LC PUFA composition and dose (EPA, DHA, AA and GLA) (as also suggested by Schuchardt et al., 2010; Richardson, 2004a; Stordy, 2000; R. Taylor & Connock, 2007).

The following is a brief summary of this chapter. It will not re-iterate the discussion of the results but rather state all key findings.

Summary of discussion

The TWFT was used to investigate fluency and spelling. Retrospective G* Power calculations (Erdfelder et al., 1996) identified that the investigation was adequately powered and no significant effect of ω-3 PUFA supplementation was identified. These findings do not suggest that dietary supplementation with LC ω-3 PUFA influences fluency ability of primary school children.

Using the words generated when testing for verbal fluency a trend was evident across the spelling of both *S* words and *C* words. The children receiving ω-3 PUFA supplementation had a greater spelling accuracy rate compared to those in the placebo group. This was at a significant level with the younger 8-9 year old children in the ω-3 PUFA group when they underwent the difficult task of generating *C* words. Retrospective G* Power calculations (Erdfelder et al., 1996) identified that this study was underpowered to investigate spelling differences using *S* and *C* words. Even though significant improvements in spelling ability attributed to ω-3 PUFA supplementation were identified in 8-9 year olds, only 47% power was evident to detect these changes.

When considering these significant results and the trend of improvements in spelling accuracy across all ages and words, the notion that dietary supplementation with LC ω-3 PUFAs is able to influence spelling ability of primary school children is supported but only when considering a small subgroup of 8-9 year old children. These findings support the spelling results of Richardson and Montgomery (2005), and Dalton (2009) and highlight the need for futher research involving younger children.

Retrospective G* Power calculations (Erdfelder et al., 1996) identified that this study was significantly underpowered to investigate differences in asTTle reading ability. No significant differences between treatment groups were identified with asTTle reading tests. These findings therefore do not indicate that dietary supplementation with LC ω-3 PUFA influences reading ability, as measured by asTTle reading, in primary school children.

No significant differences between treatment groups were identified for addition, subtraction or multiplication basic facts recall. Further research is recommended using these basic facts but with younger ages and ability groups which are yet to reach recall. A retrospective G* Power calculation (Erdfelder et al., 1996) identified that this study had only 46% power to investigate small effect sizes when undertaking division testing. Significant differences however were identified in children's ability to recall division basic facts but only with specific sub-groups of children. Children with high literacy ability who received ω-3 PUFA supplementation were able to recall more division basic facts than those in the placebo groups (n=66). This was also true for those children who were highly literate and highly numerate (n=37), highly literate and 8-9 years old (n=22), highly literate and male (n=24). Research investigating changes in maths ability due to ω-3 supplementation is very scarce and therefore little is available to support these findings. For this reason only limited support is provided to the notion that dietary supplementation with LC ω-3 PUFAs is able to influence retention of basic facts knowledge (i.e. recall of division basic facts ability) in primary school children but only in a small subgroup of highly literate and numerate children. These findings warrant further research.

Parents, teachers and children were not able to accurately predict the children's treatment grouping. Justification for predication did not focus on taste or fishy burps as

found in other studies where unblinding was believed to have occurred (Moscucci et al., 1987; Stoll et al., 1999). More children in the ω-3 PUFA group were accurate with their predictions. The main reasons given for their choice focussed on improved maths and spelling ability and they often stated their division had improved. These were the only areas where subjective measures had identifed significant differences attributed to ω-3 PUFA supplementation.

There was a trend whereby more children in the ω-3 PUFA group felt they had improved (in behaviour, attitude and ability to remember) whilst more in the placebo group who felt they had regressed. After 4 weeks of supplementation significantly more children felt their behaviour towards others had improved compared with those in the placebo group. This was still significant after 15 weeks although less pronounced. Children in the ω-3 PUFA group also felt their schoolwork had improved compared to the children in the placebo group although this did not quite reach signifciant levels. The above findings provide limited support for the notion that dietary supplementation with LC ω-3 PUFAs produce a noticeable improvement on the playground and in the classroom detectable by primary school children.

Findings from the parents' questionnaires support the notion that dietary supplementation with LC ω-3 PUFAs do not produce a change in behaviour at home in primary school children which is noticeable by their parents.

When considering the teachers' questionnaires a trend similar to that of the children's questionnaires became apparent. More children in the ω-3 PUFA group were believed to have improved (in general behaviour, attitude and learning) whilst more children in the placebo group were thought to have regressed. Teachers identified more children in the ω-3 PUFA group to have improved in their attitude to school than in the placebo group. This however was not at a level of significance . The above findings suggest that dietary supplementation with LC ω-3 PUFAs do not produce a change in children's behaviour in the playground and in the classroom which is detectable by primary schoolteachers. The trend and near significant finding as well as the finding of 7 out of 10 of the teachers believing an overall improvement in the classroom and school had

occurred, highlight the need to ensure further research includes investigating opinions of child, parent and teachers.

The diversity of assessment measures and the inclusion of parents, teachers and children's views has meant that numerous calculations have been undertaken, therefore greatly increasing the chance of a Type 1 error. Any significant findings have involved only a subgroup of children or aspects which greatly reduced the power to detect meaningful results.

The over-arching holistic research question.
Does dietary supplementation with LC ω-3 PUFAs influence academic achievement in mainstream primary school children? *Analysis using a MANOVA suggested that dietary supplementation with LC ω-3 PUFAs does not influence academic achievement in mainstream primary school children.*

1. Does dietary supplementation with LC ω-3 PUFAs influence fluency ability in primary school children? *Dietary supplementation with LC ω-3 PUFA does not influence word fluency, in primary school children.*
2. Does dietary supplementation with LC ω-3 PUFAs influence spelling ability in primary school children? *Dietary supplementation with LC ω-3 PUFAs may influence spelling ability in primary school children, with a greater potential of improvements in subgroups of children (8-9 year olds).*
3. Does dietary supplementation with LC ω-3 PUFAs influence reading ability in primary school children? *Dietary supplementation with LC ω-3 PUFAs does not influence primary school children's reading ability (as assessed by the asTTle reading test.*
4. Does dietary supplementation with LC ω-3 PUFAs influence retention of basic facts knowledge in primary school children? *Dietary supplementation with LC ω-3 PUFAs may influence retention of division basic facts knowledge in primary school children but only in small subgroups of children (those with high literacy, 8-9 year olds with above average literacy, males with above average literacy, and children above average at both literacy and numeracy).*
5. Is the effect of LC ω-3 PUFA supplementation influenced by the age or gender of primary school children? *Age appears to influence the effect of LC ω-3 PUFA supplementation as mentioned above. The youngest children in this study (8-9 year olds) at times demonstrated a more pronounced effect than their older (10-13 year old) counterparts (as mentioned above). Gender had minimal effect other than in the division findings stated above.*
6. Does dietary supplementation with LC ω-3 PUFAs produce a change in behaviour at home, on the playground and in the classroom in primary school children, as assessed by parents, teachers and children*? Findings in this study suggest dietary supplementation with LC ω-3 PUFAs may produce an improvement in behaviour towards others and possibly also towards their school work which is noticeable by children. Teachers demonstrated a trend in their ability to observe these improvements in children's behaviour on the*

playground and in the classroom, however parents did not perceive improvements in their child's behaviour at home

The following Conclusion chapter will outline what these findings may mean for NZ children, parents, teachers and researchers.

Chapter 6

Conclusion

Chapter 6: Conclusion

The majority of ω-3 PUFA research has been based on traditional internationally developed neuropsychological and IQ based tests (Fergusson et al., 1982; Horwood & Fergusson, 1998; Rogan & Gladen, 1993). Scoring highly in these tests does not automatically equate to a similar score in achievement tests (Reis & McCoach, 2000), just as standardised achievement tests may not be an indicator of classroom performance. Pirozzo (1982) believed that over half the gifted children who scored in the top 5% of intellectual ability on individualised IQ tests do not demonstrate comparable school achievement. Therefore teachers and parents are less interested in slight increases in these scores and more interested in how these are reflected in classroom practice (Paris & McEvoy, 2000). Is their child better at reading, spelling or maths? Are they better behaved and more focussed? These are the aspects of research which have meaning to them. Until these questions are answered they are at the mercy of advertising companies.

In this unique NZ study changes to both cognitive and academic ability were investigated. Traditional word fluency tests were included to add rigor in case the tests proved to be not sensitive and less than adequate over the time span. Classroom and national standardised testing were also included and the mixture of these two was shown to be effective.

The development of literacy and numeracy is seen as the 'fundamental role of school' (Ministry of Education, 2012) and as such occupies a large proportion of the child's school day. The ability to read and write has a major influence on all aspects of education (Miles & Stipek, 2006; Worthy & Viise, 1996). For this reason investigating possible improvements in aspects of literacy due to ω-3 PUFA supplementation, is extremely valuable. It is also likely that anything that affects literacy has the potential to affect other curriculum areas as well.

In this NZ study no significant changes in fluency and reading asTTle scores were associated with ω-3 PUFA supplementation. Significant improvements in 8-9 years olds spelling ability however were associated with ω-3 PUFA supplementation. If supplementation is able to have an impact on spelling the flow-on effect of this could be profound. Spelling is often the first thing noticed when reading someone's written work and is considered an indicator of intellectual and academic ability. Increasing the

accuracy of spelling can help address academic, vocational and social needs, and may also positively impact on their motivation to communicate through writing (Berninger & Richards, 2002).

This heavy emphasis on literacy and numeracy in NZ (Ministry of Education, 2012) has reflected a call for "back to basics" and with it a focus on the learning of basic maths facts (Yates, 2009). These basic facts are seen as the foundation for arithmetic and therefore ensuring children have total recall of all maths basic facts is seen as crucial (Gray, 1991). It is believed that by accurately and rapidly producing answers to basic facts the load on working memory is reduced enabling a person to develop more complex problem solving skills (Tronsky & Royer, 2002). Proficiency with basic facts may place you at an advantage compared with others. Identifying changes in basic facts ability requires more power than this NZ study provided in most areas. Where more power was provided (division) significant differences were evident. The findings in this NZ study identified division improvements associated with ω-3 PUFA supplementation, in 8-9 years olds and also those who were highly numerate and/or literate. In light of these findings and recognising parental interest in this area further investigation into the effect of ω-3 PUFA supplementation on basic facts recall is highly recommended.

Learning is socially-constructed. By influencing a single child's behaviour you can not only influence that one child's learning, their relationships with others, and their self-esteem, but also the dynamics of the classroom and therefore the learning of others. This research has demonstrated that ω-3 PUFA supplementation can influence a child's perception of behaviour and relationships with others and may influence parents' and teachers' perceptions of the child's attitude to others and schoolwork. Whether this is due to the direct effect of ω-3 PUFA or indirectly due to changed expectations warrants further investigation.

Trends and significant findings amongst subgroups in this study have highlighted numerous directions and foci for future studies however this study has generated more questions than answers. Its methodology was seen a strength and enabled the identification of new analysis strategies, new variables and trends. It highlighted the need and benefits for involving children with both high and low ability who are mainstreamed rather than with special needs, across a wide range of learning areas. Asking parent, teachers and children specific and open ended questions ensured a rich

base of data was sourced. Full school and community engagement enabled a good compliance rate and minimised the loss of valuable learning time. The success of this study is largely due to the commitment of a school staff keen to find ways to make a difference for children. The study identified no clear benefits for ω-3 supplementation but identified the need for the funding of further research in this area.

Any future research would benefit from adapting this study's basic design to incorporate the changes recommended in previous chapters.

LC ω-3 PUFA's are among the most crucial molecules that determine a brain's integrity and ability to perform (C. Chang et al., 2009). For this reason identifying the impact of omega-3 supplementation on academic achievement, using tests which parents and teachers are familiar with such as spelling and basic maths facts will enable parents to make informed choices about dietary changes and/or purchases of supplementation. It will also provide teachers with additional suggestions, for parents keen to support to children's progress, based on NZ research rather than marketing and promotional material.

Over one hundred years ago Kirkpatrick stated

> Unlike other machines, the brain is always in process of construction, always being modified and never completed...Every time the mind does a thing it becomes a different mind; hence the factors of nature and nurture are almost inextricably mingled in physical development. The question is often asked whether certain characteristics are native or acquired. The answer might be in nearly every case, "They are both". (Kirkpatrick, 1908, pp. 8-9)

Numerous trials have identified gains due to ω-3 PUFA supplementation. Little research however has been undertaken to investigate how these culminate into academic gains. Identification and utilisation of classroom academic tests as well as longitudinal studies are needed to investigate the effects of ω-3 PUFA supplementation on general academic outcomes. When considering the final application of this knowledge, what the parent or teacher wants to know is "how might ω-3 PUFA help this child?" This study has taken a step forward in answering this question.

With the immense worldwide emphasis for schools to go 'back to the basics' (Kalantzis & Cope, 2008; Luke, Woods, & Weir, 2013) further research is needed into the effects

of ω-3 PUFA and academic achievement in numeracy and literacy ability. Traditionally a great deal of money has been spent on assisting those children struggling to gain average grades often at the expense of those more able children. Perhaps a more unconventional way may be a dietary change or supplementation.

The majority of research into ω-3 PUFA is undertaken by researchers, scientists and doctoral students. No literature was found identifying ω-3 PUFA research with children, undertaken by a teacher or educationalist. It is no surprise then, that research findings are routinely published in food and nutrition, psychological, neuropsychological and scientific journals. An educationalist can see the importance of passing findings back to the sectors which enabled the research to be undertaken. With parents of children especially those at primary school tending to be in close contact with teachers (J. Epstein & Dauber, 1991; Stevenson & Baker, 1987) any findings or new knowledge which could help improve children's learning would be quickly passed on. The majority of publications which are most commonly read by teachers do not rank highly in international PBRF publication scores and hence many researchers are not willing to and do not feel it warrants the time taken to rework, adapt and gain approval from the original publishers to publish articles in lowly ranked 'practitioner magazines/journals'. Yet in fact do we not have an ethical obligation to pass on findings to the communities which supported the initial research? The results of this research will be disseminated through educational forums.

If we are able to influence children's academic success by changing nutritional intake then children and society as a whole must benefit. Conversely if we know that those people with reduced levels of ω-3 PUFA consumption may be disadvantaged and at an academic disadvantage and we choose not to encourage dietary changes should we not be held accountable? However if we find ω-3 PUFA supplementation does not clearly influence academic achievement then are we also not obliged to tell the public? Overall the findings of this study did not support the notion that dietary supplementation with LC ω-3 PUFAs results in increased academic achievement in primary school children. It did however highlight that further research is needed as the results were not 'cut and dry'.

The question is… when can you say it definitely does/does not influence academic achievement? More research is obviously needed and possibly my post-doctoral journey begun.

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Appendix

Participants' sketches of ω-3 researchers

Appendix

A1… Staff Information Sheet

A2… Parent Information Sheet

A3… Child Information Sheet

A4… Screening Form

A5… Parent Food Frequency Form

A6… Fish Intake log

B1 … asTTle Test level 2

B2… asTTle Test level 3

B3… asTTle Test level 4

B4… asTTle Reading Achievement Objective

B5… asTTle Background Information Questions

B6… asTTle Practice Questions

B7… asTTle Results Sheet

B8… Basic Facts Tests

B9… Child Questionnaire (4 week)

B10… Child Questionnaire (15 week)

B11… Parent Questionnaire (15 week)

B12… Teacher Questionnaire (15 week)

D2… Reference Studies

D3… Explanation of Educational Tests