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Conditioning of Manila clam broodstock on natural and artificial diets.

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CONDITIONING OF MANILA CLAM BROODSTOCK ON NATURAL AND ARTIFICIAL DIETS

By

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Abstract

Two trials in 1991 and another two in 1992 were carried out on the broodstock conditioning of Manila clams, *Tapes philippinarum*. The main objective was to manipulate the lipid and polyunsaturated fatty acid content of Manila clam eggs by maintaining broodstock in different dietary regimes. The second objective was to assess dried algae as alternative diets for conditioning broodstock.

Clams were brought into the laboratory from the natural environment early in the year, before gametogenesis had started. Supplements of cultured live (*Dunaliella tertiolecta*, *Skeletonema costatum, Tetraselmis suecica* and *Isochrysis galbana*) and dried algae (*T. suecica)* diets were fed to the broodstock, usually in a range of mixed diets, at rations equivalent to 3% or 6% of the initial dry meat weight of the broodstock in dry weight of algae per day. The microalgae differed in their long-chain polyunsaturated fatty acid content (PUFA). Unfed control clams received only the organic material which remained in the sea water after sand filtration. The nutritional value of these diets in relation to gametogenesis, fecundity, quality of eggs, and viability and growth of larvae were assessed.

Dry *T. suecica* was the same food value as live *T. suecica* but Manila clams produced more eggs if supplements of live algae were added. The requirement for conditioning Manila clams (32 mm shell length) to spawn with live or dry T. *suecica+S. costatum* was 500 to 700 "day-degrees" (D°). With dry T. *suecica* on its own or mixed with *I. galbana, S. costatum* and *D. tertiolecta* it was 500 to 600 D° (44 mm shell length). In one trial clams spawned in the tanks (equivalent to 462 D°) before the first attempt to spawn them was made.

Successful spawning was dependent on the quantity and quality of the algal diet during gametogenesis. With a 6% food ration, clams fed dry *T. suecica+S. costatum* or dry *T. suecica+I. galbana* produced the highest number of eggs (an average of 3.2 and 4.5 million eggs per female, respectively). The average fecundity was 83% lower when the diet was reduced to a 3% food ration. The dry meat weight, condition index and fecundity of fed broodstock were significantly higher than for unfed animals.

The quantity of lipid in the eggs, usually between 4 and 9 ng egg^{-1}, was similar whatever the broodstock diet. However, levels of the essential polyunsaturated fatty acids 20:5w3 and 22:6w3 in the eggs were low if the broodstock diet was deficient in these PUFAs.

Even though diet manipulation caused changes in the fatty acid composition of the eggs, growth and survival of Manila clam larvae was not reduced in a hatchery situation.

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Chapter 1

Chapter 1 - Introduction

1.1. Bivalve production

The bivalve industry is of great importance in Europe and in the U.K. where it is a traditional, well-established profession with a strong social structure. The culture of bivalves has the advantage over commercial production of other food species, in that bivalves feed on the natural phytoplankton of an area which reduces production costs. The site selected must have a good water quality, high primary production, and moderate currents to supply food and oxygen and remove wastes (Walne,1974). Usually bivalves are cultured in the intertidal zone or in suspended culture in the sea with good accessibility from land or, under certain conditions in polyculture utilising the effluents of a fish/crustacean farm.

The economic importance of bivalve culture varies between countries, as does the market demand for these products. Countries such as Japan, France, Italy, Spain and Portugal, with shellfish eating habits, are the principal producers but also prime consumers. The high market demand for bivalves in these countries maximizes the utilization of natural resources as well as the culture of a variety of species, both to increase supply and to compensate the depleted natural stocks.

In 1983 the production of more than 500,000 tons live weight of Manila clam was shared between Japan, U.S.A., France, Italy, Great Britain, Canada and Germany (Anon, 1985), of which 80% was cultured and the remainder harvested from natural populations. Japan accounts for 60% of the clam production with an annual tonnage of 240,000 tons of Manila clams. The British shellfish industry farmed output, spread over more than 300 sites in 1986 and the estimated output for 1991 are given in Table 1.1.

Table 1.1. Annual production of cultivated shellfish in England and Wales (Multi-Annual Guidance Programme).

Species	Production 1986	(tonnes) 1991
Ostrea edulis	100	200
Crassostrea gigas	300	452
Mytilus edulis	3,500	9,400
Clams	10	50
Scallops	30	30

There are about 16,000 hectares of public ground in the U.K. which are suitable for bivalve cultivation. The annual production per hectare is estimated at 15 t for oysters, and 10 - 25 t for clams. In Britain, about 75 years ago 5,000 t of oysters per year were eaten but nowadays it is around 400 t per year. Although home consumption has decreased it is speculated that there is much scope for an increase in demand. Prices of oysters are £2,000-3,000 tonne $^{-1}$ and of clams £4,000-6,000 tonne $^{-1}$. There is potential for export to European countries to increase as well: in 1986, 543 t of oysters were exported and according to Piquion (1985), the Spanish market had been short of clams for the previous 10 years.

In the U.K. the overfishing, the decline of natural oyster beds and the prohibition of the importation of Portuguese oysters (*Crassostrea angulata*) in 1962 stimulated interest in the Pacific oyster *(Crassostrea gigas*) which was introduced in 1964. The Manila clam (*T. philippinarum*) was introduced in 1980 and the American oyster (*Crassostrea virginica*) in 1984. For these non-native species it is necessary to rear spat in hatcheries for distribution to farmers, to maintain and increase bivalve culture and production for home consumption and for export.

The efficiency of the hatchery operation has been improved recently with the introduction of nursery stages. Spat can be reared from settlement to planting size in upwelling recirculation systems which result in higher overall survival.

1.2. Species history and importance

The present work studied the introduced clam species, *Tapes philippinarum* (Adams and Reeve, 1850). The Manila, Pacific or Japanese little-neck clam *T. philippinarum* was introduced from the Pacific to European coastal waters for its improved qualities of resistance, adaptation and faster development relative to the over-exploited native palourde, *Tapes decussatus*. In a number of European countries, including France (Latrouite and Claude, 1976) it is presently reared in great quantities in some hatcheries (Parache, 1982). It appears to have good potential for cultivation on a commercial scale (Piquion, 1985; Utting 1987a,b) by virtue of its tolerance to salinity, temperature, disease, TBT and handling (Loosanoff and Davis, 1963; Utting, 1987 b). Trials comparing the performance of the native palourde with the Manila clam confirm its superior growth, survival and condition factor (Spencer, 1987).

This clam is also known by the generic names of *Venerupis* or *Ruditapes* and has been reported under a number of synonyms. Bernard (1983) regards *Ruditapes* as a sub-genus of the genus *Tapes*. He also considers the genus *Venerupis* as a distinct taxon, concluding that *Tapes philippinarum* (Adams and Reeve, 1850) is the correct name and the oldest of this group of names. On the other hand, Partridge (1977) argues that the proper name is

Tapes semidecussatus Reeve and refers to this name as the grammatically correct form of *Tapes semidecussata* Reeve, 1864.

The Manila clam was accidentally introduced from Japan to the Pacific Coast of North America with seed of the Pacific oyster, *C. gigas* (Thunberg). It was first recorded in Ladysmith Harbour in 1936 (Quayle, 1938, 1941) from where it soon spread. During the 1950s and 1960s Manila clams were of little commercial interest. Great development occurred in the mid-1970s and Manila clams are now fished in commercial quantities from British Columbia to California (Magoon and Vining, 1981). This clam is now the largest fishery on the west coast of North America, especially in Washington State and British Columbia. Manila clams appear to grow in the same habitat as Pacific oysters (Chew, 1990). Production and demand for this clam species extended to the European market. Manila clam broodstock from the west coast of North America were introduced into Western Europe (Great Britain, France and Italy) and in 1990 there were approximately 18 hatcheries producing seed (Chew, 1990).

In France,the Manila clam was introduced in the mid - 70s, owing to its superior growth and viability when compared to the endemic species *T. decussatus*. Preliminary investigations of this species have been made by a number of French workers, who have mainly concentrated on the ongrowing of spat imported from the Pacific. In Italy, in areas such as Venice Lagoon by virtue of regular introductions and natural setting, subsequent commercial harvest of Manila clams commenced in the late 1980s. The Manila clam was introduced to Spain in 1980 where most of the work has concentrated on the on-growing of spat (Perez Camacho, 1980; Perez Camacho and Cuna, 1985). The Spanish market imports approximately 1,500 tonnes of clams mainly from natural fisheries in Portugal, France and Tunisia. In countries such as Japan, Korea and Portugal clam production plays an important part in their economy (Korringa, 1976).

Manila clam broodstock was first introduced into the U.K. under quarantine from Washington State, U.S.A. in 1980. The stock was spawned upon receipt and the F1 generation reared in quarantine at the Ministry of Agriculture, Fisheries and Food (MAFF), Fisheries Laboratory, Conwy, Wales (Millican and Williams, 1985). The original broodstock was then destroyed. This procedure followed the guidelines as defined by the International Council for the Exploration of the Sea (1988). After 12 months of disease checks by the Fish Diseases Laboratory, MAFF, Weymouth, the F1 generation was offered to the commercial hatcheries as a disease-free broodstock. Hatchery production of Manila clam seed is essential in the U.K. because it is highly improbable that this clam will successfully spawn and establish a self-recruiting population. This is because *T. philippinarum* breeds at temperatures above those normally found in U.K. inshore waters (Utting, 1987a). Research on the hatchery rearing of larvae as a source of seed for cultivation has been conducted at Conwy (Lovatelli, 1985; Spencer *et al*, 1991; Utting and Spencer, 1991).

The expansion of the Manila clam industry in Britain has been slower than expected. This may be due to the different culture techniques compared to oysters since clam seed must either be covered with plastic netting of 6-13 mm mesh size or be placed in cages (Utting, 1987a; Spencer 1990). As the species is non-native some UK conservation groups (English nature, Joint Nature Conservancy Council) have expressed concern about the possibility of this species spawning in the wild and competing with native clams (*Tapes* spp.). The release or escape into British coastal waters of non-native shellfish, unless under licence, constitutes a contravention of the 1981 Wildlife & Countryside Act (Great Britain Parliament, 1985).

1.3. Hatchery culture techniques

Many studies have been carried out on hatchery techniques for larvae and spat production of a range of bivalve species (Belding, 1910, 1912, 1930; Wells, 1920, 1927; Bruce, Knight and Parke, 1940; Davis,1950; Loosanoff and Davis, 1963; Walne, 1963, 1974; Epifanio, 1975; Helm and Millican, 1977; Pillsbury, 1985; Enright, Newkirk, Craigie and Castell, 1986; Laing and Millican, 1986).

Shellfish research at Conwy began in 1914 and a range of species, spawned and reared in the hatchery, were tested for commercial farming development. These included the New Zealand oyster, *Tiostrea lutaria*, the Chilean oyster, *Ostrea chilensis*, the Pacific oyster, *C. gigas*, the Portuguese oyster, *C. angulata*, the American hard shell clam, *Mercenaria mercenaria*, the European palourde, *Tapes decussatus*, the Chilean mussel, *Choromytilus chorus* and later the Manila clam, *T. philippinarum* and the American oyster, *C. virginica*.

The production of spat in hatcheries is a fundamental part of commercial production in many countries of the world. Hatchery operation requires continuous improvement of methods and techniques. Three principal lines in the production of hatchery-reared bivalves have been outlined (Walne,1974): good quality broodstock, suitable sea water quality, and sufficient quantities of good quality algae. The diagram in Table 1.2 shows the basic culture scheme for bivalve seed production.

At present, in the U.K., two commercial hatcheries are in active production: these are at Guernsey (Channel Islands) and Whitstable (Kent). Now they produce up to 100 million juvenile Pacific oysters and Manila clams per year (Spencer, 1990). In 1988 75% of the

U.K. bivalve hatchery output was sold to Spain, France, Italy and South Africa, and in 1990 to Spain, Ireland and South Africa (Spencer, pers. comm.).

Table 1.2. Basic culture scheme for bivalve seed production

Broodstock	
⇓	Conditioning
Maturity	
⇓	Temperature shock?
Spawning	
⇓	
Larval rearing	
⇓	Metamorphosis
Spat	
⇓	
Ongrowing of seed in the sea	

The major problem to the viability of commercial hatcheries throughout Europe is mass mortality of bivalve larvae during summer months. These mortalities can affect production. Growth and survival of marine invertebrates can be adversely affected by poor water quality (Wilson, 1951, 1981; Millar and Scott, 1968; Helm, 1971; Utting and Helm, 1983). Abnormal mortalities at specific times in the year may also be related to larval quality and to nutritional value of food species. Helm (1971) and Utting and Helm (1983) suggested that the presence of metabolites and decomposition products of blooms, may be a contributary factor for mass mortalities of larvae after spring phytoplankton blooms in a hatchery sea water supply.

The quality of the sea water used in a hatchery is an important factor in bivalve culture. At the Conwy laboratory, the sea water is taken from the Conwy estuary and is consequently subject to natural fluctuations in quality. Bivalve larvae may be affected by excessive turbidities caused by suspended silt, detritus or unicellular organisms (Davis, 1953; Loosanoff, 1954, 1958; Berg, 1971; Walne, 1970). Changes in bivalve larval growth and development have also been related to natural and artificial pollutants, metabolic exudates of dinoflagellate blooms, organic constituents in the water liberated from bottom sediments (Davis and Chanley, 1956; Loosanoff, 1958; Millar and Scott, 1968; Thain and Watts, 1984), heavy metal toxicity (Fujiya, 1960; Calabrese *et al.*, 1973; Martin *et al.*, 1981) and bacterial contamination (Tubiash *et al.*, 1985).

The level of food reserves in bivalve eggs at spawning is critical to successful seed production and can be influenced by the nutritional quality of particulates and dissolved organic material in the water, as well as the level of essential reserves in adults before gametogenesis or accumulated during gametogenesis. In molluscan larvae, embryogenesis is characterized by extensive morphological change and a loss in energy reserves. Pandian (1969) with the slippershell, *Crepidula fornicata* Say, calculated that oxidation of lipid reserves during embryogenesis was 65.3% of the total energy expended while 18.8% and 6.3% were from protein and carbohydrate respectively. Helm *et al.* (1973) demonstrated that adults of the European flat oyster, *Ostrea edulis* L., receiving a food supplement of the unicellular alga *Tetraselmis suecica* (Kylin) Butch., during laboratory conditioning for spawning, produced more broods of larvae than oysters receiving only the natural phytoplankton in the sea water supply. Larvae were liberated sooner, grew at an enhanced rate and provided greater spat production than broods from the control stock.

1.4. Natural environment and hatchery gametogenesis

In bivalves in temperate latitudes the natural gametogenic cycle is linked to cycles of glycogen storage and subsequent synthesis of lipids during vitellogenesis in the spring. Interruption of this cycle by artificial conditioning at elevated temperature may force the development of eggs before sufficient glycogen has accumulated for the synthesis of lipid. The consequence of this would be the production of fewer, poor quality eggs (Gallager and Mann, 1986).

The objective of broodstock conditioning in the laboratory /hatchery is to extend the natural breeding season of cultured species, producing larvae of high quality to sustain seed production.

Breeding in bivalves is controlled largely by temperature and by food supply. An adequate food supply is necessary for the broodstock to accumulate reserves of glycogen and lipid for utilization during gonad development and gametogenesis.The quality and quantity of phytoplankton available to the broodstock is of great importance in providing the best conditioning environment prior to gamete production and liberation. It is usual to select potential breeding stock based on their condition, determined as condition index and levels of glycogen reserves.

In the hatchery routine artificial conditioning of bivalve broodstock can be achieved by manipulating the temperature and phytoplankton supply to bivalve stocks removed from the natural environment at different times of the year (Loosanoff and Davis, 1963; Walne, 1970).Conditioning regimes to reduce adult stress and maximize the viability and survival of larvae have been described by Bayne (1972) and Lannan *et al.*, (1980). Broodstock conditioning in bivalves ensures that the broodstocks are at the same state of gametogenesis and will produce eggs in the optimum state of development and ensure maximum larval survival (Lannan, 1980; Lannan *et al.*, 1980). Bivalves in good condition with high levels of lipid and carbohydrate reserves (Quayle, 1969; Walne, 1970) have higher fecundity than animals of similar size in poor condition (Walne, 1964). Well-conditioned broodstocks produce eggs that go through meiosis synchronously after fertilization (Allen *et al.*, 1989).

During extended conditioning periods, the initial lipid content of released larvae and subsequent survival declined (Gallager and Mann, 1986). Bayne (1972) and Bayne *et al.* (1975) showed reduced growth of *M. edulis* larvae that developed from nutritionally stressed adults.

Spawning of *T. philippinarum* in the U.K. hatcheries needs thermal stimulation, requiring the water temperature to be raised well above 20°C. Cahn (1951) reported that spawnings in Hokkaido, Japan, occurred between temperatures of 20 and 23°C, and Loosanoff and Davis (1963) stated that spawning in the Manila clam usually occurred at temperatures between 20°C and 27.5°C. Millican and Williams (1985) reported no natural spawning in *T. philippinarum* in the summer of 1984 in North Wales when peak sea water temperatures exceeded 20°C in August.

1.5. Importance of lipid in diet quality

Numerous studies have suggested the importance of lipid in adult marine bivalves, for the formation of gametes and as an energy reserve (Walne, 1970; Beukema and De Bruin, 1977; Holland, 1978; Taylor and Venn, 1979; Beninger and Lucas, 1984). Rogers (1983) demonstrated that *O. edulis* preconditioned in phytoplankton enhanced water at low temperatures accumulated significantly higher reserves of carbohydrates and lipid than control oysters of the same origin maintained in the sea. It is known that oocytes are rich in lipids (Gabbott, 1975; Holland, 1978) and a large proportion of adult bivalve lipids consist of phospholipids (Lubet and Longcamp, 1969; Krishnamoorthy *et al.*, 1978).

Beninger (1984) also refers to the importance of the phospholipids in the cell membrane of the oocytes that can be used as an energy reserve.

The importance of lipid reserves in *O. edulis* during the period from liberation to metamorphosis has been clearly shown by Millar and Scott (1967) and Holland and Spencer (1973). Helm *et al.* (1973) found that viability and survival of cultured larvae was significantly correlated to the initial neutral lipid content at the time of liberation. Gallager and Mann (1986), working on *C. virginica* and *M. mercenaria* also found significant correlation between initial egg lipid content and survival to both straight hinge and pediveliger larvae stages.

The lipid composition of the diet is important in determining the growth and development of bivalve larvae (Holland and Spencer, 1973). The lipid content in bivalve larvae can be used as an indicator of their physiological condition and potential for successful metamorphosis (Gallager *et al.*, 1986).

1.6. Essential Fatty acids

The effect of specific polyunsaturated acid (PUFA) deficiencies in algal diets fed to broodstock during gametogenesis in *O. edulis* and *T. philippinarum* was investigated by Utting *et al.* (1988). The quality of clam eggs or oyster larvae, defined in terms of their PUFA content, was mainly a reflection of the composition of the cultured algae fed to the broodstock during conditioning.

Utting *et al.* (1988) suggested that the development of the larvae was related to the quality of the reserves accumulated during gametogenesis, especially long chain PUFA's.

The fatty acids of marine organisms has been studied extensively, since the introduction of capillary-column gas chromatography (Ackman *et al.*, 1974). One of the most important factors that determines the quality of a diet for bivalve larvae is the fatty acid composition (Chuecas and Riley, 1969; Holland and Gabbott, 1971; Holland and Spencer, 1973; Helm *et al.*, 1973; Creekman, 1977; Langdon and Waldock, 1981; Wikfors *et al.*, 1984; Pillsbury, 1985).

Two long-chain PUFAs, eicosapentanoic acid, 20:5w3 and docohexanoic acid, 22:6w3 are the most important. The fatty acid 20:5w3 was shown to be essential for growth and development of *C. gigas* juveniles (Webb and Chu, 1981) and clams require 20:5w3 or 22:6w3 (Helm and Laing, 1987).

1.7. Selection and production of suitable algae for hatchery culture

The selection of suitable species of algae is important as this will shorten the period for rearing the bivalves and thereby reduce the cost. From about 50 microalgal species tested, only about 10 are regularly used in hatcheries.

Cultured marine algal species are used as the principal food supply during conditioning. Useful species that can be cultured on a large scale are *T. suecica*, *Thalassiosira pseudonana* and *Skeletonema costatum*. A mixed algal diet is better than a single species diet. Multi-species algal diets at a ratio equivalent to 6% of the initial dry meat weight of the broodstock in dry weight of algae fed per day provide good results (Davis and Guillard, 1958; Walne and Spencer, 1968; Calabrese and Davis, 1970; Helm, 1977).

Many authors have described the nutritional value of different algal species to a range of bivalve species at all stages of development (Davis, 1950; Loosanoff and Davis, 1963;

Walne, 1964, 1965, 1974; Rodhouse *et al.*, 1983; Ukeles *et al.*, 1984; Helm and Laing, 1987; Laing and Millican, 1986). The nutritional value of the main cultured species is: *Chaetoceros* sp.> *Chroomonas* sp.> *Isochrysis* sp.> *Skeletonema* sp.> *Tetraselmis* sp.

There are several criteria determining the nutritional value of algae: the cells must be easily ingested (cell size less than 10 μm diameter) especially by the larvae, non toxic, easy to culture, have a digestible cell wall and good nutritional quality (biochemical composition of the cell contents) (Haven and Morales-Alamo, 1970; Epifanio *et al.*, 1981; Webb and Chu, 1983). In particular, the presence of the long-chain PUFAs 20:5w3 and 22:6w3 is considered to be essential (Chu and Webb, 1984; Langdon and Waldock, 1981). Diatoms have long been considered as important for bivalve growth. They have a rigid valve impregnated with silica but enzymes in the bivalve stomach can lyse this outer covering (Reid, 1983).

Walne (1970) obtained high growth rates for *M. mercenaria* spat fed with *S. costatum* and *C. calcitrans*. Enright *et al.* (1985) fed *O. edulis* spat with 16 phytoplankton species and showed that the value was: *Chaetoceros gracilis*> *C. calcitrans*> *S. costatum*> *T. pseudonana*. Later Laing and Millican (1986) feeding *O. edulis* spat, found *C. calcitrans* better than *S. costatum* and Laing *et al.* (1987) confirmed *S. costatum*, *T. pseudonana* and *C. calcitrans* as all being suitable for rearing *T. philippinarum* spat although many algal diets have proved to be ideal for clam larvae, juveniles and broodstock (Kilada, 1985; Laing *et al.*, 1987; Kersuzan, 1989).

The production of sufficient quantities of suitable algal species is technically the most difficult and relatively costly part of bivalve rearing in a hatchery (De Pauw, 1981; Helm and Laing, 1981; Laing, 1985). The feed may represent up to 40% of the total operation cost in a hatchery (Laing, pers. comm.), and the spat produced can account for 30% of the production cost of a marketable oyster (Spencer, pers. comm.). Algal culture techniques have been extensively developed, and growth enrichment media, isolation techniques and culture are well described (Aquacop, 1983; Fox, 1983).

There have been many attempts to substitute phytoplankton by artificial diets for marine bivalves (see review by Langdon *et al.*, 1985). These diets, to be acceptable must remain in suspension, be non-toxic and be easily assimilated and digested (Laing *et al.*, 1990). Inert diets such as yeast or ground macrophytes have failed (Masson, 1977; Epifanio, 1975). Microcapsulated diets have been developed and tested but these diets were unsuitable for bivalve larvae, and produced only moderate growth in spat (Castell and Trider, 1974; Chu *et al.*, 1982; Laing, 1987; Martin, 1987), although some authors reported oyster growth which was up to 73% growth rate of control animals (Langdon and Seigfried, 1984).

Encapsulation is a process where liquid or particulate materials are enclosed in a special artificial membrane wall or shell made of natural polymers (gelatin), synthetic polymers (ethyl cellulose, polyvinyl alcohols) or polyethylene. The use of microencapsulated nutrients for aquatic filter-feeders was first described by Jones *et al.* (1974) and later used to study the nutritional requirements of crustacean larvae, *Penaeus japonicus* (Jones *et al.*, 1979) and *Artemia* sp. nauplii (Sakamoto *et al.*, 1982).

Spray-dried unicellular algae produced by the Micro Algae Research Institute of Japan were used first as food for hard shell clam (*M. mercenaria*) larvae by Hidu and Ukeles (1962). They reared clam larvae successfully to metamorphosis, although the growth rate was lower than that of larvae fed live algae. Celsys (Cell Systems Ltd.) from Cambridge, UK, has developed dried algae, heterotrophically grown in the dark with sugars. However the company went into liquidation and the licence for producing spray-dried algae is now held by British Technology Group. Preliminary results (Laing, 1989a,b; Janke, 1988;

Foster, 1989) showed that these dried algal diets, could have a promising future as live algal replacement diets for bivalves.

1.8. Objectives of this study

The aim of this research is to assess the hatchery warm water conditioning of Manila clam (*T. philippinarum*) broodstock fed with live or spray-dried alga diets in terms of gonad development, gametogenesis, fecundity, quality of eggs and viability and growth of larvae and spat. The main objective of the present work was to manipulate the lipid and polyunsaturated fatty acid content of Manila clam eggs by maintaining broodstock in different dietary regimes.

To maintain a food supply at the optimum ration over long periods of time requires large volumes of cultured algae, and for this reason the secondary objective of the study was to assess the use of alternative, dried alga diets for conditioning Manila clam broodstock.

Chapter 2

Chapter 2 - Materials and Methods

2.1. Hatchery sea water quality

2.1.1. Sea water supply

The sea water used in this study was pumped at high tide from the Conwy estuary (North Wales, UK) into a concrete storage tank for sedimentation for 24 h. Lacron sand-filters were used to prefilter the water before it was pumped as required into fibreglass tanks in the loft of the laboratory.

2.1.2. Particle content of sand-filtered sea water

a) Number of particles

The concentration of suspended particles in the sand-filtered sea water supply was estimated once per week from a sample of approximately 100 ml taken from the overflow of the indoor sea water storage tank. The number of particles of two size groups (2.5 - 5 μm and 5 - 10 μm diameter), was determined with a Coulter Counter (Model ZM) fitted with a 70 μm orifice head. This provided data on the availability of potential food particles in the water.

b) Dry weight and ash-free dry weight of suspended particles

i. Supra-micron particles

Two samples, each of 1 l were filtered under a vacuum not exceeding 0.35 kg cm^{-2} through separate Whatman glassfibre filter papers, grade GF/C (4.7 cm diameter).

The ash-free dry weights of the filters had been pre-recorded after they had been kept for a minimum of 4 h at 450^oC in a muffle furnace. The filter paper, with filtered material, was washed with isotonic (3% w/v) ammonium formate and dried at 60^oC for 48 h after which dry weight was determined using an Electrobalance. Ash weight was similarly determined after a minimum of 24 h at 450^oC in a muffle furnace. Dry and ash weights of particles in mg l^{-1} of test water were calculated by subtracting the ash-free dry weight of the GF/C paper. Ash-free dry (organic) weight of suspended particles was calculated by subtracting the ash weight from the dry weight.

ii. Sub-micron particles

Millipore cellulose acetate filters, pore size 0.22 µm, were dried for a minimum of 48 h over silica gel in a desiccator and weighed on a pan balance. Two 100 ml sub-samples of the GF/C filtered test water were passed through the filters under a vacuum not exceeding 0.35 kg cm^{-2}. The filtrate and the filter were washed with isotonic (3%) ammonium formate and then dried, as before, for a further 48 h. The dry weight of the particles in mg particles per 100 ml of test water were calculated by subtracting the dry weight of the filter paper.

c) Particulate lipid determination

Duplicate 1 l samples of sand-filtered sea water, were filtered on to Whatman GF/C filter papers. After washing with isotonic (3% w/v) ammonium formate to remove salt, the filters were transferred to labelled, graduated test tubes, sealed with parafilm and stored at -20^oC for later analysis.

Particulate lipid was measured using a charring method (Marsh and Weinstein,1966). To extract the lipid, 3 ml of 2:1 (v/v) chloroform/methanol were added to each tube. Standards of 0 (reagent blank), 25, 50, 75 and 100 µg cholesterol ml^{-1} were prepared from a freshly made cholesterol stock solution. To prepare the cholesterol stock solution, 0.1 g cholesterol was dissolved in 100 ml of chloroform/methanol (2:1 v/v) in a volumetric flask. From this solution 10 ml was pipetted and diluted to 100 ml with chloroform/methanol. The standards were prepared in graduated glass centrifuge tubes from 0, 0.5, 1.0, 1.5 and 2.0 ml of the stock solution to which 2:1 chloroform/methanol solution was added, if necessary, to make up to 2 ml total volume. Sample tubes were covered with aluminium foil and left in the dark at 20^{o}C for 2 h to extract the lipid.

After 2 h, 0.6 ml of 0.7% (w/v) sodium chloride solution was added to each standard and each sample (Folch et al., 1957). The contents of the tubes were vortex-mixed. The tubes were recapped and placed at 4^{o}C in a refrigerator overnight to complete extraction and separation of the solvent phases. Following this samples and standards were removed from the refrigerator and centrifuged at 3,000 rpm for 10 min. The volume of the lower solvent phase (chloroform), containing extracted lipid, was recorded for each sample and standard against the tube calibration marks, together with a reference number. A volume of 1 ml was pipetted from the lower solvent phase of each tube and transferred to thick borosolicate glass, boiling tubes. The boiling tubes were placed in a water bath at 80^{o}C, and the chloroform evaporated to dryness. Tubes were then cooled to 20^{o}C and 2 ml of concentrated sulphuric acid was added to each. The tubes were placed in an alumminium heating block at 200^{o}C for 15 min to char the residue, and were then transferred quickly through a hot water bath (100^{o}C) to a cold water bath (20^{o}C) where they were left for 10 min.

After the addition of 3 ml of distilled water, the samples and standards were shaken throroughly. Two ml of the resulting solution were transferred from each sample and standard to a plastic 1 cm path length cuvette. The optical density of the standards were measured against the reagent blank on a spectrophotometer at 375 nm wavelength. A single wavelength programme on a BBC microcomputer was used to calculate a linear regression (calibration equation) with which the absorbance of each sample, measured against the reagent blank, was converted to concentration of cholesterol, as $\mu g\ ml^{-1}$. Concentrations were recorded by a printer. These values were converted to total cholesterol μg in each original sample by multiplying by the appropriate volume (in ml) of the chloroform phase at the end of the extraction period. Total cholesterol values were then converted to total lipid by multiplying by 1.25 (Barnes and Blackstock, 1973). Lipid content of particles in the sea water was expressed as a percentage of the ash-free dry weight:

$$\text{Lipid, as \% of ash-free dry weight} = \frac{\text{Lipid.}10^{-6}\text{ particles} \quad \times 100}{\text{Ash-free dry weight.}10^{-6}\text{ particles}}$$

d) Chlorophyll a

A 1 l sample of sea water was filtered on to a Whatman GF/C paper under a vacuum not exceeding 0.35 kg cm^{-2}. The filter paper, with particles, was placed in 35 ml of 90% (v/v) acetone in a polypropylene centrifuge tube and stored overnight in the dark at $4^{o}C$. After centrifuging at 3000 rpm for 5 min to concentrate the particles from the filter paper, the supernatant was then poured into a 10 cm glass cuvette. Absorbance readings from 630-750 nm were measured against a reference blank of 90% (v/v) acetone on a spectrophotometer and recorded by a printer.

Chlorophyll a concentration, in mg m^{-3}, was determined using the formula below, derived from Strickland and Parsons (1968):

$$\text{Chl } a = \frac{11.6\ (E665\text{x}3.5) - 1.31\ (E645\text{x}3.5) - 0.14\ (E630\text{x}3.5)}{\text{Volume of filtered sample, l}}$$

where, E_{665} = Absorbance reading at 665 nm, E_{645} =Absorbance reading at 645 nm and E_{630} = Absorbance reading at 630 nm. Corrected absorbance values for each wavelength were determined as:

$$\text{Corrected value} = \text{Recorded value at } E_x - \text{Recorded value at } E_{750} - \text{Recorded value at } E_{xb}$$

where, E_x = Wavelength for which absorbance value is to be corrected, E_{750} = Absorbance reading at 750 nm of the same sample (Subtracted to remove error due to absorbance by filter paper remnants), E_{xb} = Absorbance reading at E_x of the acetone blank (Subtracted to remove error due to absorbance of cuvette and acetone).

2.2. Broodstock supply and conditioning

Adult *T. philippinarum,* approximately two years old, were obtained from the MAFF bivalve on-growing site at Tal-y-foel, Anglesey (Menai Strait, North Wales, UK). Clams were collected from the beach before the start of gametogenesis (Tables 2.1 and 2.2). A random sample of 30 individuals was removed from the batch and preserved to establish the initial condition of the broodstock. The remaining clams were divided randomly into three arbitrary groups of large, medium and small animals. These groups were divided equally between experimental treatments, to avoid a greater number of animals of any one size in a particular tank. Sixty clams were stocked in each of the tanks, with approximately equal total live weight of clams in each tank per experiment.

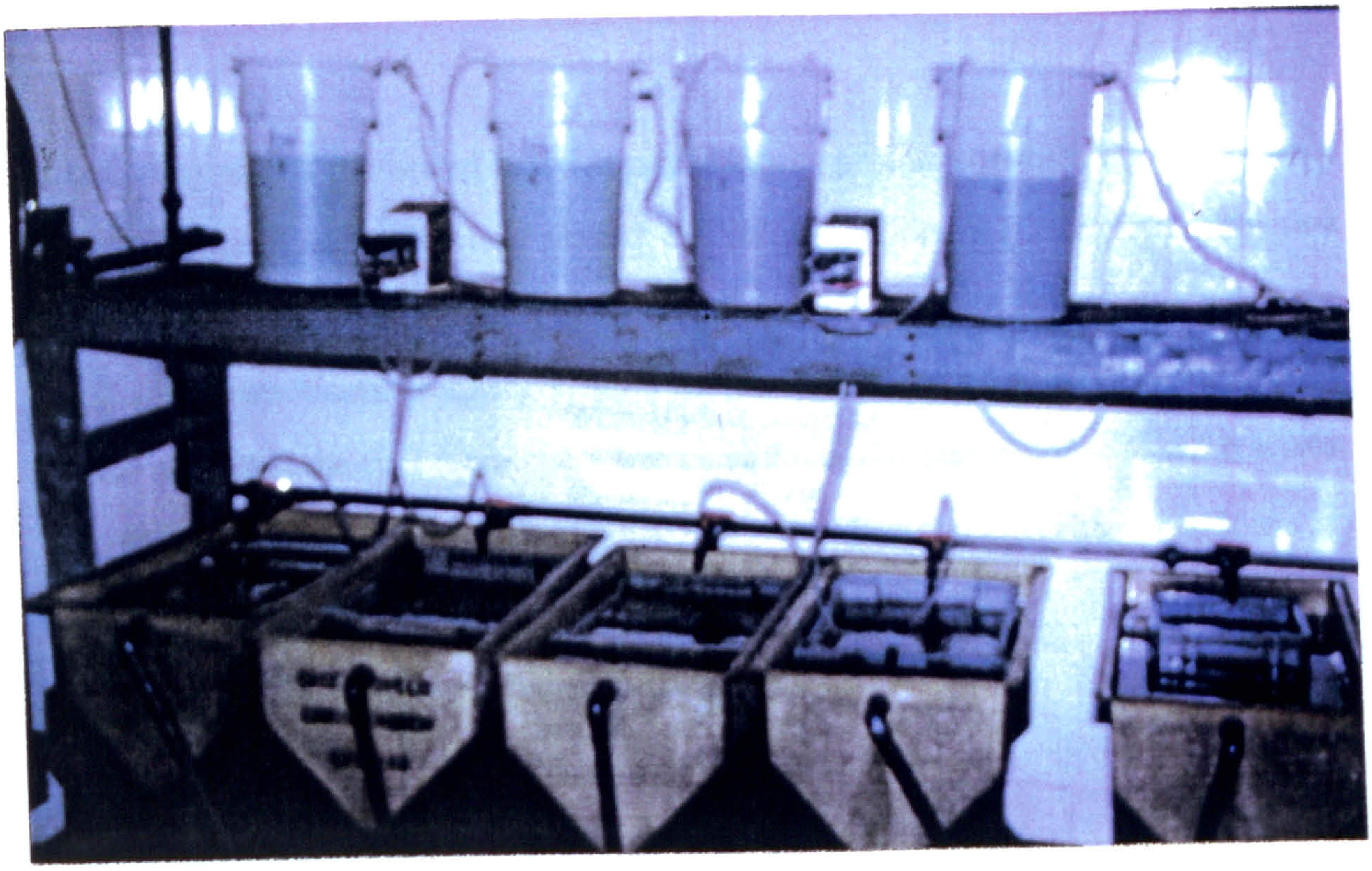
The time required for bivalves to reach spawning condition in the hatchery was dependent on temperature and was calculated as "day degrees" (D°): $D° = d\ (t-t_0)$, where d = the number of days of hatchery-conditioning, t = the temperature (°C) of the hatchery sea water supply and t_0 = the threshold temperature to initiate gonad development ie 10 °C, (Mann, 1979).

Table 2.1. Six treatments for 1991 broodstock conditioning. T. philippinarum broodstock were induced to spawn after 6, 7 and 8 weeks (1st broodstock 1991) and after 5, 7 and 9 weeks (2nd broodstock 1991).

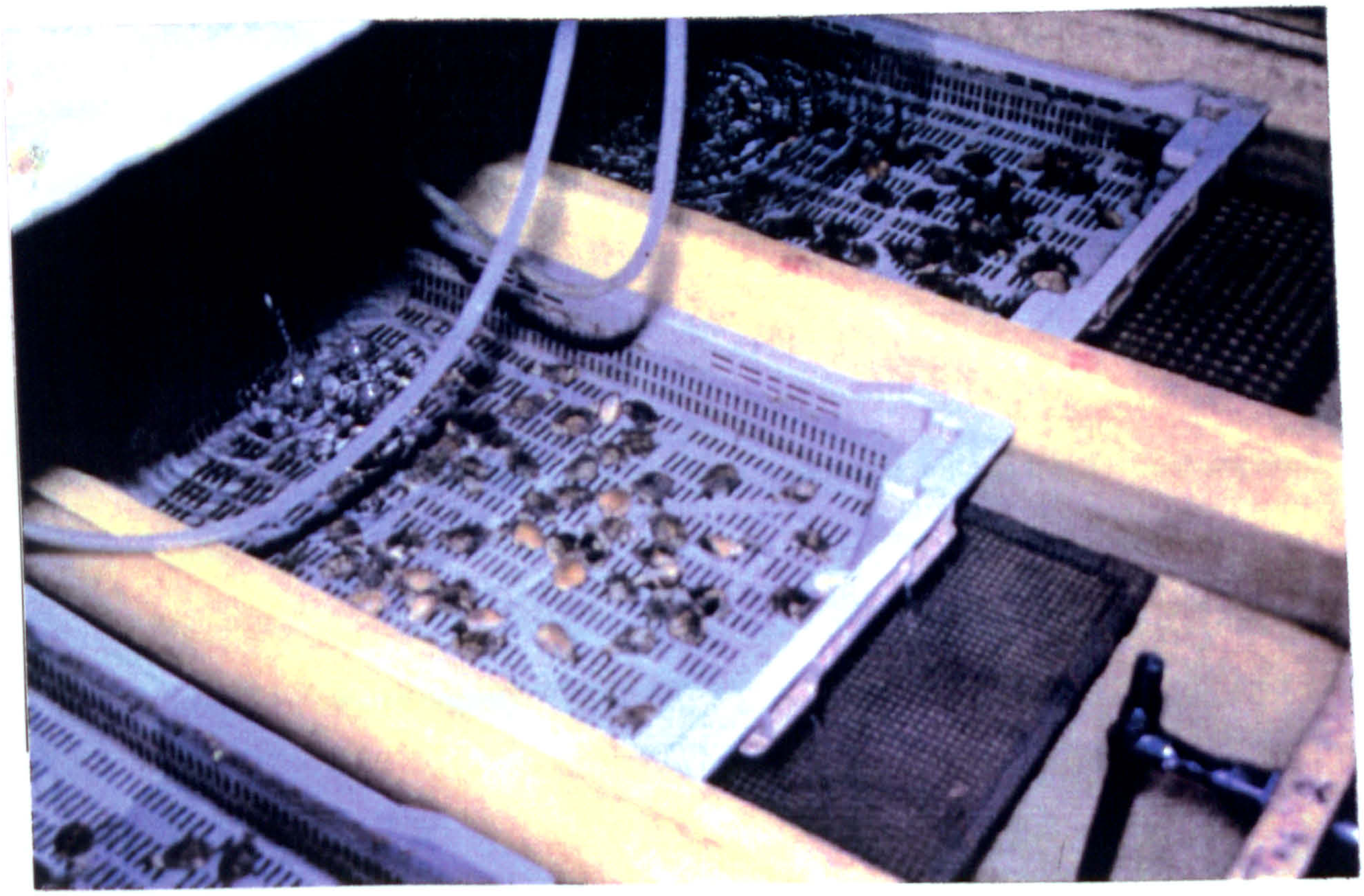
Broodstock	Conditioning dates Start	Conditioning dates Final	Tank number	Stocking density, g	Mean Individual live weight, g	Food supplement
						Live food at 3%
1st broodstock 1991	21.01.91	19.03.91	1	494.50	8.24	100% D. tertiolecta
			2	494.55	8.24	"
			3	494.98	8.25	100% S. costatum
			4	494.27	8.24	"
			5	494.82	8.25	None
			6	494.78	8.25	"
						Live and Dried food at 6 %
2nd broodstock 1991	17.04.91	17.06.91	1	566.40	9.44	30% S. costatum + 70% T. suecica
			2	566.90	9.45	"
			3	566.90	9.45	30% S. costatum + 70% Dry T. suecica
			4	566.20	9.44	"
			5	566.40	9.44	None
			6	566.60	9.44	"

Table 2.2. Five treatments for 1992 broodstock conditioning. T. philippinarum broodstock were induced to spawn after 6, 7 and 8 weeks (1st broodstock 1992) and after 5, 6 and 7 weeks (2nd broodstock 1992).

Broodstock	Conditioning dates Start	Conditioning dates Final	Tank number	Stocking density, g	Mean individual live weight, g	Food supplement
						Dried and live food
1st broodstock 1992	21.01.92	16.03.92	1	1255.20	20.92	70% Dry T. suecica + 30% D. tertiolecta
			2	1255.20	20.92	70% Dry T. suecica + 30% S. costatum
			3	1256.70	20.95	70% Dry T. suecica + 30% I. galbana
			4	1255.40	20.92	100% Dry T. suecica
			5	1254.30	20.91	None
						Dried and live food (replicate)
2nd broodstock 1992	16.03.92	04.05.92	1	1036.90	17.28	70% Dry T. suecica + 30% D. tertiolecta
			2	1036.40	17.27	70% Dry T. suecica + 30% S. costatum
			3	1036.70	17.28	70% Dry T. suecica + 30% I. galbana
			4	1037.00	17.28	100% Dry T. suecica
			5	1036.10	17.27	None



a)



b)

Plate 2.1. Broodstock conditioning tanks of one hundred and twenty litres.

a) Broodstock tanks received a through flow of sand - filtered sea water.
Food was added with a peristaltic pump

b) Manila clams supported in plastic trays with perforated base.

plastic tray with a perforated base. Any biodeposits produced, which attract bacteria, did not, therefore, accumulate around the animals (Plate 2.1). In 1991, the first broodstock was conditioned for periods of 6, 7 and 8 weeks before attempts were made to spawn clams. Respective times for the second broodstock were 5, 7 and 9 weeks. In 1992, the first broodstock was conditioned for periods of 6, 7 and 8 weeks and for the second broodstock respective times were 5, 6 and 7 weeks.

2.2.1 Routine maintenance of the broodstock

2.2.1.1. Temperature and salinity

Daily records were kept of temperature and salinity. The temperature of the sea water was maintained at 20 - 22°C by a heat exchange unit. The salinity was measured with an optical refractometer (Reichert Ts Model 10419).

2.2.1.2. Flow rates

The sea water flow rate through each tank was set at 1 - 1.2 l min^{-1} and adjusted daily if necessary.

2.2.1.3. Food supply

i. Algal culture

Five species of live algae and one freeze-dried alga were used for broodstock conditioning and larvae culture. The live algae were:

Dunaliella tertiolecta (Butcher) - Family *Chlorophyceae*
Skeletonema costatum (Greville) - Family *Bacillariophyceae*

Tetraselmis suecica (Kylin) - Family *Prasinophyceae*

Chaetoceros calcitrans (Paulsen) - Family *Bacillariophyceae*

Isochrysis galbana T-ISO - Family *Prymnesiophyceae*

The culture of algae at Conwy followed the techniques described by Walne (1966, 1974) and modified by Laing (1991). *C. calcitrans* was cultured in 3 l of autoclaved sea water in 3 l glass flasks. Fresh cultures were set up daily and used after four days. The other species were cultured in a semi-continuous system of 20 l glass flasks in sea water filtered to remove particles larger than 2 µm. Volumes of 25% of each culture were harvested three times each week. New cultures were established every 4 to 6 weeks. The cultures were illuminated continuously by fluorescent tubes, and maintained at a temperature of 18 - 20°C. Air containing 1% by volume of carbon dioxide was bubbled through the cultures to maintain a water pH of 7.8.

Algal cell densities at harvest were determined with a compound microscope and haemacytometer slide. The range of cell densities in the experiments was:

Algal culture	Cells per µl (at harvest)
I. galbana	5,000 - 25,000
C. calcitrans	50,000 - 80,000
T. suecica	1,200 - 3,200
D. tertiolecta	1,000 - 3,760
S. costatum	4,000 - 13,000

The freeze-dried alga, Celsys Algal 161, was supplied by Cell Systems Ltd. This company went into liquidation in 1991 and supplies of freeze-dried algae are no longer available. This alga, *T. suecica*, was grown under heterotrophic conditions. Before feeding to broodstock the required ration of the algal powder was shaken for 30 seconds with filtered, U. V. sterilised sea water and then mixed in a domestic blender for approximately 10 secs.

ii. Food supply

Cultured marine microalgae and freeze-dried algae were added as the food supply in all broodstock experiments. The live unicellular algae species used in the broodstock diets were *D. tertiolecta* and *S. costatum* (1st broodstock) and *S. costatum* mixed with live or dried *T. suecica* (2nd broodstock) experiment in 1991. The spray-dried *T.suecica* was the principal food supply in both broodstock experiments in 1992 (Tables 2.1 and 2.2). These algae differed in their relative quantities of lipid and PUFAs (Laing et al., 1987). In mixed diets 70% of the diet was dry *T. suecica* as used by Laing (1991) for feeding Manila clam spat.

The broodstock was fed from 25 l bins each of which contained the daily ration of the appropriate food species. The food was pumped into the sea water supply to each respective tank through peristaltic pumps at a rate of 1 l hr^{-1}. The pumps were switched off and on automatically with a timer. The bins which contained live algae were aerated, and those with dried food were stirred continually with a mixer to prevent settlement of the *T. suecica* cells. Food bins and pipes were washed every day, and fresh food was added. The bins were filled up with sea water, to a total volume of 12 l in order to provide food for 12 h in each day.

In each experiment, the quantity (in weight) of food fed initially was the same in all the tanks. It was calculated as 3% (1st broodstock in 1991) and 6% (all other broodstocks) of the initial dry meat weight of the broodstock in dry weight of algae per day. The relative amounts fed in 1991 were 0.6 g (1st broodstock) and 1.2 g per day (2nd broodstock) (in both experiments dry meat weight = 19 g and 22g, assumed to be 5% of the total live weight). In 1992 quantities were 2.9 g (1st broodstock) and 2.2 g per day (2nd broodstock). The volume of live algae which was required was calculated from the cell density of the algae culture, and the individual cell weight of each species:

Total live weight of clam x 5% = dry meat weight

Daily ration = 3% and 6% dry meat weight

Weight of *D. tertiolecta* = 95 pg/cell

S. costatum = 32 pg/cell

I. galbana = 20 pg/cell

T. suecica = 200 pg/cell (Laing and Milican, 1986)

The quantity of food changed during each experiment because total biomass of clams was reduced after individuals were removed for each spawning. When 50% of the clams were removed to induce spawning, the feeding was not reduced by 50% but only by 25%, in order to maintain a sufficiently high cell density to encourage efficient filtration activity and thereby avoid stress in the remaining animals.

Clams in tanks with no food supplement received only the organic material which had not been removed from the sea water by the sand filter. The whole broodstock conditioning system was drained once a week and thoroughly rinsed with fresh water.

2.2.1.4. Filtration rate of clams

The filtration rate of the clams was measured weekly by collecting samples of water from the inlet and outlet of each broodstock conditioning tank. The water flow rate through each tank was measured daily using a measuring cylinder and a stopwatch. This provided data to calculate the percentage filtration of the clams in relation to specific flow rate. The particle number in the water samples (approximately 100 ml) were counted using a Coulter Counter (Model ZM) fitted with a 70 μm orifice head. Particles of two sizes groups (2.5 - 5 and 5 - 10 μm diameter) were counted.

The percentage filtration (P) of particles (2.5 - 10 µm diameter) by the clams in each tank was calculated from the formula: $P = 100 (C_o - C_1) / C_o$, where C_o and C_1 were the concentration of particles in the inflowing and outflowing water respectively (Spencer, 1988). The filtration rate (F) was calculated by: $F = P \times V / 100$, where V was the volume of water flowing per minute per gram live weight of clams. F was equivalent to the volume of water filtered clear of particles per unit time and per unit live weight (ml $min^{-1}g^{-1}$).

2.2.2. Condition factor determination

In each experiment, an initial sample of 30 clams from the bivalve on-growing site and a sample of 12-15 clams from each treatment after each spawning attempt were stored at -20^{o}C for subsequent analysis.

The clams, after thawing, were individually numbered on the shell and then their weight, length and breadth were measured. The internal volume of the clams was calculated as the difference between the total volume and the shell volume (Rodhouse, 1977). The volumes were measured by the water displaced, each animal was weighed when immersed in water. The weight measured (g) was equal to the volume of the animal (ml), since the density of freshwater is 1. Each clam was then opened with an oyster knife, the meat was removed and placed in a pre-weighed, 33x51 mm plastic tube and frozen for 24 h. The meats were freeze-dried at -35°C for 24 h, and reweighed. This gave the dry meat weight of clams. The condition index (C.I.) for each adult was calculated as described by Walne (1970):

$$\text{C.I. (Volume)} = \frac{\text{Dry meat weight, g} \times 1000}{\text{Internal volume, ml}}$$

2.2.3. Analytical methods for meat samples

2.2.3.1. Dry weight

Dry meat weight was measured as described immediately above. The meats were then ground to a fine powder before biochemical analysis.

2.2.3.2. Ash weight and ash free dry weight

Samples of approximately 20 mg ground dry meat were weighed and placed in previously tared aluminium foil pots. Samples were left for 24 h at 450°C, to burn off all the organic matter and reweighed. The weight of the ash was calculated by subtracting the weight of the pot, and then calculated as a percentage of the original sample weight.

The ash-free dry weight (Afdwt) was obtained by subtraction of the ash weight from the dry weight.

2.2.3.3. Lipid analysis

For the analysis of total lipid in the broodstock, a gravimetric method was used.

Samples of approximately 20-25 mg of ground dry meat were weighed in triplicate into numbered, graduated 15 ml glass test tubes. A volume of 3 ml of 2:1 (v/v) chloroform/methanol was added to each tube and samples were left for 15-20 min at 20°C. The samples were then purified by adding 0.6 ml of 0.7% (w/v) sodium chloride solution. Each tube was vortex mixed for 5 seconds. The samples were left to separate into 2 phases for 5 min, and then centrifuged at 3000 rpm for 10 min. The volume of the lower phase, containing the chloroform and extracted lipid, was recorded. From this lower phase 1 ml was transferred into dry, pre-weighted teflon pots. A clean pipette tip was used for each of the triplicate samples. The teflon pots were left in a drying cabinet at 60°C for 2 h to evaporate the chloroform, leaving the lipid as a residue. The teflon pots were then reweighed on a Cahn Electrobalance and the tare weight subtracted to give the lipid weight. Total sample lipid was obtained by multiplying lipid weight by the volume of the sample lower solvent phase. The percentage of lipid was calculated from the weight of meat in the sample and expressed as a percentage of the Afdwt.

2.2.3.4. Carbohydrate analysis

The anthrone method was used for the determination of carbohydrate (Strickland and Parsons, 1968).

The reagents used were prepared as follows: The trichloroacetic acid (TCA) solution was prepared with 25 g of TCA crystals dissolved in 500 ml distilled water in a volumetric flask, shaken and stored at 4°C. For the anthrone, 1.0 g recrystallised anthrone was dissolved in a mixture of 40 ml ethyl alcohol and 150 ml distilled water in a 1 l glass beaker. The beaker was placed in a cold water bath in a fume cupboard and gradually 500 ml ANALAR concentrated sulphuric acid was added, with continuous stirring using a glass rod. Before being used the solution was left in the cold water bath to cool. A glucose standard solution was made and kept at 4°C before use. One gram of D-glucose was dissolved in 100ml distilled water in a volumetric flask and mixed thoroughly. For calibration standards, 1 ml of this solution was made up 100 ml with distilled water in a clean volumetric flask. This solution contained 100 μg glucose ml^{-1}.

Triplicate samples of 2-5 mg dry meat were weighed and placed in graduated test tubes and 2 ml of 5% (w/v) TCA were added. The tubes were covered with foil, to prevent excess evaporation and heated for 1 h at 90°C in a thermostatically controlled water bath. They were centrifuged at 3000 rpm for 5 min and the volumes in the tubes were recorded. Then 0.5 ml of TCA supernatant (with extracted carbohydrate) was pipetted into a thick-walled glass Pyrex boiling tube. Standards were prepared by pipetting 0.5 ml of a glucose solution into 5 replicate boiling tubes. The reagent blank was 0.5 ml of distilled water. To each tube were added 4 ml of anthrone reagent. The contents of the tubes were vortex mixed. All the tubes were placed for 7 min in a water bath at 100°C, and then cooled for 10 min in a cold water bath.

Standard and sample solutions were placed in disposable cuvettes and absorbancy read in a spectrophotometer at 620 nm wavelength. The concentration of carbohydrates was calculated against the standards and blank by a multiple wavelength computer programme (forced zero mode) coupled to the spectrophotometer. The concentration of glucose per ml was multiplied by the volume of the TCA fraction, to give ug of carbohydrate per sample. Carbohydrate was expressed as a percentage of meat ash-free dry weight.

2.2.3.5. Fatty acid analysis

The extraction of total lipids and esterification of the constituent fatty acids was carried out by the MAFF laboratory's biochemist (A.R.Child) using the method of Lepage and Roy (1984).

Analysis of the fatty acid composition of the adult clams, before and after spawning was carried out by gas chromatography. Total lipid was extracted with chloroform/methanol. Transesterification of the lipid was then achieved by reaction with methanol in the presence of an acetyl chloride catalyst to form fatty acid methyl esters (FAMEs). After extraction in hexane, chromatography was carried out with a Carlo Erba flame ionisation detector. FAME separation was on a 5 polyethylene glycol fused silica column, temperature programmed from 80° - 200°C, using hydrogen as the carrier gas. FAME standards were supplied by Sigma Chemical Co. Ltd. The description can be found in Appendix 1.

The percentages of saturated (14:0, 16:0 and 18:0), monoenoic (16:1w9, 16:1w7, 18:1w9, 18:1w7, 20:1w9 and polyenoic (18:2w6, 18:3w3, 18:4w3, 20:4w6, 20:5w3, 22:5w3 and 22:6w3) FAMEs were calculated.

2.2.4. Gametogenesis determination

2.2.4.1. Treatment of material for histological examination

Five clams from each broodstock treatment were sampled for gonad tissue before each spawning. Microscopic analyses were made on clam gonads which had been preserved in Davidson's fixative (Shaw and Battle,1957). This process was quick, simple and particularly suitable for materials containing high levels of lipid. The material was treated as follows:

1. Fixation
   - The tissue was fixed for 24 h in Davidson's fixative
   - It was washed with 70% alcohol and stored for later treatment

2. Dehydration
   - The piece was immersed in:
     - 90% alcohol (2 h)
     - 100% alcohol (2 h)

- 50% xylene + 50% alcohol (2 h)
- 100% xylene (30 min)
- 50% parafin wax + 50% xylene (2 h)
- 100% paraffin wax (2 h)

3. Embedding

- Two L-shaped pieces of brass to mould the paraffin wax (Leukart's embedding moulds) were used for embedding the clam pieces.

4. Section cutting

- The paraffin wax block was mounted in block holders.
- Cut in the microtome (6 μm)

5. Staining

- Several sections were stained in porcelain staining trough with eosin (1 min) and haematoxylin (4 - 5 min).

6. Mounting

-The clam sections were mounted on slides with D.P.X. mountant.

2.2.4.2. Analysis of slides

Gonadal stages have been determined for clams and related bivalves by many researchers. In this study six gonadal stages were chosen to describe the reproductive cycle of the Manila clam based on studies by Holland and Chew (1974) and Spencer personal communication). The stages of female and male gonad were: stage I (inactive), stage II (early gametogenesis), stage III (late gametogenesis), stage IV (mature), stage V (partially spawned) and stage VI (spawned).

2.3. Spawning and fertilization

The required number of conditioned clams from each diet treatment were selected from the conditioning tanks and placed in a 100 l glassfibre trough, measuring 140x50x16 cm. Black polyvinyl chloride (PVC) sheets were used to provide a contrasting background to aid the detection of gamete liberation.

Methods to induce spawning in bivalves are well established (Loosanoff and Davis, 1963) and in this study the method of temperature shock treatment was used in the weeks shown in Tables 2.1 and 2.2.

Spawning was induced by subjecting the adults to cyclic temperature shocks of 20°C and 30°C. This was achieved by changing the sea water at 15 min intervals. Algae were added to the water to induce filtration activity, followed by the addition of egg suspension from an opened female as an additional stimulant (Loosanoff and Davis, 1963). This treatment was repeated until spawning occurred. When each individual clam began to spawn, it was removed and placed in a separate clean 1 l beaker where it was allowed to complete gamete liberation. The beaker contained 500 ml filtered sea water, at ambient salinity (30 - 34 psu) heated to 25°C and passed over an ultra-violet light source. The sex of each animal was determined as early as possible by observation of the gametes. Females liberated eggs which dispersed into granular suspension while males produced a thick, non-granular stream of sperm.

The eggs from each female were kept separate in one or two clean 3 l glass beakers, depending on the number of eggs obtained. Sperm from the males in each treatment was pooled but in a 1 l glass beaker. Fertilization was effected by mixing 2 ml of the dense sperm suspension to every litre of egg suspension. Each suspension of fertilised eggs was filtered through a 90 μm nylon mesh sieve in order to remove any large debris and the volume of the suspension made up to 2.5 l. The egg numbers in a known volume were estimated after fertilization by sampling, and counting them with a Coulter Counter model ZM fitted with a 200 μm orifice head.The concentration per ml was multiplied by the volume in the beaker to give the total number of fertilized eggs. All the broodstock clams were numbered on the shell, placed in plastic bags according to sex and diet treatment, and saved for determination of condition and for biochemical analyses.

2.4. Egg quality

2.4.1. Egg sampling for biochemical analysis

From each treatment, a sample of eggs was saved for later analysis of dry weight, ash free dry weight, lipids and PUFAs.

2.4.1.1. Dry weight and ash-free dry weight

Duplicate samples of a known number of eggs (usually between 50,000 and 100,0000) were taken and placed, with 2-3 drops of 6% neutralized formalin, into graduated, labelled, conical-based, 15 ml test tubes. The sea water was removed using an autopipette and the eggs washed twice in isotonic (3%) ammonium formate to remove inorganic salts (Strickland and Parsons, 1968).

Samples were then transferred on to separate pre-ashed (at 450°C) 4.7 cm diameter glassfibre, Whatman GF/C filter papers which were held on a Millipore filter, under 0.35 kg cm^{-2} vacuum. Each filter paper was placed on a numbered aluminium sheet and dried in an oven at 60°C for 48 hours. Filter papers were placed in a desiccator before being weighed (dry weight). Samples were covered with another filter paper and ashed in a Carbolite furnace at 450°C for 24 h. The samples were then removed to a desiccator and allowed to cool to room temperature before being re-weighed (ash weight). Dry weights and ash-free weights were expressed on a per egg basis (ng). The ash-free dry weight per egg was calculated by subtracting the ash from the dry weight.

2.4.1.2. Lipid analysis

From each female, triplicate samples containing a known number of between 17,000 - 25,000 eggs were placed, into 15 ml centrifuge tubes. All the tubes were centrifuged at 2,500 rpm for 2 min, and the water removed with a vacuum pipe. The eggs were rinsed with 3% ammonium formate to remove the salts and centrifuged at 2,500 rpm for another 2 min. The ammonium formate solution was removed with a vacuum pipe as before. Samples were stored at -75°C in the graduated tubes until numbers were sufficient for analysis. Total lipid was measured as described previously (Section 2.1.2.c). Division of total lipid values by the number of eggs per sample provided a measure of the mean total lipid per egg which was then expressed as a percentage of ash-free dry weight.

2.4.1.3. Fatty acid analysis

One sample of eggs per female, containing a known number of 30,000 to 50,000 eggs was placed with 2 - 3 drops of 6% neutralized formalin, in 15 ml centrifuge tubes. Samples were collected and cleaned with ammonium formate as described in the lipid section immediately above, before 2 ml of chloroform/ methanol (2:1 v/v) containing an antioxidant, 2,6 di-tert-butyl p-cresol, at a concentration of 0.005%, were added.

The sample was sealed in a glass vial, then stored at -75°C. FAMES analysis by gas chromatography was carried out as described previously (Section 2.2.3.5.).

2.5. Embryo bioassays

To test the viability of eggs from individual females, bioassays were carried out in Zaroogian artificial sea water, since it proved to be the best of the artificial sea waters tested. Water was filtered under a vacuum not exceeding 0.35 kg cm^{-2} through Whatman filters GF/C to remove suspended particulate material. The water was aerated overnight and kept at 4°C for later use. The chemical EDTA was added at 1 mg l^{-1}.

Aliquots of 1500 embryos were transferred to each of a series of non-toxic, transparent, polystyrene vials containing 25 ml of the test waters heated to 25°C. The contents were made to 30 ml with the test water giving a final concentration of 50 embryos ml^{-1}. Each treatment was made in quadruplicate. After incubation overnight at 25°C, the water in each vial was mixed thoroughly to ensure an even distribution of embryos. Two ml subsamples were taken from each vial and placed on gridded slides with two drops of 6% formalin. The number of well-formed D-larvae in each subsample was determined using a binocular microscope. It was usual to count the D-larvae in 3 of the 4 replicates. The fourth replicate was sampled only when there was disparity in counts. Results were expressed as the mean number of D-larvae per 2 ml, effectively the percentage yield.

2.6. Embryo development

Fertilized eggs from each treatment were placed in 45 or 125 l cylindrical polyethylene culture bins, as appropriate, in order to give a stocking density of 100 eggs ml^{-1}. The sea water in the bins was filtered, heated to 25°C, sterilized through a UV lamp unit, treated with EDTA (1ppm) to improve the water quality (Utting and Helm, 1985) and 2 ppm of chloramphenicol to prevent excessive bacterial numbers. The bins were covered with a circular sheet of clear PVC and left for 24 h for the embryos to hatch.

The cultures were not aerated since the turbulence created would have been detrimental to the early development of the embryos (Helm and Spencer, 1972).

2.7. Rearing of larvae

D-larvae were present in the polyethylene culture bins 24 h after fertilization. The larvae were collected by siphoning the bin contents through a 35 μm mesh sieve. Larvae were washed from the sieve into a 1 l beaker, and a 1 ml sample observed under a microscope. The larvae were separated into three groups (35, 45 and 61 μm sieve meshes). The larvae retained by a 61 μm mesh were good D-larvae. The percentage of D-larvae retained in 45 and 35 μm meshes was very small and these larvae, which were usually abnormal, were discarded. The number of larvae per ml was measured using a ZM Coulter Counter. The total number of D-larvae in the 1 l beaker was calculated and expressed as percentage recovery from the total number of embryos incubated.

2.7.1. Larval culture

Larvae, at an initial density of 10 ml^{-1}, were reared in cylindrical, flat-bottomed polyethylene bins, each of which was covered with a circular transparent PVC sheet. The bins were filled with filtered, heated and UV light sterilised natural sea water. The temperature and salinity ranged between 24 - 26^{o}C and 30 - 33 psu respectively. Larvae were grown for 14 or 16 days to assess their growth and survival to metamorphosis in relation to initial egg quality.

Three times each week on Mondays, Wednesdays and Fridays, sea water in the bins was changed and the inside of the vessels washed (Walne, 1974). Larvae were collected and graded, using stacked sieves each with a different mesh aperture ranging from 61 µm to 265 µm. Larvae were concentrated on the sieves and they were washed with a jet of water to remove detritus (pseudo-faeces, surplus food, shell fragments, etc). At each water change larvae were sampled to determine mean size and survival. The larvae were then replaced in clean sea water in the culture vessels and 1 mg EDTA l^{-1} and 2 mg chloramphenicol l^{-1} were added.

2.7.2. Feeding

In all bin trials, larvae were fed initially on a mixture of 50 *I. galbana* and 50 *C. calcitrans* or with 100 *C. calcitrans* cells μl^{-1}. When larvae were large enough to ingest larger algae *T. suecica* was generally added (Tables 2.3 and 2.4). The algal clearance rate was measured every day by counting the algal numbers in a 100 ml sample taken from each bin. Counts were made using a Coulter Counter fitted with a 50 µm head and set to count particles of between 2 - 5 µm for *I. galbana* and *C. calcitrans* and 5-10 µm for *T.suecica*. It was necessary to increase food rations if more than 75% of the cells were cleared in 24 h.

Table 2.3. Algal species and quantities supplied to T. philippinarum larvae during the 1991 feeding trial (D. tertiolecta=Dun, S. costatum=Ske, T. suecica=Tet, Dry T. suecica=Dry Tet, C. calcitrans=Chaet, I.galbana=Iso and replicate=R)

Broodstock diet	Duration		Larval diet	Feeding regime cells/µl
1st broodstock 1991				
Spawning III				
Dun	Day 0-5	* Day 0-4	Iso/Chaet	50;50
Ske*	Day 5-16	Day 4-14	Iso/Chaet	100 ; 100
2nd broodstock 1991				
Spawning II				
Tet+Ske	Day 0-5		Chaet	100
Dry Tet+Ske	Day 5-16		Chaet/Tet	200 ; 2
Fsw				
Fsw-R				
Spawning III				
Tet+Ske	Day 0-5	*Day 0-4	Chaet	100
Tet+Ske-R	Day 5-16	Day 4-14	Chaet/Tet	200 ; 2
Dry Tet+Ske				
DryTet+Ske-R*				
Fsw *				
Fsw-R*				

1st Broodstock 1991 - Spawning I - clams did not spawn
Spawning II - not enough D-larvae
2nd Broodstock 1991 - Spawning I - not enough D-larvae
* In these treatments the duration was day 0-4 and day 4-14 instead of day 0-5 and day 5-16.

Table 2.4. Algal species and quantities supplies to T. philippinarum larvae during the 1992 feeding trial. For abbreviation see Table 2.3.

Broodstock diet	Duration	Larval diet	Feeding regime cells/µl
1st broodstock 1992			
Spawning I			
Dry Tet + Dun	Day 0-5	Chaet	100
Dry Tet + Ske	Day 5-19	Chaet / Iso / Tet	100 ; 100 ; 2
Dry Tet + Iso			
Dry Tet			
Spawning III			
Dry Tet + Dun	Day 0-5	Chaet / Iso	50 ; 50
Dry Tet + Ske	Day 5-19	Chaet / Iso /Tet	100 ; 100 ; 2
Dry Tet + Iso			
Dry Tet			
2nd broodstock 1992			
Spawning III			
Dry Tet + Dun	Day 0-5	Chaet / Iso	50 ; 50
Dry Tet + Ske	Day 5-14	Chaet / Iso / Tet	100 ; 100 ; 2
Dry Tet + Iso			
Dry Tet			

1st Broodstock 1992 - Spaw. II - D-larvae only utilised for bioassay
2nd Broodstock 1992 - Spaw. I - clams did not spawn
Spaw. II - clams did not spawn

The amount of food required each day was determined by the following formula:

$$\text{Quantity of algal culture to feed (ml)} = \frac{\text{Required cell density (cells } \mu m\ l^{-1}) \times \text{Rearing vessels volume (ml)}}{\text{Harvested algae cell density (cell } \mu m^{-1})}$$

2.7.3. Estimation of larval growth and survival

At each water change, larvae were washed with filtered sea water and concentrated into a 1 l graduated cylinder. Triplicate 1 ml samples were taken with an adjustable volume automatic pipette, placed in ruled perspex dishes with a few drops of 6% neutralised formalin and counted under a microscope. From the mean of the three counts an estimate of the total number of larvae in the graduated cylinder was obtained.

Mean shell length was calculated for larvae in each treatment. Measurements of the axis bisecting the anterior and posterior margins were made with a binocular microscope fitted with a calibrated eyepiece. The numbers of larvae measured was 25, for larvae less than 100 µm, and 50, for larvae larger than 100 µm. Larvae were scored in 10 µm size classes, from which the mean shell length and the standard error was calculated.

Because larval shell length was not always measured at similar times in all experiments, growth coefficients (G) were determined to compare daily growth rates of larvae. For larvae grown in natural sea water they were based on measurement of shell length and assessed as:

$$G = \frac{\ln Vt - \ln Vo}{t}$$

where Vo = initial growth variable value, Vt = final growth variable value and t = time in days.

2.7.4. Analysis of larvae

Methodologies for the assessment of dry weight, ash-free dry weight, lipid and PUFAs were as described previously (see section 2.4.1).

The numbers of larvae used were: dry and ash free dry weight 20,000 - 100,000, lipid 20,000 - 25,000, carbohydrate 22,000 - 24,000 and fatty acid 20,000 - 50,000. Larvae were placed, with 2 - 3 drops of 6% neutralized formalin into a graduated labelled conical based 15 ml test tubes. Sea water was removed with a vacuum pipe and larvae washed with 3% ammonium formate. Techniques for further analysis were as described above (see section 2.4.1).

2.7.5. Salinity experiment

A salinity experiment was carried out with Manila clam D-larvae, one-day-old, obtained from the 2nd broodstock 1992. Broodstock were conditioned with either 100% spray-dried *T. suecica* or 70% spray-dried *T. suecica* with a supplement of 30% live algae (*D. tertiolecta, S. costatum* or *I. galbana*). Larvae were reared in beakers at salinities of 10, 15 and 30 psu. All experiments had replicates per treatment. Growth and survival were calculated after 7 and 14 days.

2.8. Rearing of spat

At the end of the pelagic larval phase and at the onset of the peliveliger phase, larvae were transferred into recirculating downwelling sea water systems, similar to those described by Laing and Millican (1986). These consisted of 50 l polypropylene reservoir tanks (57 x 33 x 25 cm), containing a transparent acrylic constant head tank (32 x 25 x 20). The latter tank contained one acrylic downwelling cylinder (15 cm diameter by 20 cm height) fitted with a monofilament nylon mesh bottom (140 μm mesh size) raised 4 cm from the bottom of the cylinder. A flow of 1.5-2 l min^{-1} through the downwelling tube was maintained by an air lift device which raised sea water from the outer reservoir to the constant head tank (Utting and Spencer 1991).

This type of system was operated in a similar way to larval vessels. Water was changed at 48 h intervals and the tank was supplied daily with equal quantities, on a weight basis of *T. suecica*, *I. galbana* and *C. calcitrans*.

2.9. Statistical Analysis

The statistical analysis was carried out using two statistical packages, which were:

1. SAS statistical package (SAS Institute, SAS Circle, P.O. Box 8000, Carr, N. 27512-8000).
2. EXCEL statistical functions (Microsoft Excel Function Reference, Microsoft Corporation, Redmond, WA 98052-6399).

A variety of statistical tests were available with each package. One way and two way analysis of variance (ANOVA) was carried out on many of the parameters measured and analysed. For the parameters in which the results of ANOVA were significant, t-test (LSD) were done for comparison of each groups between treatments. Linear regression was calculated for the parameters measured in the initial broodstocks. Some descriptive statistics e.g. mean, standard error were also calculated.

Chapter 3

CHAPTER 3. RESULTS

3.1. BROODSTOCK FED ON LIVE ALGAE (3 % DIET, 1st BROODSTOCK 1991)

3.1.1 Biochemical analysis of the cultured algae

The gross biochemical composition of the cultured algae fed to the 1st 1991 broodstock is presented in Table 3.1. *S. costatum* contained more lipid than *D. tertiolecta*. It was not possible to analyse the carbohydrate of these two live algae because the samples were contaminated (dirty material in the test tubes). The qualitative fatty acid composition of the live algae, *D. tertiolecta* and *S. costatum* used in the 1st broodstock is presented in Table 3.2. The level of long chain polyunsaturated fatty acids (PUFAs) varied with species. *D. tertiolecta* had high levels of 18C fatty acids, particulary 18:3w3 and no 20C or 22C PUFAs while *S. costatum* had no 18:3w3 and a high level of 20:5w3.

3.1.2. Broodstock conditioning

3.1.2.1. Temperature and salinity

Sea water temperature during conditioning ranged from 21.0 to 24.8 ^{o}C (spawning I and II) and 20.8 to 24.8 ^{o}C (spawning III). Mean temperature values for each experiment are shown in Table 3.3. Temperature differences between broodstock tanks were not significantly different [$F_{5,348} = 0.31$, $p = 0.91$].

The salinity ranged from 27 to 32 psu during the course of the three experiments in the 1st 1991 broodstock (Table 3.4). Salinity between tanks was not significantly different [$F_{5,348} = 0.005$, $p = 0.99$].

Table 3.1. Ash free dry weight (Afdwt, mg per million cells and as a % of the dry weight) and biochemical composition of diets in the 1st broodstock 1991. Protein values were obtained by subtraction of lipid and carbohydrate percentages. Standard error in parentheses.

Diet species	Afdwt (mg) / million cells	% Afdwt	Lipid (% Afdwt)	Carbohydrate (% Afdwt)	Protein (% Afdwt)
1st broodstock 1991					
D. tertiolecta	0.16 (±0.02)	51.97 (±5.50)	15.81 (±2.78)	«	«
S. costatum	0.03 (±0.004)	54.08 (±2.67)	26.73 (±1.82)	«	«

« contaminated sample

Table 3.2. Fatty acid composition of live algae used on the 1st broodstock 1991. Values are percentages of total fatty acids.

	1st broodstock 1991	
Algae	*D. tertiolecta*	*S. costatum*
Fatty acid		
14:0	25.90	32.67
16:0	27.31	19.77
18:0	7.41	6.04
16:1w9	1.68	1.03
16:1w7	1.62	10.12
18:1w9	15.51	11.04
18:1w7	1.06	0.45
20:1w9	0.00	0.00
18:2w6	6.60	2.53
18:3w3	11.88	0.00
18:4w3	1.02	1.85
20:2w6	0.00	2.50
20:3w3	0.00	0.14
20:4w6	0.00	0.00
20:5w3	0.00	11.63
22:5w3	0.00	0.00
22:6w3	0.00	0.24
Saturated	60.62	58.48
Monoenoic	19.87	22.64
Polyenoic	19.50	18.89

Table 3.3. Mean temperature and day-degrees in *T. philippinarum* broodstock tanks (1st broodstock 1991). Standard error in parentheses. Dun=*D. tertiolecta*; Ske=*S. costatum*; Fsw= Filtered sea water and R=replicate.

Spawning	Week	Broodstock treatment					
		Dun	Dun-R	Ske	Ske-R	Fsw	Fsw-R
1st broodstock 1991							
I	6	22.64 (±0.12) 556	22.66 (±0.12) 557	22.68 (±0.11) 558	22.55 (±0.12) 552	22.53 (±0.11) 551	22.70 (±0.10) 559
II	7	22.60 (±0.10) 642	22.67 (±0.10) 646	22.68 (±0.09) 647	22.58 (±0.11) 641	22.55 (±0.09) 640	22.69 (±0.09) 647
III	8	22.50 (±0.11) 738	22.57 (±0.11) 742	22.56 (±0.10) 741	22.48 (±0.11) 737	22.42 (±0.10) 733	22.55 (±0.10) 740

Table 3.4. Mean salinity in T. philippinarum broodstock tanks (1st broodstock 1991). Standard error in parentheses. For abbreviations see Table 3.3.

Spawning	Week	Broodstock treatment					
		Dun	Dun-R	Ske	Ske-R	Fsw	Fsw-R
1st broodstock 1991							
I	6	31.10 (±0.14)	31.08 (±0.15)	31.10 (±0.15)	31.09 (±0.15)	31.08 (±0.15)	31.08 (±0.15)
II	7	30.87 (±0.16)	30.86 (±0.16)	30.88 (±0.16)	30.86 (±0.16)	30.86 (±0.16)	30.87 (±0.16)
III	8	30.64 (±0.16)	30.64 (±0.17)	30.65 (±0.17)	30.63 (±0.17)	30.62 (±0.17)	30.63 (±0.17)

3.1.2.2. Filtration rate of clams

The percentage filtration (P) and the filtration rate (F) of the clams from the 1st broodstock 1991 is shown in Table 3.5. From the results of ANOVA and "t" test, there was no significant difference between the percentage filtration or filtration rate of clams fed *D. tertiolecta* and *S. costatum* but the unfed broodstock had a significantly lower percentage filtration compared with the other treatments [$F_{5,42} = 13.74$, $p < 0.0001$].

3.1.2.3. 1st broodstock conditioning 1991

I. Initial broodstock

The gross biochemical composition and condition indices of the initial broodstock are shown in Table 3.6 and Appendix 2. There were significant relationships between many of the measured variables eg. the live weight and the shell length, breadth and meat dry weight of the clams. Values for the regression equations which described the relationships between the different parameters of the initial broodstock are given in Table 3.7.

As a summary of general trends, all the meat dry weight, condition index, lipid and carbohydrate results, from the initial broodstock and after conditioning for 6, 7 and 8 weeks, are summarised in Figures 3.1-3.4.

II. Broodstock conditioning for 6 weeks

None of the fed or unfed broodstock, spawned after 6 weeks (556 D^o) of conditioning. The gross biochemical composition and condition of the broodstock after

Table 3.5. Percentage filtration and filtration rate (particles between 2.5 - 10 µm) of <u>T. philippinarum</u> broodstock (1st broodstock 1991). For abbreviations see Table 3.3.

Date	Percentage filtration						Filtration rate					
	Dun	Dun-R	Ske	Ske-R	Fsw	Fsw-R	Dun	Dun-R	Ske	Ske-R	Fsw	Fsw-R
1st broodstock 1991												
(25.01.91)	62.77	32.01	45.14	60.93	12.00	11.97	376.64	192.04	270.85	365.57	72.00	71.84
(4.02.91)	53.46	63.05	84.35	73.36	54.59	53.46	320.77	378.28	506.08	440.14	327.55	320.79
(11.02.91)	79.43	81.26	83.85	77.99	38.42	45.60	476.61	487.58	503.08	467.95	230.54	273.61
(18.02.91)	74.54	74.69	53.49	65.27	31.42	25.81	447.26	448.15	320.94	391.63	188.52	154.89
(25.02.91)	66.89	50.27	59.02	71.25	29.75	29.34	401.35	301.62	354.09	427.51	178.51	176.06
(4.03.91)	76.19	76.92	57.24	73.55	38.14	37.55	457.13	461.49	343.41	441.32	228.86	225.29
(11.03.91)	52.77	55.37	71.88	57.01	34.94	28.03	316.59	332.23	431.30	342.06	209.63	168.17
(18.03.91)	52.02	55.47	71.03	58.23	19.46	9.12	312.11	332.82	426.20	349.35	116.76	54.73
Mean	64.76	61.13	65.75	67.20	32.34	30.11	388.56	366.78	394.49	403.19	194.05	180.67
SE	3.97	5.78	5.05	2.80	4.55	5.40	23.83	34.68	30.32	16.78	27.29	32.39

Table 3.6. Mean values for condition indices and biochemical composition of T. philippinarum. 1st broodstock 1991 conditioned from 21.01.91 to 21.03.91. Values are given for Initial and 6, 7 and 8 weeks of conditioning. Protein values were calculated by difference. Standard error in parentheses. For abbreviations see Table 3.3. Afdwt = ash-free dry weight

Broodstock diet	Live weight g	Length mm	Breadth mm	Meat dry weight, g	Condition index	% Afdwt	Lipid (% Afdwt)	Carbohydrate (% Afdwt)	Protein (% Afdwt)
Initial									
N=30	8.21 (±0.28)	31.75 (±0.52)	23.20 (±0.34)	0.32 (±0.01)	85.20 (±1.47)	73.76 (±0.55)	5.43 (±0.12)	5.42 (±0.23)	89.15 (±0.26)
6 weeks									
N=15									
Dun	8.55 (±0.16)	32.67 (±0.33)	24.27 (±0.27)	0.27 (±0.02)	66.80 (±4.55)	83.25 (±0.52)	15.76 (±0.42)	5.93 (±0.23)	78.31 (±0.39)
Dun-R	9.31 (±0.38)	33.60 (±0.57)	24.93 (±0.60)	0.33 (±0.02)	74.28 (±1.91)	83.29 (±0.50)	14.25 (±0.64)	6.03 (±0.30)	79.72 (±0.75)
Ske	8.89 (±0.26)	32.53 (±0.27)	24.33 (±0.29)	0.32 (±0.01)	76.19 (±1.96)	82.84 (±0.75)	9.23 (±0.32)	5.26 (±0.44)	85.51 (±0.47)
Ske-R	9.24 (±0.26)	33.33 (±0.40)	24.93 (±0.37)	0.32 (±0.01)	73.96 (±2.43)	81.63 (±0.54)	8.13 (±0.18)	4.49 (±0.29)	87.39 (±0.34)
Fsw	9.23 (±0.37)	33.93 (±0.60)	24.93 (±0.34)	0.24 (±0.01)	54.13 (±2.16)	79.34 (±0.54)	7.51 (±0.10)	3.24 (±0.09)	89.24 (±0.16)
Fsw-R	8.99 (±0.22)	33.33 (±0.36)	24.67 (±0.23)	0.23 (±0.01)	52.84 (±1.29)	78.66 (±0.55)	7.93 (±0.38)	3.36 (±0.12)	88.70 (±0.32)

(continued)

Table 3.6. (continued)

7 weeks									
N=14 * N=15									
Dun	6.95 (±0.17)	31.43 (±0.40)	23.21 (±0.37)	0.29 (±0.01)	81.31 (±5.48)	83.12 (±1.10)	12.85 (±0.40)	3.95 (±0.24)	82.28 (±0.47)
Dun-R	7.49 (±0.32)	30.80 (±0.42)	23.27 (±0.47)	0.29 (±0.02)	71.63 (±3.95)	80.60 (±0.40)	10.90 (±0.19)	4.36 (±0.39)	84.74 (±0.50)
N=15									
Ske	7.53 (±0.30)	31.40 (±0.41)	23.67 (±0.41)	0.30 (±0.02)	71.26 (±4.08)	80.62 (±0.54)	10.80 (±0.30)	3.34 (±0.16)	85.86 (±0.38)
Ske-R	8.01 (±0.21)	32.60 (±0.34)	24.67 (±0.43)	0.29 (±0.01)	64.83 (±2.62)	81.64 (±0.52)	9.22 (±0.23)	3.13 (±0.17)	87.65 (±0.30)
Fsw	7.81 (±0.31)	32.13 (±0.41)	23.73 (±0.28)	0.22 (±0.01)	59.75 (±3.00)	73.71 (±0.78)	10.08 (±0.20)	2.47 (±0.11)	87.45 (±0.19)
Fsw-R	7.79 (±0.28)	31.80 (±0.50)	23.53 (±0.38)	0.23 (±0.02)	62.34 (±3.53)	73.87 (±0.61)	8.02 (±0.13)	2.34 (±0.10)	89.77 (±0.08)
8 weeks									
N=12 * N=14									
Dun	7.14 (±0.36)	30.67 (±0.48)	23.00 (±0.35)	0.25 (±0.01)	80.84 (±4.38)	80.83 (±0.56)	10.84 (±0.16)	8.65 (±0.44)	80.51 (±0.52)
Dun-R	7.32 (±0.36)	32.07 (±0.43)	23.36 (±0.32)	0.24 (±0.01)	63.09 (±2.52)	80.57 (±0.55)	10.41 (±0.20)	6.26 (±0.36)	83.33 (±0.49)
N=13									
Ske	8.25 (±0.20)	32.00 (±0.30)	23.31 (±0.13)	0.30 (±0.01)	79.13 (±2.59)	80.66 (±0.60)	11.28 (±0.26)	5.99 (±0.45)	82.72 (±0.42)
Ske-R	7.92 (±0.53)	31.77 (±0.70)	22.69 (±0.50)	0.30 (±0.02)	80.73 (±2.98)	80.60 (±0.90)	13.73 (±0.39)	4.78 (±0.35)	81.49 (±0.72)
N=12 * N=15									
Fsw	7.41 (±0.42)	31.50 (±0.68)	23.25 (±0.39)	0.14 (±0.01)	39.83 (±2.81)	75.13 (±0.56)	9.07 (±0.15)	2.57 (±0.15)	88.36 (±0.20)
Fsw-R	7.93 (±0.39)	32.07 (±0.86)	23.20 (±0.45)	0.18 (±0.01)	49.43 (±1.99)	73.77 (±0.88)	9.55 (±0.18)	2.83 (±0.09)	87.63 (±0.19)

Table 3.7. Linear regression equations of the initial 1st broodstock conditioning 1991. The data were not transformed. The linear relationship was significant because of the limited size range of animals used. Y=A+Bx, A=intercept; B=slope; r=correlation coefficient; d.f.=degrees of freedom; S=significance; n.s.= not significant;
* significant = p < 0.05 > 0.01;
* * significant = p < 0.01 > 0.001;
* * * significant = p < 0.001.

1st broodstock 1991 (initial)

Parameters estimated (y x)	Intercept (A)	Slope (B)	r	d.f.	S
Live weight x length	-4.398	0.397	0.730	29	* * *
Live weight x breadth	-10.054	0.787	0.934	29	* * *
Live weight x meat dry weight	0.929	23.075	0.942	29	* * *
Length x breadth	5.568	1.129	0.728	29	* * *
Length x meat dry weight	20.290	36.341	0.807	29	* * *
Breadth x meat dry weight	14.954	26.149	0.900	29	* * *

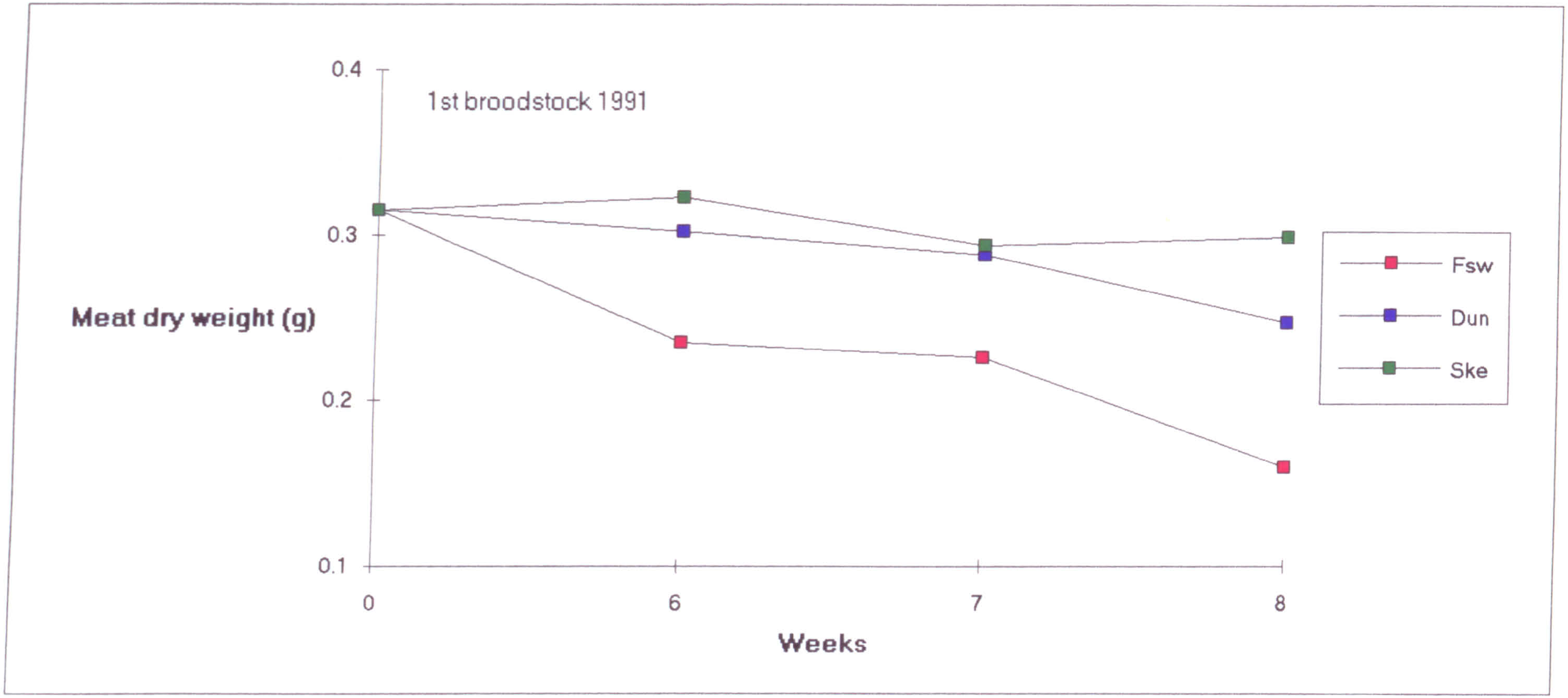


Figure 3.1. Mean meat dry weight of the initial <u>T. philippinarum</u> 1st broodstock 1991, and conditioned for 6 (spawning I), 7 (spawning II) and 8 (spawning III) weeks with different diets. For clarity, standard errors (given in Table 3.6) are not included in this Figure. For abbreviations see Table 3.3.

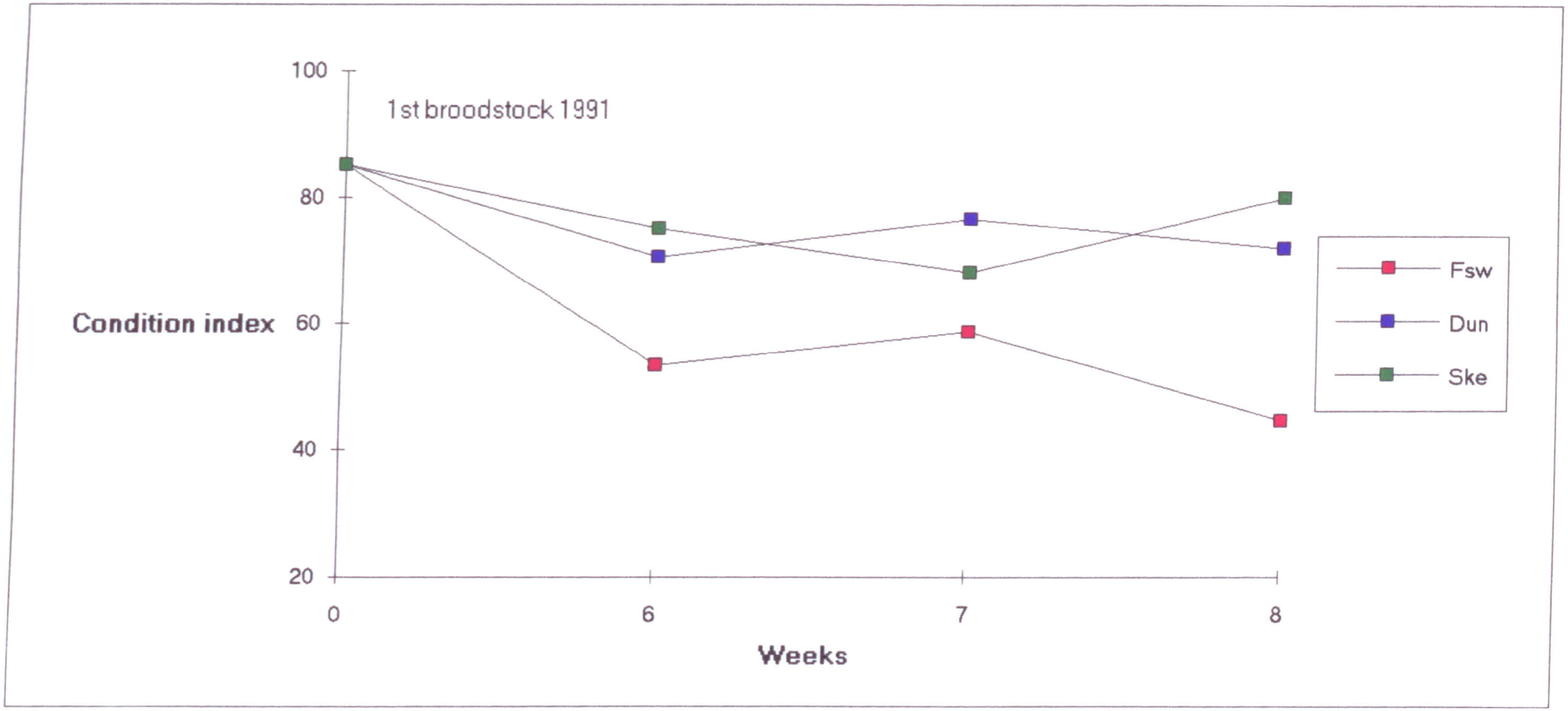


Figure 3.2. Mean condition index of the initial _T. philippinarum_ 1st broodstock 1991, and conditioned for 6 (spawning I), 7 (spawning II) and 8 (spawning III) weeks with different diets. For clarity, standard errors (given in Table 3.6) are not included in this Figure. For abbreviations see Table 3.3.

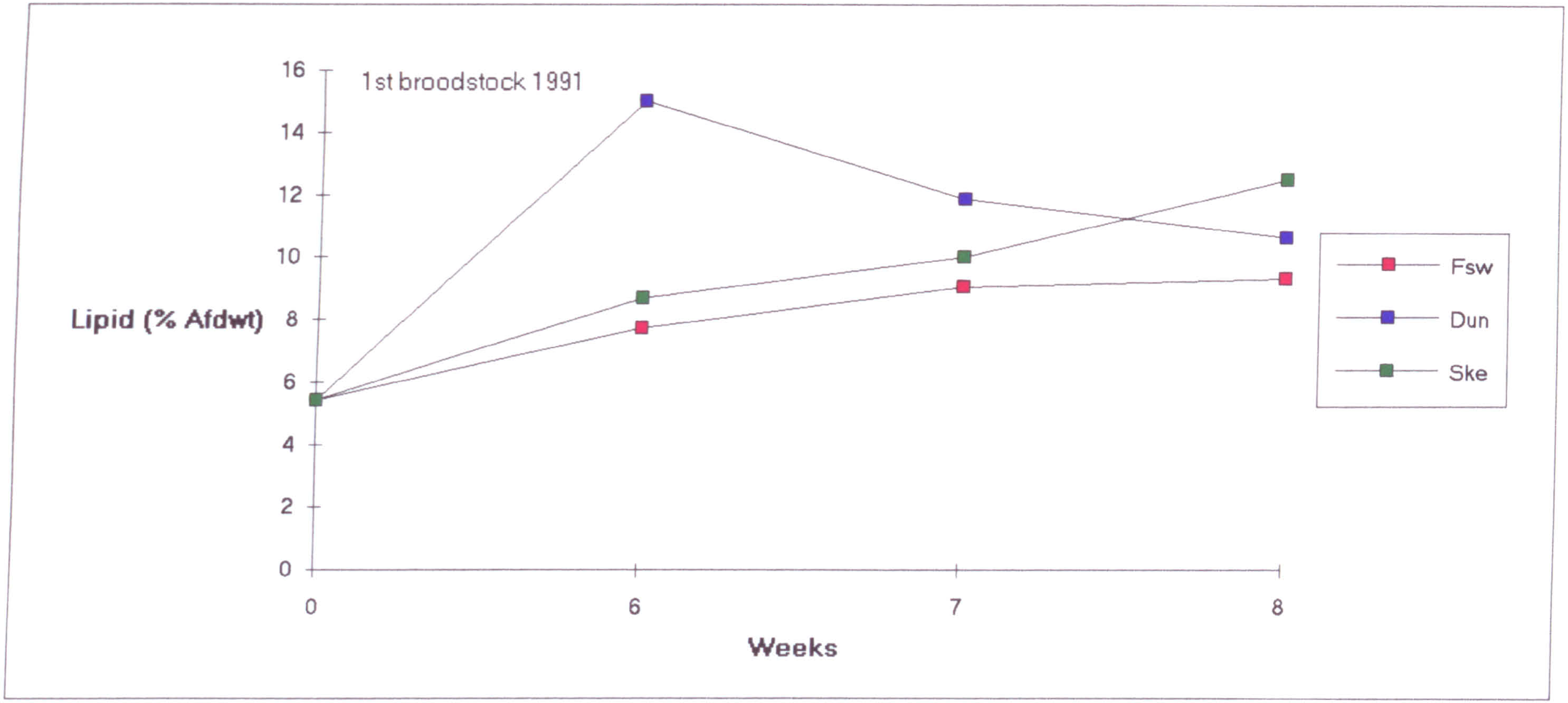


Figure 3.3. Mean lipid content of the initial _T. philippinarum_ 1st broodstock 1991, and conditioned for 6 (spawning I), 7 (spawning II) and 8 (spawning III) weeks with different diets. For clarity, standard errors (given in Table 3.6) are not included in this Figure. For abbreviations see Table 3.3.

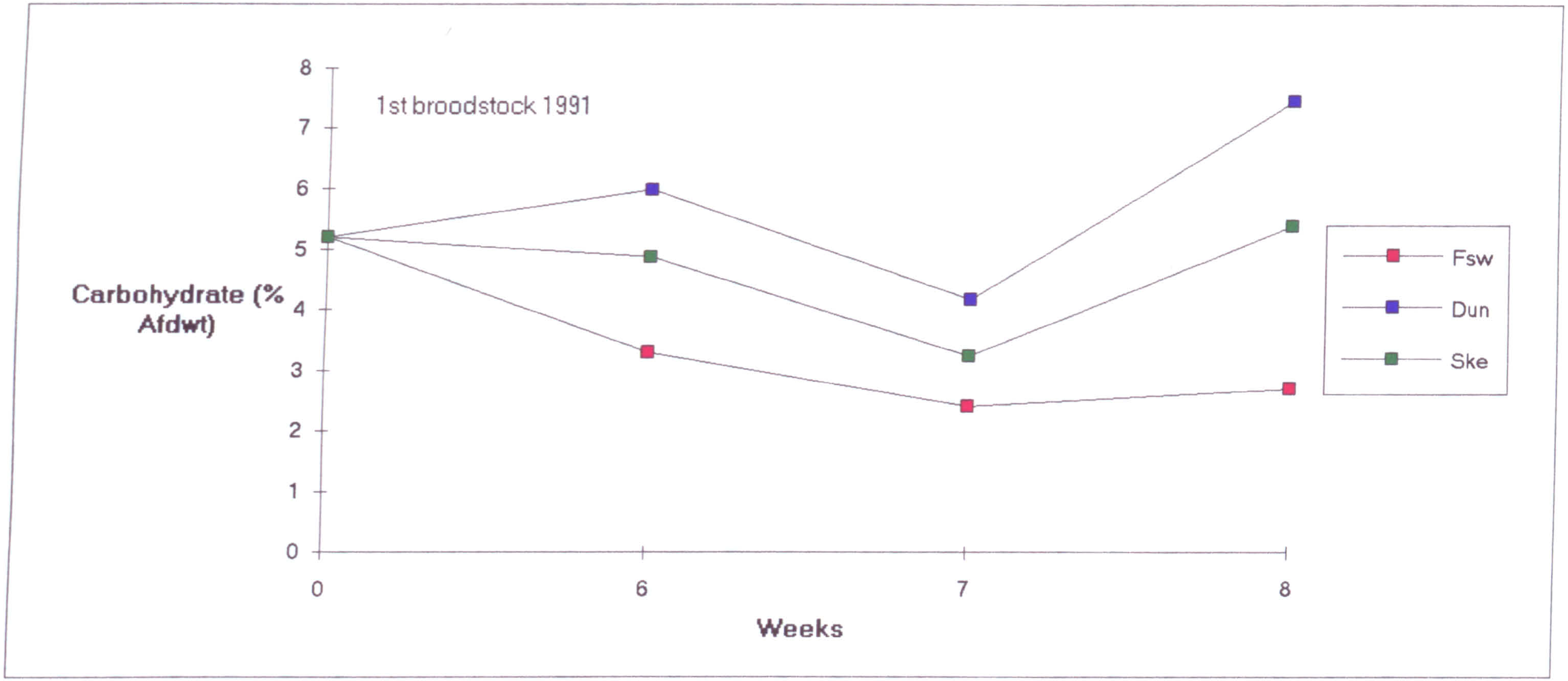


Figure 3.4. Mean carbohydrate content of the initial <u>T. philippinarum</u> 1st broodstock 1991, and conditioned for 6 (spawning I), 7 (spawning II) and 8 (spawning III) weeks with different diets. For clarity, standard errors (given in Table 3.6) are not included in this Figure. For abbreviations see Table 3.3.

6 weeks of conditioning are presented in Table 3.6 and Appendix 2a. For ANOVA the data sets for the replicates within diet treatments were combined because there were no significant differences between replicates. From the results of ANOVA, there was no significant difference in live weight, length and breadth among the different diet groups. There were highly significant differences ($p < 0.001$) between the diet treatments for all the other variables (Table 3.8). The meat weight, condition index and ash-free dry weight of clams fed *D. tertiolecta* and *S. costatum* were not significantly different but they were significantly higher than for clams in the unfed treatment (Table 3.8a). Compared with the initial broodstock, the condition index and the meat dry weight (with the exception of *S. costatum)* had decreased at this stage, especially in the filtered sea water treatment (Table 3.6 and Figure 3.1 and 3.2). The lipid content of clams in the unfed broodstock was significantly lower than the lipid of clams fed with either of the live algae. Clams fed *D. tertiolecta* accumulated more lipid than clams fed *S. costatum* (Figure 3.3 and Table 3.8a). There were significant differences in the carbohydrate such that *D. tertiolecta*>*S. costatum*>unfed broodstock. Carbohydrate in unfed broodstock was significantly lower than in the two other fed groups (Table 3.8a). The carbohydrate dropped between weeks 6 and 7 of conditioning, suggesting that carbohydrate was converted into lipid during gametogenesis (Figure 3.4). However, clams were not yet ready for spawning.

III. <u>Broodstock conditioning for 7 weeks</u>

After 7 weeks (644 D^{O}) *D. tertiolecta* and *S. costatum* broodstock produced an insignificant number of eggs (Table 3.11). Clams from the unfed treatment did not spawn. The gross biochemical composition and condition of the broodstock after 7 weeks of conditioning are presented in Table 3.6 and Appendix 2b. From the results of ANOVA, as in week 6, there was no significant difference in live weight, length and breadth among the different diet groups but there were highly significant differences ($p < 0.001$) between the diet treatments for all the other variables (Table 3.9).

Table 3.8. Analysis of variance for variation in condition indices and biochemical composition of T. philippinarum conditioned for 6 weeks with different nutritional diets in the 1st broodstock 1991. For other details see Table 3.7.

1st broodstock 1991

6 weeks conditioning

Source of variation	d.f.	Sum of squares	Mean square	F Value	P	S
Live weight						
Treatment	2	5.23682E-01	2.61841E-01	0.21	0.8121	n.s.
Residual	87	1.09215E+02	1.25535E+00			
Total	89	1.09739E+02				
Length						
Treatment	2	7.80000E+00	3.90000E+00	1.32	0.2714	n.s.
Residual	87	2.56300E+02	2.94598E+00			
Total	89	2.64100E+02				
Breadth						
Treatment	2	6.88889E-01	3.44444E-01	0.17	0.8461	n.s.
Residual	87	1.78967E+02	2.05709E+00			
Total	89	1.79656E+02				
Meat dry weight						
Treatment	2	1.21520E-01	6.07600E-02	22.96	0.0001	* * *
Residual	87	2.30280E-01	2.64690E-03			
Total	89	3.51800E-01				

Table 3.8. (continued)

Condition index						
Treatment	2	7.77611E+03	3.88805E+03	37.72	0.0001	***
Residual	87	8.96671E+03	1.03066E+02			
Total	89	1.67428E+04				
Ash free dry weight						
Treatment	2	2.97308E+02	1.48654E+02	30.1	0.0001	***
Residual	87	4.29676E+02	4.93880E+00			
Total	89	7.26984E+02				
Lipid						
Treatment	2	9.39053E+02	4.69527E+02	191.59	0.0001	***
Residual	87	2.13206E+02	2.45064E+00			
Total	89	1.15226E+03				
Carbohydrate						
Treatment	2	1.08612E+02	5.43060E+01	48.88	0.0001	***
Residual	87	9.66654E+01	1.11110E+00			
Total	89	2.05277E+02				

Table 3.8a. Results of the t-tests (LSD) for condition indices and biochemical composition of T. philippinarum conditioned for 6 weeks with different nutritional diets in the 1st broodstock 1991 For abbreviations and other details see Table 3.3 and 3.7.

1st broodstock 1991

6 weeks conditioning

	Dun	Ske
Meat dry weight		
Ske	n.s.	
Fsw	*	*
Condition index		
Ske	n.s.	
Fsw	*	*
Ash free dry weight		
Ske	n.s.	
Fsw	*	*
Lipid		
Ske	*	
Fsw	*	*
Carbohydrate		
Ske	*	
Fsw	*	*

Table 3.9. Analysis of variance for variation in condition indices and biochemical composition of T. philippinarum conditioned for 7 weeks with different nutritional diets in the 1st broodstock 1991. For other details see Table 3.7.

1st broodstock 1991

7 weeks conditioning

Source of variation	d.f.	Sum of squares	Mean square	F Value	P	S
Live weight						
Treatment	2	6.10280E+00	3.05140E+00	2.76	0.0689	n.s.
Residual	86	9.50740E+01	1.10551E+00			
Total	88	1.01177E+02				
Length						
Treatment	2	1.51527E+01	7.57633E+00	2.86	0.0626	n.s.
Residual	86	2.27656E+02	2.64717E+00			
Total	88	2.42809E+02				
Breadth						
Treatment	2	1.27473E+01	6.37367E+00	2.73	0.0706	n.s.
Residual	86	2.00444E+02	2.33074E+00			
Total	88	2.13191E+02				
Meat dry weight						
Treatment	2	8.56046E-02	4.28023E-02	13.56	0.0001	***
Residual	86	2.71404E-01	3.15586E-03			
Total	88	3.57009E-01				

Table 3.9. (continued)

Condition index						
Treatment	2	3.43975E+03	1.71987E+03	7.69	0.0008	* * *
Residual	86	1.92394E+04	2.23714E+02			
Total	88	2.26792E+04				
Ash free dry weight						
Treatment	2	1.17958E+03	5.89792E+02	80.30	0.0001	* * *
Residual	86	6.31649E+02	7.34476E+00			
Total	88	1.81123E+03				
Lipid						
Treatment	2	1.62202E+02	8.11009E+01	36.87	0.0001	* * *
Residual	86	1.89151E+02	2.19943E+00			
Total	88	3.51353E+02				
Carbohydrate						
Treatment	2	4.59368E+01	2.29684E+01	33.34	0.0001	* * *
Residual	86	5.92389E+01	6.88825E-01			
Total	88	1.05176E+02				

Table 3.9a. Results of the t-tests (LSD) for condition indices and biochemical composition of T. philippinarum conditioned for 7 weeks with different nutritional diets in the 1st broodstock 1991. For abbreviations see Table 3.3 and 3.7.

1st broodstock 1991

7 weeks conditioning

	Dun	Ske
Meat dry weight		
Ske	n.s.	
Fsw	*	*
Condition index		
Ske	*	
Fsw	*	n.s.
Ash free dry weight		
Ske	n.s.	
Fsw	*	*
Lipid		
Ske	*	
Fsw	*	*
Carbohydrate		
Ske	*	
Fsw	*	*

As in week 6, the meat weight and ash-free dry weight of clams in *D. tertiolecta* and *S. costatum* treatments were not significantly different and both were higher than in the unfed broodstock treatment. The condition index of clams in the unfed broodstock was significantly lower than the condition index of clams fed with *D. tertiolecta* but not significantly different from clams fed with *S.costatum*. There was a significant difference between the fed treatments such that *D. tertiolecta*>*S. costatum*. The lipid and carbohydrate were significantly higher in clams fed *D. tertiolecta* than *S. costatum* Lipid and carbohydrate in unfed broodstock was significantly lower than in the two other fed groups (Table 3.6 and 3.9a).

IV. Broodstock conditioning for 8 weeks

The gross biochemical composition and condition of the broodstock after 8 weeks of conditioning are presented in Table 3.6 and Appendix 2c. After 8 weeks (738 D^{o}) of conditioning, all fed broodstock spawned but clams in the unfed treatment did not spawn (Table 3.11). From the results of ANOVA, as in weeks 6 and 7, there were no significant differences in live weight, length and breadth among the different diet groups. There were highly significant differences ($p < 0.001$) between the diet treatments for all the other variables (Table 3.10). The meat weight of clams in the unfed broodstock was significantly lower than in the two other fed groups and clams fed *S. costatum* had a higher meat weight than those fed *D. tertiolecta*. Clams in the *S. costatum* treatment had significantly higher lipid and significantly lower carbohydrate than clams in the *D. tertiolecta* treatment Lipid and carbohydrate in the unfed broodstock treatment were significantly lower than the other fed treatments (Table 3.6 and 3.10a). Clams at week 8 accumulated a high amount of carbohydrate in both fed treatments. From week 6 to 8, the condition index and lipid increased in *S. costatum*, but decreased slightly in *D. tertiolecta*. Although condition index decreased in the filtered sea water treatment lipid content was maintained.

Table 3.10. Analysis of variance for variation in condition indices and biochemical composition of T. philippinarum conditioned for 8 weeks with different nutritional diets in the 1st broodstock 1991. For other details see Table 3.7.

1st broodstock 1991

8 weeks conditioning

Source of variation	d.f.	Sum of squares	Mean square	F Value	P	S
Live weight						
Treatment	2	9.24806E+00	4.62403E+00	2.38	0.0990	n.s.
Residual	76	1.47374E+02	1.93913E+00			
Total	78	1.56622E+02				
Length						
Treatment	2	3.22972E+00	1.61486E+00	0.32	0.728	n.s.
Residual	76	3.85074E+02	5.06676E+00			
Total	78	3.88304E+02				
Breadth						
Treatment	2	7.63226E-01	3.81613E-01	0.20	0.8165	n.s.
Residual	76	1.42705E+02	1.87770E+00			
Total	78	1.43468E+02				
Meat dry weight						
Treatment	2	2.55429E-01	1.27714E-01	53.29	0.0001	***
Residual	76	1.82146E-01	2.39666E-03			
Total	78	4.37575E-01				

Table 3.10. (continued)

Condition index						
Treatment	2	1.74481E+04	8.72404E+03	62.2	0.0001	* * *
Residual	76	1.06597E+04	1.40259E+02			
Total	78	2.81077E+04				
Ash free dry weight						
Treatment	2	7.02869E+02	3.51435E+02	53.87	0.0001	* * *
Residual	76	4.95763E+02	6.52320E+00			
Total	78	1.19863E+03				
Lipid						
Treatment	2	1.34806E+02	6.74029E+01	52.92	0.0001	* * *
Residual	76	9.68069E+01	1.27378E+00			
Total	78	2.31613E+02				
Carbohydrate						
Treatment	2	2.88898E+02	1.44449E+02	72.99	0.0001	* * *
Residual	76	1.50416E+02	1.97915E+00			
Total	78	4.39314E+02				

Table 3.10a. Results of the t-tests (LSD) for condition indices and biochemical composition of T. philippinarum conditioned for 8 weeks with different nutritional diets in the 1st broodstock 1991. For abbreviations see Table 3.3 and 3.7.

1st broodstock 1991

8 weeks conditioning

	Dun	Ske
Meat dry weight		
Ske	*	
Fsw	*	*
Condition index		
Ske	*	
Fsw	*	*
Ash free dry weight		
Ske	n.s.	
Fsw	*	*
Lipid		
Ske	*	
Fsw	*	*
Carbohydrate		
Ske	*	
Fsw	*	*

3.1.3. Fecundity

Three attempts were made to spawn the clams fed on a 3% food ration (spawning I, II and III) after 6 (556 D^o), 7 (644 D^o) and 8 (738 D^o) weeks of conditioning in the 1st broodstock 1991. Spawning condition was reached within 644 to 738 degree days of conditioning. The results are given in Table 3.11 and Figure 3.5. At the first attempt (spawning I) after 6 weeks, the clams did not spawn, which suggested insufficient conditioning. None of the clams from the filtered sea water spawned, even after 8 weeks of warm water conditioning and there was a loss of condition index in the adults from this treatment. Analysis of the gonad slides showed that the unfed clams were at stage I (inactive) after 2 and 5 weeks of conditioning (Appendix 3).

In all spawnings none of the treatments produced many eggs. The mean number of eggs per female ranged from 58,000 to 437,000 (spawning II) and from 358,000 to 966,000 eggs (spawning III). The fecundity of the females was very variable (Appendix 4).

After 7 weeks, diet had no significant effect on the number of eggs produced [$F_{3,16} = 2.43$, $p > 0.1$] (Table 3.12). The number of eggs after 8 weeks was similar in the *D. tertiolecta* and *S. costatum* treatments. There was no significant difference in the number of eggs between diets [$F_{2,13} = 1.89$, $p > 0.2$] (Table 3.12). However the actual numbers of eggs spawned after 8 weeks was higher than after 7 weeks (Appendix 4). The percentage of clams which spawned in the *D. tertiolecta* treatment ranged from 33 to 71% and in the *S. costatum* treatment from 39 to 60% at week 7 and 8 respectively.

Table 3.11. Fecundity of T. philippinarum after being conditioned for 6 (Spawning I), 7 (Spawning II) and 8 (Spawning III) weeks on 3% diets of D. tertiolecta and S. costatum. Standard error in parentheses. For abbreviations see Table 3.3.

1st Broodstock conditioning - 1991

Spawning	Treatment	Nº of clams induced	Total nº spawned	Female/male spawning	Total nº of eggs produced (in millions)	Eggs mean per female (in millions)
I	Dun	15	0	0/0	0	0
	Dun - R	15	0	0/0	0	0
	Ske	15	0	0/0	0	0
	Ske - R	15	0	0/0	0	0
	Fsw	15	0	0/0	0	0
	Fsw - R	15	0	0/0	0	0
II	Dun	14	5	4/1	0.231	0.058 (± 0.01)
	Dun - R	15	5	4/1	0.645	0.161 (± 0.09)
	Ske	15	9	6/3	2.623	0.437 (± 0.13)
	Ske - R	15	8	6/2	1.058	0.176 (± 0.10)
	Fsw	15	0	0/0	0	0
	Fsw - R	15	0	0/0	0	0
III	Dun	12	6	4/2	1.433	0.358 (± 0.18)
	Dun - R	14	10	9/1	8.697	0.966 (± 0.18)
	Ske	13	6	3/3	2.298	0.766 (± 0.37)
	Ske - R	13	5	0/5	0	0
	Fsw	12	0	0/0	0	0
	Fsw - R	15	0	0/0	0	0

Figure 3.5. Mean fecundity of T. philippinarum in relation to diet (3%) after being conditioned for 6 (spawning I), 7 (spawning II) and 8 (spawning III) weeks (1st broodstock 1991). For clarity, standard errors (given in Table 3.11) are not included in this Figure. For abbreviations see Table 3.3.

Table 3.12 - Analysis of variance for variation in number of eggs per female of T. philippinarum conditioned with different nutritional diets from the 1st broodstock 1991. For abbreviations and other details see Table 3.7.

1st broodstock 1991

7 weeks conditioning

Source of variation	d.f.	Sum of squares	Mean square	F Value	P	S
Treatment	3	4.12548E+11	1.37516E+11	2.43	0.1035	n.s.
Residual	16	9.07295E+11	5.67059E+10			
Total	19	1.31984E+12				

8 weeks conditioning

Source of variation	d.f.	Sum of squares	Mean square	F Value	P	S
Treatment	2	1.02478E+12	5.12392E+11	1.89	0.1901	n.s.
Residual	13	3.52180E+12	2.70908E+11			
Total	15	4.54659E+12				

3.1.4. Egg and larvae quality

3.1.4.1. Survival of D-larvae

The percentage survival of D-larvae after 24 h of incubation in natural sea water in the 1st broodstock 1991 is shown in Table 3.13. The percentage survival of D-larvae after 7 weeks in the *S. costatum* treatment was 19.13% and after 8 weeks in the *D. tertiolecta* and *S. costatum* treatments was 53.13% and 41.10% respectively. It was not possible carry out any statistical analysis because there were not enough data.

The quality of the egg, expressed as percentage survival of D-larvae in artificial sea water, increased from week 7 (644 D^o) to week 8 (738 D^o) in both treatments. After 7 [$F_{3,8} = 0.32$, $p = 0.81$] and 8 weeks [$F_{2,13} = 1.21$, $p = 0.33$] there was no significant difference in the percentage survival of D-larvae from the treatments (Table 3.14). From pooled data, there was no significant difference in percentage survival of D-larvae from week 7 [$F_{1,10} = 1.18$, $p = 0.30$] or week 8 [$F_{1,14} = 0.54$, $p = 0.48$] in all treatments. This was probably due to the fact that a 3% diet is an insufficient food ration.

3.1.4.2. Lipid and fatty acid analysis

The ash-free dry weight and the lipid content of *T. philippinarum* eggs and D-larvae which developed from them, after the II and III spawnings in the 1st broodstock 1991 is shown in Table 3.15. These larvae were used to start semi-commercial scale trials. The mean shell length of D-larvae derived from the different diet treatments is also given (Table 3.15).

In the 1st broodstock 1991, the lipid content of eggs spawned by *T. philippinarum* from the different broodstock diets was between 6.5 and 8.8 ng egg^{-1} (20 to 36% of

Table 3.13. Percentage survival of T. philippinarum D-larvae after 24 h of incubation from the 1st broodstock 1991. Clams were conditioned for 7 (Spawning II) and 8 (Spawning III) weeks with a diet of live D. tertiolecta and S. costatum. For abbreviations see Table 3.3.

Spawning	Treatment	No. embryos incubated (in millions)	No. D-larvae recovered (in millions)	% survival from D-larvae
1st broodstock 1991				
II	Dun	*	*	*
	Dun-R	*	*	*
	Ske	2.338	0.442	18.89
	Ske-R	0.527	0.102	19.37
	Fsw	-	-	-
	Fsw-R	-	-	-
III	Dun	*	*	*
	Dun-R	5.921	3.166	53.48
	Ske	1.343	0.551	41.10
	Ske-R	^	^	^
	Fsw	-	-	-
	Fsw-R	-	-	-

* not enough embryos to incubate
^ only males spawned

Table 3.14. Percentage survival of T. philippinarum D-larvae after 24 h of incubation in artificial sea water in the 1st broodstock 1991. Clams had been conditioned for 6 (Spawning I), 7 (Spawning II) and 8 (Spawning III) weeks with a diet of live D. tertiolecta or S. costatum. For abbreviations see Table 3.3.

Spawning	Treatment	Females, nº 1	2	3	4	5	6	7	8	9	Mean	SE
1st broodstock 1991												
I	_	_	_	_	_	_	_	_	_	_	_	_
II	Dun	27.2	*	1.0	3.4	_	_	_	_	_	10.53	8.36
	Dun-R	*	4.0	*	16.0	_	_	_	_	_	10.00	6.00
	Ske	*	4.7	1.5	68.7	_	_	_	_	_	14.98	16.95
	Ske-R	49.3	46.5	4.0	1.3	_	_	_	_	_	20.22	11.71
	Fsw	_	_	_	_	_	_	_	_	_	_	_
	Fsw-R	_	_	_	_	_	_	_	_	_	_	_
III	Dun	24.8	15.7	33.3	64.3	_	_	_	_	_	34.53	10.56
	Dun-R	22.5	63.0	55.7	64.8	41.8	44.0	46.5	48.0	54.3	48.96	4.26
	Ske	60.4	39.8	7.8	_	_	_	_	_	_	36.00	15.30
	Ske-R	^	^	^	^	^	^	^	^	^	^	^
	Fsw	_	_	_	_	_	_	_	_	_	_	_
	Fsw-R	_	_	_	_	_	_	_	_	_	_	_

_ clams did not spawn
* insufficient quantity of embryos to incubate
^ only males spawned

Afdwt) from the two spawnings. In spawning II lipid ranged from 6.5 to 7.6 ng egg^{-1} (28 to 36% of Afdwt). In spawning III, lipid ranged from 7.7 to 8.8 ng egg^{-1} (20 to 27% of Afdwt). However, the highest and lowest values for lipid as ng egg^{-1} did not always correspond with the highest and lowest values for lipid as % Afdwt. The lipid content of D-larvae was between 3.3 and 5.6 ng $larvae^{-1}$ from the two spawnings. In spawning II and III, lipid as ng per egg was not significantly different between treatments [$F_{1,7} = 0.02$, $p = 0.91$ and $F_{1,14} = 0.58$, $p = 0.46$ respectively], or spawnings [$F_{1,23} = 0.05$, $p = 0.83$]. An assessment of the lipid energy reserves utilized during development from the egg to the D-larvae stage, in the first 24h before feeding, was determined (Table 3.15). In spawning III, the percentage utilization of lipid between and within the diet treatments was similar. The utilization of lipid was around 35% compared with 50% in spawning II.

Changes in the lipid content of *T. philippinarum* larvae (ng per larva) during development (spawning III) are presented in Table 3.17, from 5 to 26 days in *D. tertiolecta* and from 3 to 14 days in *S. costatum*. Changes in the quantity of lipid (ng $larva^{-1}$) during development are shown in Figure 3.6. Larvae from the two broodstock treatments were grown in natural sea water and measurements made on the same days. In larvae from the *D. tertiolecta* treatment, there was an increase in the quantity of lipid, until larvae reached 243 μm (from day 5 to day 12), followed by a decrease. In the *S. costatum* broodstock treatment, there was an increase in the quantity of lipid until larvae reached 220 μm (from day 5 to day 10), followed by a decrease. The decrease in lipid probably indicated that larvae had reached the stage of metamorphosis and utilised lipid as an energy source (Figure 3.6). At day 10, lipid content was not significantly different between treatments [$F_{1,3} = 0.69$, $p = 0.49$]. It was interesting that larvae from both the *S. costatum* and *D. tertiolecta* treatments accumulated the same amount of lipid (148 ng) before a drop in lipid was observed.

Table 3.15. Ash-free dry weight (Afdwt) and lipid content of eggs and D-larvae of T. philippinarum in the 1st broodstock 1991. For abbreviations see Table 3.3.

	Egg			D-larvae				% utilization of lipid during egg development
Spawning/ Treatment	Afdwt (ng egg)	Lipid (ng egg)	Lipid (% Afdwt)	Mean larval size (µm)	Afdwt (ng D-larva)	Lipid (ng D-larva)	Lipid (% Afdwt)	
I (6 weeks)	_	_	_	_	_	_	_	_
II (7 weeks)								
Dun	23.32	7.33	31.43	*	*	*	*	*
Dun-R	27.24	7.63	28.01	*	*	*	*	*
Mean	25.28	7.48	29.72	*	*	*	*	*
Ske	22.17	7.91	35.68	90.0	62.77	*	*	*
Ske-R	22.22	6.47	29.12	91.1	*	3.27	*	49.46
Mean	22.20	7.19	32.40	90.6	62.77	3.27	*	49.46
Fsw	_	_	_	_	_	_	_	_
Fsw-R	_	_	_	_	_	_	_	_
III (8 weeks)								
Dun	34.22	8.80	25.72	*	*	*	*	*
Dun-R	38.85	7.68	19.77	94.7	42.01	5.01	11.93	34.77
Mean	36.54	8.24	22.75	94.7	42.01	5.01	11.93	34.77
Ske	31.47	8.61	27.36	94.3	54.15	5.59	10.32	35.08
Ske-R	^	^	^	^	^	^	^	^
Mean	31.47	8.61	27.36	94.3	54.15	5.59	10.32	35.08
Fsw	_	_	_	_	_	_	_	_
Fsw-R	_	_	_	_	_	_	_	_

_ clams did not spawn

* Insufficient quantity of D-larvae

^ Only males

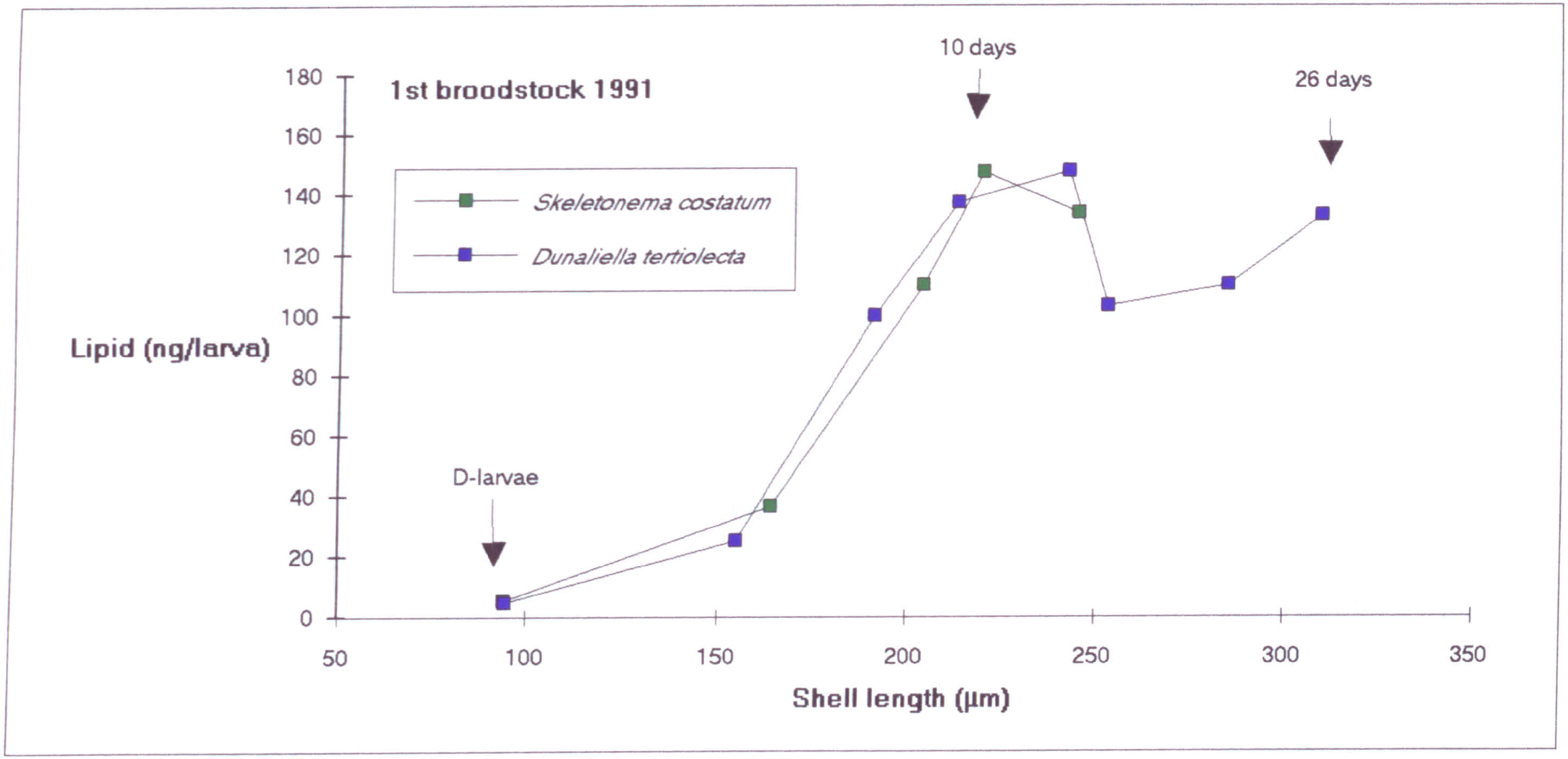


Figure 3.6. Lipid content of T. philippinarum larvae from the 1st broodstock 1991 conditioned on 3% diet of D. tertiolecta or S. costatum. Points on each line represent, from left to right, day 0, 5, 7, 10, 12, 16, 21, 26 (D. tertiolecta) and day 0, 5, 7, 10 and 14 (S. costatum).

Table 3.16. Fatty acid composition of eggs and larvae produced by T. philippinarum broodstock fed on 3% of D. tertiolecta and S. costatum. Values are percentages of total fatty acid.

1st broodstock 1991			Age of larvae								
	Eggs	**D-larvae**	5	7	9	10	12	14	16	21	26
D. tertiolecta											
Spawning II											
18:3w3	5.62	*	-	-	-	-	-	-	-	-	-
20:5w3	1.56	*	-	-	-	-	-	-	-	-	-
22:6w3	0.60	*	-	-	-	-	-	-	-	-	-
Polyenoic	15.35	*	-	-	-	-	-	-	-	-	-
Spawning III											
18:3w3	5.12	4.38	2.71	2.71	1.67	-	2.81	-	1.49	1.23	1.13
20:5w3	0.86	1.19	4.10	2.63	4.57	-	5.23	-	4.11	2.40	2.36
22:6w3	0.00	0.00	11.51	6.71	7.79	-	10.71	-	7.90	4.31	3.41
Polyenoic	12.89	12.19	32.22	20.89	24.16	-	30.47	-	22.43	13.93	12.53
S. costatum											
Spawning II											
18:3w3	0.00	0.00	-	-	-	-	-	-	-	-	-
20:5w3	9.25	5.36	-	-	-	-	-	-	-	-	-
22:6w3	4.08	3.04	-	-	-	-	-	-	-	-	-
Polyenoic	17.82	11.74	-	-	-	-	-	-	-	-	-
Spawning III											
18:3w3	0.00	0.00	0.00	1.72	-	1.03	-	1.20	-	-	-
20:5w3	6.11	3.01	0.00	3.92	-	3.02	-	5.40	-	-	-
22:6w3	1.47	0.00	0.00	6.33	-	4.54	-	4.25	-	-	-
Polyenoic	10.01	6.49	0.00	20.3	-	17.73	-	17.17	-	-	-

* not enough eggs to incubate

The fatty acid composition of eggs and D-larvae (as % of total fatty acids) from spawning II in the 1st broodstock 1991 is shown in Table 3.16 and Appendix 5. In spawning III, the fatty acid composition of eggs, D-larvae and 5 to 26 day-old larvae from *D. tertiolecta* is shown in Table 3.16 and and Appendix 5a. The fatty acid composition of eggs, D-larvae and 5 to 14 day-old larvae from *S. costatum* is shown in Table 3.16 and Appendix 5b. The fatty acid composition of the lipid reserves in eggs was generally dependent on diet quality, ie levels of 18:3w3 and the essential PUFAs 20:5w3 and 22:6w3 were low in the eggs if the broodstock diet was deficient in these fatty acids.

In spawning II and III, eggs from the *D. tertiolecta* treatment had a high level of 18:3w3 and low levels of 20:5w3 and 22:6w3 (reflecting the PUFA composition of this alga). Eggs from *S. costatum* fed broodstock had no 18:3w3 and higher levels of 20:5w3, reflecting the PUFA composition of this diatom. In spawning II and III, from egg to D-larva there was a decrease in the levels of 20:5w3 and 22:6w3 in the *S. costatum* treatment (Table 3.16). In spawning III, there was an increase in 20:5w3 from egg to D-larva in the *D. tertiolecta* treatment.

In spawning III, during development of larvae from the *D. tertiolecta* treatment there was a general increase in 20:5w3 and 22:6w3 till day 12 and a decrease after that day. Also there was a decrease in 18:4w3 (Appendix 5a) after day 12 but this PUFA was not present in the eggs or D-larvae.

Overall the total percentage of the polyenoic fatty acids generally decreased from egg to D-larvae then there was a general increase during larval development, from D-larvae to 12 days (in *D. tertiolecta*) or to 7 days (in *S. costatum*), which corresponded to the increasing level of lipid in larvae (when expressed as ng larvae^{-1}). There was a decrease in these fatty acids during development from day 12 to day 26 (in *D.*

tertiolecta) and from day 10 to day 14 (in *S. costatum*), and this could have been further evidence of lipid utilisation during metamorphosis.

3.1.4.3. Growth of larvae

Results for the initial shell length of the *T. philippinarum* D-larvae, larval shell length on day 5 through to day 26 (in *D. tertiolecta*) and on day 3 through to day 14 (in *S. costatum*) and the percentage survival of larvae from the 1st broodstock 1991 (spawning III) are shown in Table 3.17.

The initial shell lengths of the larvae from broodstock fed on *D. tertiolecta* or *S. costatum* were 94.7 µm and 94.3 µm respectively. By day 14, larvae from *S. costatum* and *D. tertiolecta* fed broodstock were not significantly different between treatments [$F_{1,18} = 0.03$, $p = 0.87$]. The growth coefficient of larvae to day 10 in the 1st broodstock was 0.081 and 0.085 respectively for *D. tertiolecta* and *S. costatum* broodstock treatments.

In the 1st broodstock 1991, the percentage survival of larvae at day 14 was similar in both treatments with respective values of 28% and 29% of the initial number (1.5 million) from the *D. tertiolecta* and *S. costatum* broodstock treatments. In assessing survival, the numbers of larvae removed from each treatment for measuring shell length and percentage survival were taken into consideration. At the end of all experiments many of the larvae had developed into pediveligers which tended to crawl on the bottom of the culture bins by means of a foot and attached to surfaces with byssus threads. However this fact did not affect the survival estimations.

Table 3.17 . Larval performance in natural sea water in relation to broodstock diet quality. Shell length (µm), percentage survival and lipid of T. philippinarum larvae in the 1st broodstock 1991 (spawning III). Initial D-larval size in larvae derived from D. tertiolecta and S. costatum treatments were 94.7 µm and 94.3 µm respectively. Standard error in parentheses.

1st broodstock 1991

Spawning	Days of growth	*D. tertiolecta* % survival	*D. tertiolecta* Larval shell length (µm)	*D. tertiolecta* Lipid (ng larva)	*S. costatum* % survival	*S. costatum* Larval shell length (µm)	*S. costatum* Lipid (ng larva)
Spawning III	3	_	_	_	79.1	123.9 (±0.58)	_
(8 weeks)	5	54.3	154.4 (±0.83)	25.49	67.8	164.1 (±0.63)	36.69
	7	50.0	190.6 (±0.95)	99.79	57.2	203.8 (±0.91)	110.01
	10	41.7	213.4 (±1.81)	137.49	49.0	219.9 (±1.36)	147.51
	12	32.1	242.7 (±1.36)	148.10	32.4	230.0 (±1.98)	_
	14	27.8	243.1 (±1.76)	_	29.1	245.2 (±2.23)	134.00
	16	27.3	253.3 (±1.99)	103.17	_	_	_
	21	23.9	284.4 (±2.01)	109.53	_	_	_
	26	22.5	309.8 (±2.13)	133.24	_	_	_

3.1.5. Sea water quality

3.1.5.1. Suspended particulate material

(i) Size composition and number of particles in suspension

The size distribution of suspended particulate material between 2.5 and 10 μm diameter, in the sand-filtered sea water in the 1st broodstock conditioning trial 1991 is shown in Appendix 6. The mean total number of 2.5 - 10 μm particles during the experiment was 36.55 per μl (Table 3.18). Particles of 2.5 - 5.0 μm diameter accounted for 86.3% of the total, while particles between 5.0 - 10 μm diameter represented 13.7%. The mean total values (± standard error) of three size groups of particles, 0.22 - 1, 2.5 - 5.0 and 5.0 - 10 μm diameter, is given in Table 3.18.

The number of suspended particulates between 2.5 - 5.0 and 5.0 - 10 μm, varied greatly between weeks. The number of 2.5 - 10 μm particles in the week of 11.02.91 was exceptionally high because the filters had to be switched off.

(ii) Dry weight, ash-free dry weight and lipid content of particles

The dry weights, ash-free dry weights and lipid content of particles in suspension are show in Appendix 6. The mean percentage ash-free dry weight of the suspended particulate material was 21.06%. The lipid content of sea water in the hatchery was variable and low. Mean lipid content was usually around 4% of the ash-free dry weight of particles.

Table 3.18. Mean number, weight and % lipid content of particles, and chlorophyll a in sand-filtered sea water flowing through broodstock tanks in the hatchery. Standard error in parentheses.

	Sand-filtered sea water
1st broodstock 1991	
Number of particles (/µl)	
2.5 - 5.0 µm	31.55 (± 11.77)
5.0 - 10 µm	5.00 (± 1.46)
Total dry weight (mg/l)	
0.22 - 1 µm	30.89 (± 7.71)
> 1 µm	13.19 (± 2.14)
Ash-free dry weight (mg/l)*	2.53 (± 0.34)
% ash-free dry weight*	21.06 (± 1.88)
Lipid (% ash-free dry weight)	3.85 (± 0.74)
Chlorophyll a (µg/l)	0.94 (± 0.20)

* 2.5 - 5.0 µm diameter

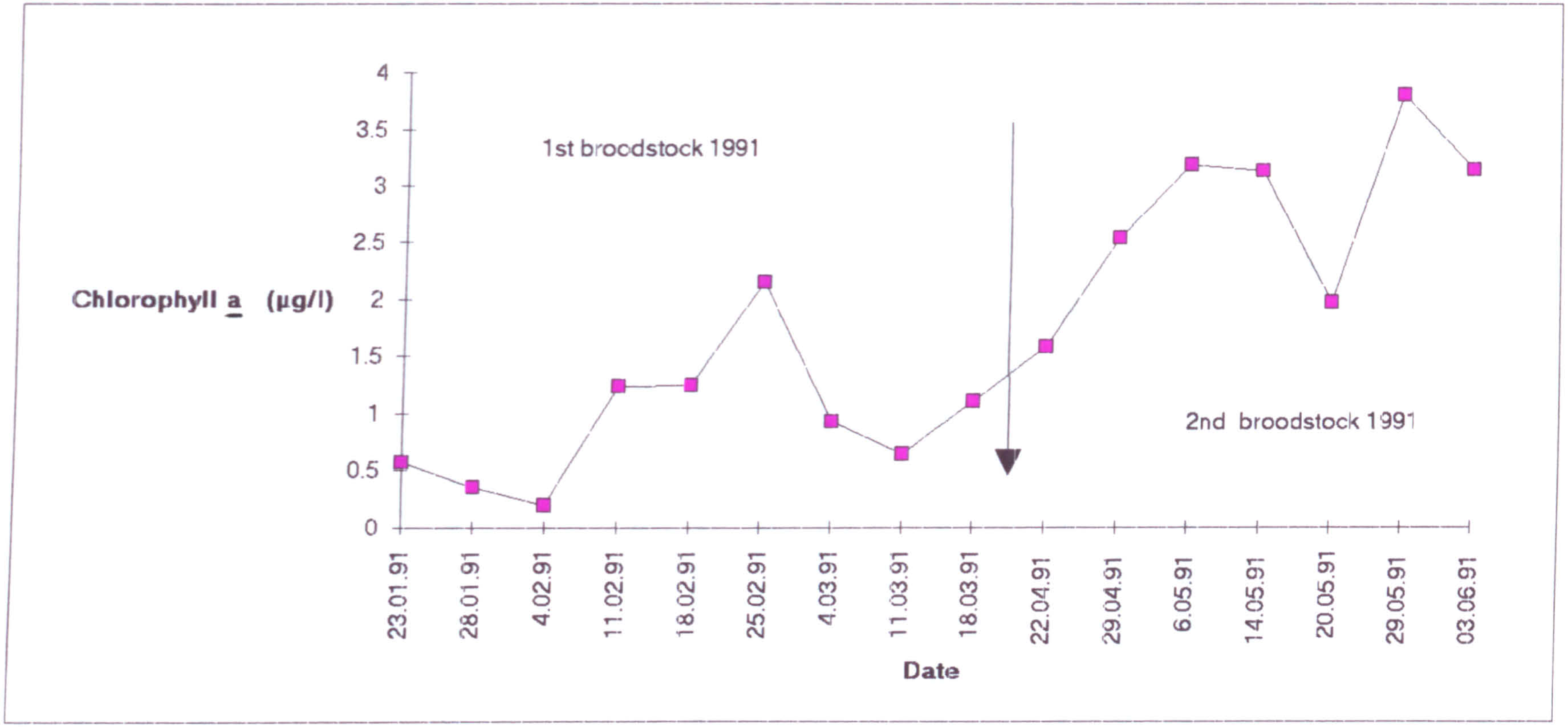


Figure 3.7. Chlorophyll a levels in sand-filtered sea water in 1st and 2nd broodstock 1991.

3.1.5.2. Chlorophyll a determination

Chlorophyll a in the sea water was variable and low (Figure 3.7 and Appendix 6). Values ranged from 0.19 to 2.15 μg per liter.

3.1.6. Summary of results

In the 1st broodstock 1991, with 3% food ration, none of the fed broodstock spawned after 6 weeks (556D^{o}) of conditioning. Spawning condition was reached within 644 to 738 D^{o} of conditioning. None of the clams from the filtered sea water spawned. There was no significant difference in the number of eggs produced in the *D. tertiolecta* and *S. costatum* treatments and in all spawnings few eggs were produced.

There were no significant differences in live weight, length and breadth of the broodstock from the different diet treatments, but there were highly significant differences ($p < 0.001$) for meat dry weight, condition index, ash-free dry weight, lipid and carbohydrate. The unfed broodstock was always significantly lower than the *D. tertiolecta* or *S. costatum* fed treatments.

The quality of the egg, expressed as percentage survival of D-larvae in artificial sea water was not significantly different in both fed treatments.

The lipid content of eggs spawned by *T. philippinarum* from the different broodstock diets was between 6.5 and 8.8 ng egg^{-1}. Lipid as ng per egg was not significantly different between treatments and spawnings. However, the fatty acid composition of the lipid reserves in eggs was generally dependent on diet quality. Eggs from the *D. tertiolecta* treatment had a high level of 18:3w3 and low levels of 20:5w3 and 22:6w3 while eggs from *S. costatum* fed broodstock had no 18:3w3 and higher levels of 20:5w3.

Lipid content of larvae at day 10 was not significantly different between treatments. Larvae from both the *S. costatum* and *D. tertiolecta* treatment accumulated the same amount of lipid (148 ng) before a drop in lipid was observed. This probably indicated that larvae had reached the stage of metamorphosis.

The growth of larvae from *S. costatum* or *D. tertiolecta* fed broodstock was not significantly different between broodstock diet treatments. The percentage survival of larvae at day 14 was similar in both treatments with respective values of 28% and 29%.

3.2. BROODSTOCK FED ON LIVE AND DRY *T. SUECICA* (6% DIET, 2nd BROODSTOCK 1991)

3.2.1 Biochemical analysis of the cultured algae

The gross biochemical composition of the cultured algae fed to the 2nd broodstock 1991 is presented in Table 3.19. *S. costatum* had more lipid than live *T. suecica* and the lipid content of dry *T. suecica* was similar to that of live *T. suecica*. The dry *T. suecica* had more carbohydrate, and correspondingly less protein than live *T. suecica*.

The qualitative fatty acid composition of the live and dry *T. suecica* used in the 2nd broodstock is presented in Table 3.20. For *S. costatum* see section 3.1.1., Table 3.2. The dry *T. suecica* was very low in total PUFAs compared with live *T. suecica*. *T. suecica* as a species, had higher levels of 18C PUFAs, than 20C or 22C PUFAs.

3.2.2. Broodstock conditioning

3.2.2.1. Temperature and salinity

Sea water temperature during conditioning in the 2nd broodstock 1991 ranged from 19.2 to 23.1 ^{o}C (spawning I) and 18.7 to 23.6 ^{o}C (spawning II and III). Mean temperature values for each experiment are shown in Table 3.21. Temperatures between broodstock tanks were not significantly different [$F_{5,372} = 0.24$, $p = 0.95$].

The salinity ranged from 28 to 32 psu during the course of the three experiments (Table 3.22). Salinities between tanks were not significantly different [$F_{5,372} = 0.02$, $p = 0.99$].

Table 3.19. Ash free dry weight (Afdwt,mg per million cells and as a % of the dry weight) and biochemical composition of diets in the 2nd broodstock 1991. Protein values were obtained by subtraction of lipid and carbohydrate percentages. Standard error in parentheses.

Diet species	Afdwt (mg) / million cells	% Afdwt	Lipid (% Afdwt)	Carbohydrate (% Afdwt)	Protein (% Afdwt)
2nd broodstock 1991					
Dry T. suecica	-	91.06 (±0.02)	3.00 (±0.17)	57.46 (±0.05)	39.54
T. suecica	0.18 (±0.03)	69.83 (±4.69)	5.02 (±0.44)	20.90 (±1.34)	74.08
S. costatum	0.03 (±0.01)	62.88 (±4.64)	24.00 (±1.92)	«	«

« contaminated sample

Table 3.20. Fatty acid composition of the live and dry algae used on the 2nd broodstock 1991. Values are percentages of total fatty acids.

	2nd broodstock 1991	
Algae	*T. suecica*	*Dry T. suecica*
Fatty acid		
14:0	12.75	4.48
16:0	27.60	32.37
18:0	4.50	5.30
16:1w9	1.00	
16:1w7	1.00	2.58
18:1w9	14.04	41.62
18:1w7	2.44	4.75
20:1w9	2.94	0.00
18:2w6	7.27	2.48
18:3w3	9.47	2.03
18:4w3	9.47	0.80
20:2w6	2.15	0.80
20:3w3	0.00	0.08
20:4w6	0.60	0.40
20:5w3	3.81	1.40
22:5w3	0.94	0.26
22:6w3	0.00	0.65
Saturated	44.85	42.15
Monoenoic	21.42	48.95
Polyenoic	33.71	8.90

Table 3.21. Mean temperature and Day-Degree in T. philippinarum broodstock tanks (2nd broodstock 1991).
Standard error in parentheses. Tet=T.suecica; Ske=S.costatum; Dry Tet=Dry T. suecica; Fsw=Filtered sea water and R=replicate.

Spawning	Week	Broodstock treatment					
		Tet + Ske	Tet + Ske-R	Dry Tet + Ske	Dry Tet + Ske-R	Fsw	Fsw-R
2nd broodstock 1991							
I	5	21.18 (±0.13) 391	21.01 (±0.13) 385	21.07 (±0.14) 388	20.92 (±0.10) 382	20.91(±0.13) 382	20.94 (±0.11) 383
II	7	21.07 (±0.15) 543	21.00 (±0.14) 539	21.12 (±0.14) 545	20.97 (±0.13) 537	20.94 (±0.14) 536	20.98 (±0.13) 538
III	9	21.07 (±0.13) 710	21.21 (±0.12) 706	21.33 (±0.12) 714	21.19 (±0.11) 705	21.17 (±0.12) 704	21.20 (±0.11) 705

Table 3.22. Mean salinity in T. philippinarum broodstock tanks (2nd broodstock 1991). Standard error in parentheses. For abbreviations see Table 3.21.

Spawning	Week	Broodstock treatment					
		Tet + Ske	Tet + Ske-R	Dry Tet + Ske	Dry Tet + Ske-R	Fsw	Fsw-R
2nd broodstock 1991							
I	5	30.93 (±0.26)	30.99 (±0.26)	30.91 (±0.26)	30.97 (±0.26)	30.97 (±0.26)	30.94 (±0.26)
II	7	31.16 (±0.19)	31.22 (±0.20)	31.18 (±0.20)	31.22 (±0.20)	31.20 (±0.20)	31.20 (±0.20)
III	9	31.33 (±0.15)	31.39 (±0.16)	31.35 (±0.16)	31.39 (±0.16)	31.37 (±0.16)	31.36 (±0.16)

Table 3.23. Percentage filtration and filtration rate (particles between 2.5 - 10 µm) of T. philippinarum broodstock (2nd broodstock 1991). For abbreviations see Table 3.21.

Date	Percentage filtration						Filtration rate					
	Tet+Ske	Tet+Ske-R	Dry Tet+Ske	Dry Tet+Ske-R	Fsw	Fsw-R	Tet+Ske	Tet+Ske-R	Dry Tet+Ske	Dry Tet+Ske-R	Fsw	Fsw-R
2nd broodstock 1991												
(22.04.91)	73.01	64.72	69.97	62.08	77.37	62.08	438.07	388.32	419.85	372.46	464.25	372.46
(29.04.91)	78.88	73.72	63.37	52.39	45.99	51.99	473.25	442.34	380.19	314.33	275.94	311.94
(6.05.91)	73.03	78.72	74.25	75.43	63.45	75.72	438.20	472.32	445.51	452.57	380.72	454.29
(14.05.91)	64.74	70.89	71.21	75.18	55.19	62.92	388.45	425.32	427.29	451.10	331.15	377.50
(20.05.91)	83.80	85.77	86.89	78.12	73.15	68.82	502.77	514.60	521.33	468.75	438.91	412.90
(29.05.91)	49.21	76.93	73.45	82.83	77.26	62.50	295.25	461.56	440.68	496.96	463.54	374.99
(03.06.91)	75.00	79.44	75.36	73.76	65.44	75.82	450.00	476.66	452.16	442.58	392.63	454.94
Mean	71.10	75.74	73.50	71.40	65.41	65.69	426.57	454.45	441.00	428.39	392.45	394.15
SE	4.26	2.55	2.68	3.96	4.44	3.20	25.55	15.29	16.11	23.75	26.61	19.20

3.2.2.2. Filtration rate of clams

The percentage filtration (P) and the filtration rate (F) of the clams from the 2nd broodstock 1991 is shown in Table 3.23. From the results of ANOVA, the percentage filtration and the filtration rate for clams fed *T. suecica+S. costatum* and dry *T. suecica+S. costatum* and the unfed broodstock were not significantly different [$F_{5,36}$ = 1.34, p = 0.27].

3.2.2.3. 2nd broodstock conditioning 1991

I. <u>Initial broodstock</u>

The gross biochemical composition and condition indices of the initial broodstock are shown in Table 3.24 and Appendix 7. There were significant relationships between many of the measured variables eg. the live weight and the shell length, breadth and meat dry weight of the clams. Values for the regression equations which described the relationships between the different parameters of the initial broodstock are given in Table 3.25. The PUFA composition of the initial broodstock meats as percentages of the total fatty acid content, is shown in Table 3.26. The highest value was for 20:5w3.

As a summary of general trends, all the meat dry weight, condition index, lipid and carbohydrate results, from the initial broodstock and after conditioning for 5, 7 and 9 weeks, are summarised in Figures 3.8-3.11.

II. <u>Broodstock conditioning for 5 weeks</u>

The gross biochemical composition and condition of the broodstock after 5 weeks of conditioning are presented in Table 3.24 and Appendix 7a. For ANOVA the data sets for the replicates within diet treatments were combined because there were no

Table 3.24. Mean values for condition indices and biochemical composition of <u>T. philippinarum</u>. 2nd broodstock 1991 conditioned from 17.04.91 to 18.06.91. Values are given for initial and 5, 7 and 9 weeks of conditioning. Protein values were calculated by difference. Standard error in parentheses. For abbreviations see Table 3.21. Afdwt = ash-free dry weight.

Broodstock diet	Live weight g	Length mm	Breadth mm	Meat dry weight, g	Condition index	% Afdwt	Lipid (% Afdwt)	Carbohydrate (% Afdwt)	Protein (% Afdwt)
Initial									
N=30	8.76 (± 0.11)	32.33 (± 0.25)	24.47 (± 0.16)	0.41 (± 0.01)	100.56 (± 2.64)	77.85 (± 0.44)	11.77(± 0.22)	11.25 (± 0.48)	76.99 (± 0.66)
5 weeks									
N=15									
Tet+Ske	11.06 (± 0.36)	34.93 (± 0.34)	26.27 (± 0.36)	0.61 (± 0.02)	119.73 (± 1.79)	85.52 (± 0.48)	10.59 (± 0.49)	19.39 (± 0.64)	70.02 (± 0.97)
Tet+Ske-R	10.88 (± 0.29)	34.67 (± 0.36)	25.93 (± 0.32)	0.60 (± 0.04)	125.29 (± 4.79)	84.33 (± 0.64)	10.92 (± 0.22)	20.31 (± 0.58)	68.77 (± 0.48)
Dry Tet+Ske	10.66 (± 0.32)	34.60 (± 0.54)	26.07 (± 0.32)	0.57 (± 0.02)	113.40 (± 2.54)	84.72 (± 0.46)	10.41 (± 0.29)	22.49 (± 0.68)	67.10 (± 0.79)
Dry Tet+Ske-R	10.76 (± 0.30)	34.87 (± 0.41)	25.93 (± 0.32)	0.57 (± 0.01)	118.09 (± 3.07)	83.89 (± 0.55)	10.02 (± 0.30)	23.62 (± 0.63)	66.36 (± 0.78)
Fsw	10.18 (± 0.33)	34.80 (± 0.50)	25.80 (± 0.33)	0.52 (± 0.02)	108.17 (± 4.59)	85.49 (± 0.60)	9.10 (± 0.23)	14.77 (± 0.70)	76.13 (± 0.72)
Fsw-R	10.13 (± 0.29)	33.33 (± 0.39)	25.20 (± 0.22)	0.51 (± 0.02)	113.70 (± 3.10)	83.76 (± 0.62)	10.98 (± 0.21)	15.81 (± 0.66)	73.21 (± 0.75)

(continued)

Table 3.24. (continued)

7 weeks									
N=15									
Tet+Ske	11.37 (± 0.37)	35.33 (± 0.53)	25.60 (± 0.39)	0.74 (± 0.03)	137.64 (± 3.20)	85.71 (± 0.38)	11.31 (± 0.30)	20.30 (± 0.80)	68.38 (± 0.82)
Tet+Ske-R	12.34 (± 0.34)	36.60 (± 0.31)	27.53 (± 0.35)	0.81 (± 0.03)	137.72 (± 4.26)	86.44 (± 0.52)	11.33 (± 0.21)	18.71 (± 0.73)	69.96 (± 0.68)
Dry Tet+Ske	10.83 (± 0.33)	35.27 (± 0.44)	25.73 (± 0.36)	0.67 (± 0.02)	134.05 (± 4.38)	85.19 (± 0.41)	11.85 (± 0.26)	23.24 (± 0.61)	64.91 (± 0.71)
Dry Tet+Ske-R	10.79 (± 0.42)	35.47 (± 0.49)	26.67 (± 0.27)	0.70 (± 0.04)	127.00 (± 3.66)	85.40 (± 0.34)	11.13 (± 0.37)	21.98 (± 0.53)	66.89 (± 0.46)
Fsw	10.73 (± 0.45)	35.00 (± 0.56)	25.33 (± 0.37)	0.66 (± 0.03)	126.34 (± 2.94)	84.15 (± 0.29)	10.79 (± 0.25)	19.95 (± 0.49)	69.26 (± 0.59)
Fsw-R	10.60 (± 0.41)	34.87 (± 0.58)	25.93 (± 0.36)	0.67 (± 0.03)	128.59 (± 3.22)	84.27 (± 0.56)	10.53 (± 0.29)	19.27 (± 0.62)	70.20 (± 0.74)
9 weeks									
N=15									
Tet+Ske	11.46 (± 0.42)	36.27 (± 0.51)	26.93 (± 0.30)	0.88 (± 0.05)	150.07 (± 5.80)	87.12 (± 0.55)	13.11 (± 0.62)	15.09 (± 0.73)	71.80 (± 0.76)
Tet+Ske-R	11.23 (± 0.46)	36.47 (± 0.50)	27.40 (± 0.49)	0.81 (± 0.04)	144.56 (± 6.49)	87.66 (± 0.36)	11.45 (± 0.33)	16.38 (± 0.82)	72.17 (± 0.76)
Dry Tet+Ske	10.81 (± 0.31)	34.73 (± 0.48)	25.93 (± 0.32)	0.67 (± 0.04)	134.00 (± 9.88)	84.31 (± 0.16)	11.92 (± 0.46)	19.58 (± 0.51)	68.51 (± 0.67)
Dry Tet+Ske-R	11.14 (± 0.36)	35.33 (± 0.42)	26.00 (± 0.35)	0.67 (± 0.04)	135.39 (± 8.52)	84.38 (± 0.26)	10.42 (± 0.25)	19.64 (± 0.66)	69.94 (± 0.76)
Fsw	10.31 (± 0.20)	35.00 (± 0.43)	25.87 (± 0.19)	0.60 (± 0.02)	116.38 (± 4.04)	83.63 (± 0.51)	10.78 (± 0.53)	17.29 (± 0.63)	71.93 (± 0.79)
Fsw-R	10.23 (± 0.28)	34.67 (± 0.41)	25.67 (± 0.27)	0.53 (± 0.02)	109.80 (± 3.22)	82.72 (± 0.55)	10.01 (± 0.26)	16.33 (± 0.68)	73.66 (± 0.71)

Table 3.25. Linear regression equations of the initial 2nd broodstock conditioning 1991. For abbreviations and other details see Table 3.7.

2nd broodstock 1991 (initial)

Parameters estimated (y x)	Intercept (A)	Slope (B)	r	d.f.	S
Live weight x length	2.422	0.196	0.426	29	*
Live weight x breadth	2.263	0.266	0.368	29	*
Live weight x meat dry weight	6.862	4.668	0.488	29	**
Meat dry weight x condition inde.	0.061	0.003	0.768	29	***
Meat dry weight x lipid	0.018	0.033	0.623	29	***
Meat dry weight x carbohydrate	0.196	0.019	0.769	29	***
Condition index x lipid	18.199	7.000	0.592	29	***
Condition index x carbohydrate	52.459	4.277	0.784	29	***
lipid x carbohydrate	8.131	0.323	0.701	29	***

Table 3.26. Fatty acid composition of T. philippinarum meats from the 2nd broodstock 1991. Initial values and broodstock fed on different diets for 7 weeks. Values are percentages of total fatty acids. For abbreviations see Table 3.21.

2nd broodstock 1991	Initial	Spawning II (7 weeks)					
Diet		Tet+Ske	Tet+Ske-R	Dry Tet+Ske	Dry Tet+Ske-R	Fsw	Fsw-R
Fatty acid							
14:0	10.98	10.67	11.58	11.70	11.61	11.68	12.86
16:0	36.18	35.53	36.18	37.29	35.29	34.10	39.81
18:0	15.29	9.78	10.15	10.08	9.33	10.70	9.82
16:1w7/9	9.20	3.70	3.88	3.88	4.25	5.93	3.31
18:1w9	5.73	13.87	13.17	14.15	14.87	14.67	15.40
18:1w7	6.26	3.95	4.12	3.86	4.20	4.73	3.53
20:1w9	3.94	5.87	5.52	5.95	6.20	5.70	5.46
18:2w6	0.64	1.00	0.74	0.74	0.76	0.61	0.69
18:3w3	0.54	1.87	1.74	1.56	1.60	1.35	1.46
18:4w3	2.00	4.06	3.69	3.32	3.84	3.89	3.06
20:2w6	2.34	1.47	1.46	1.43	1.38	1.37	1.02
20:3w3	0.00	0.41	0.30	0.32	0.31	0.00	0.23
20:4w6	0.87	0.59	0.55	0.43	0.45	0.55	0.24
20:5w3	3.61	2.65	2.67	1.85	1.94	2.08	0.66
22:5w3	0.55	0.40	0.46	0.42	0.66	0.00	0.63
22:6w3	1.86	4.18	3.80	3.04	3.30	2.64	1.82
Saturated	62.45	55.98	57.91	59.07	56.23	56.48	62.49
Monoenoic	25.13	27.39	26.69	27.84	29.52	31.03	27.70
Polyenoic	12.41	16.63	15.41	13.11	14.24	12.49	9.81

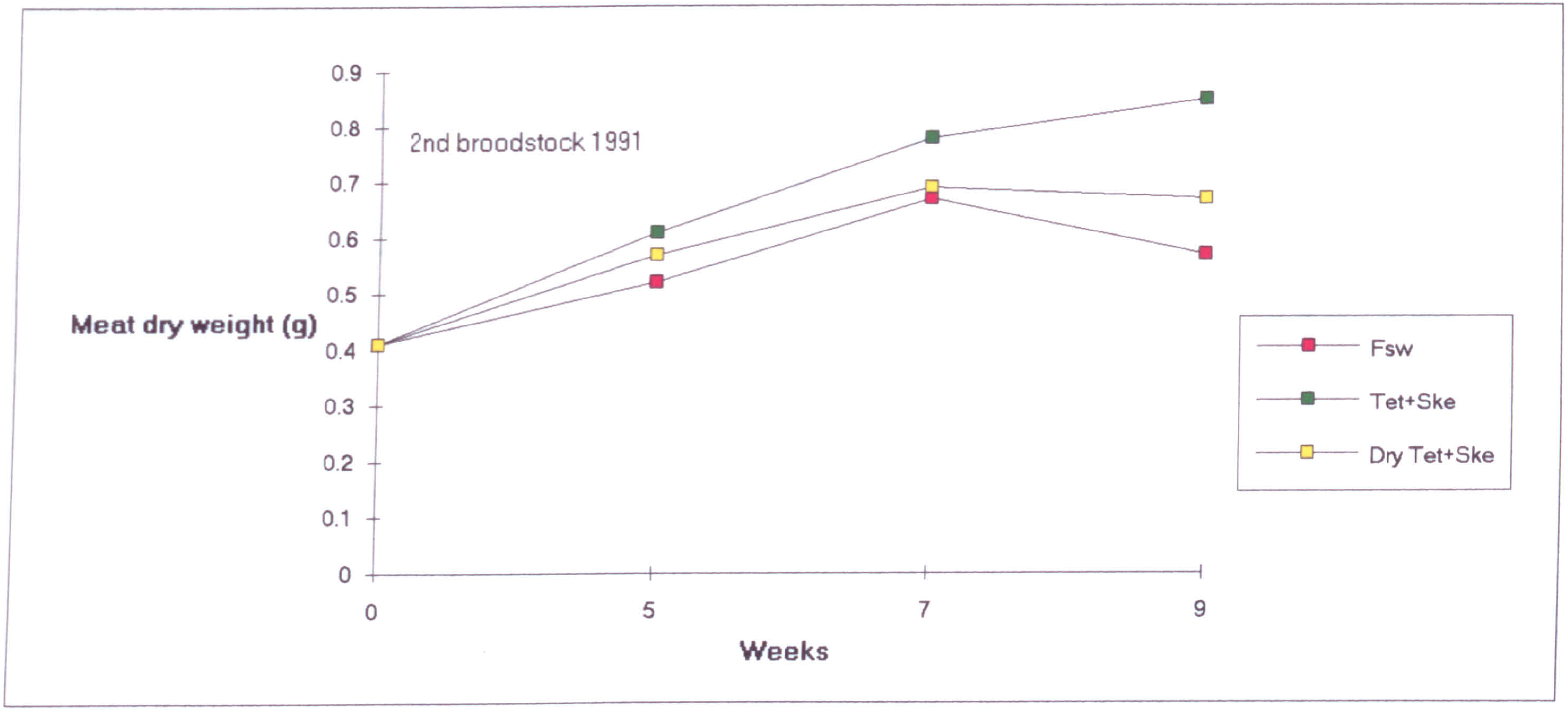


Figure 3.8. Mean meat dry weight of the initial _T. philippinarum_ 2nd broodstock 1991, and conditioned for 5 (spawning I), 7 (spawning II) and 9 (spawning III) weeks with different diets. For clarity, standard errors (given in Table 3.24) are not included in this Figure. For abbreviations see Table 3.21.

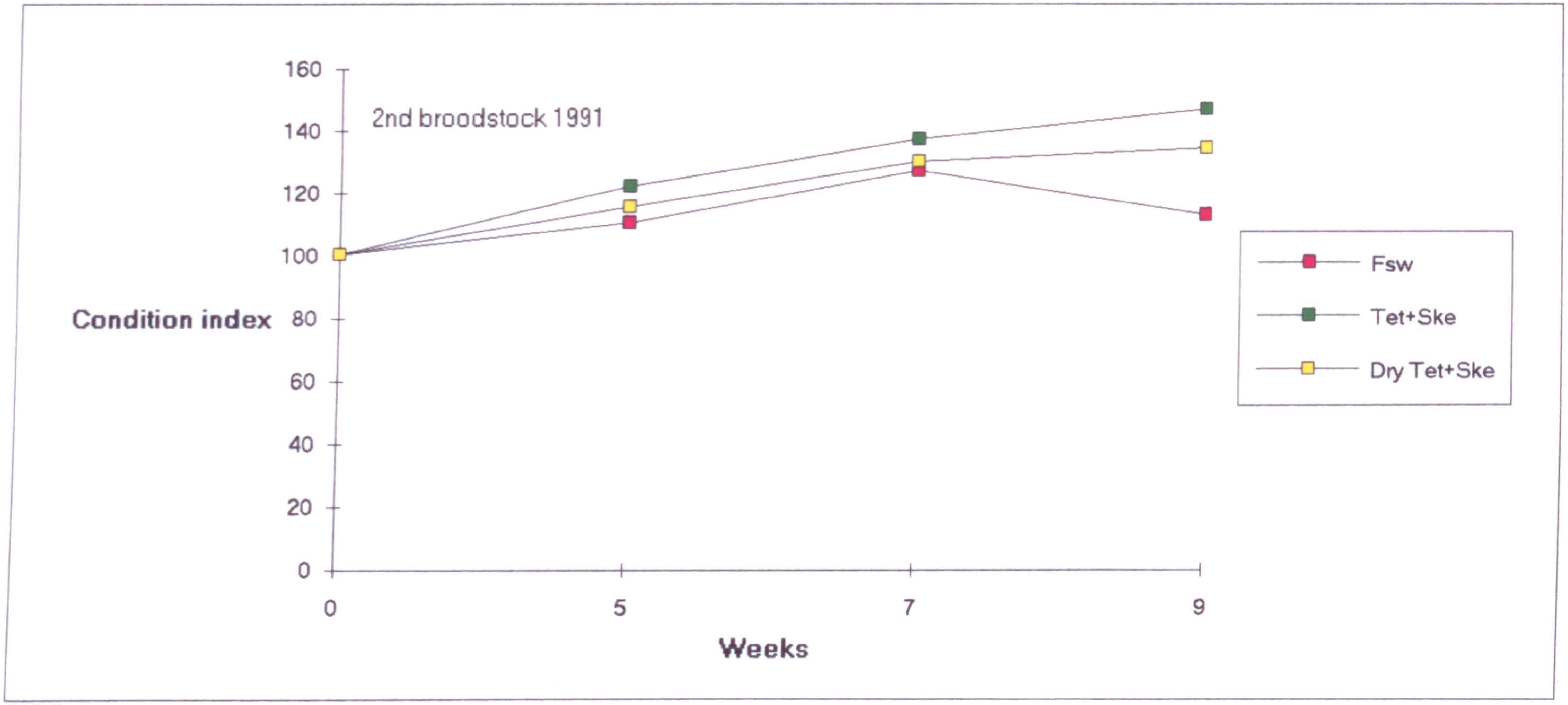


Figure 3.9. Mean condition index of the initial T. philippinarum 2nd broodstock 1991, and conditioned for 5 (spawning I), 7 (spawning II) and 9 (spawning III) weeks with different diets. For clarity, standard errors (given in Table 3.24) are not included in this Figure. For abbreviations see Table 3.21.

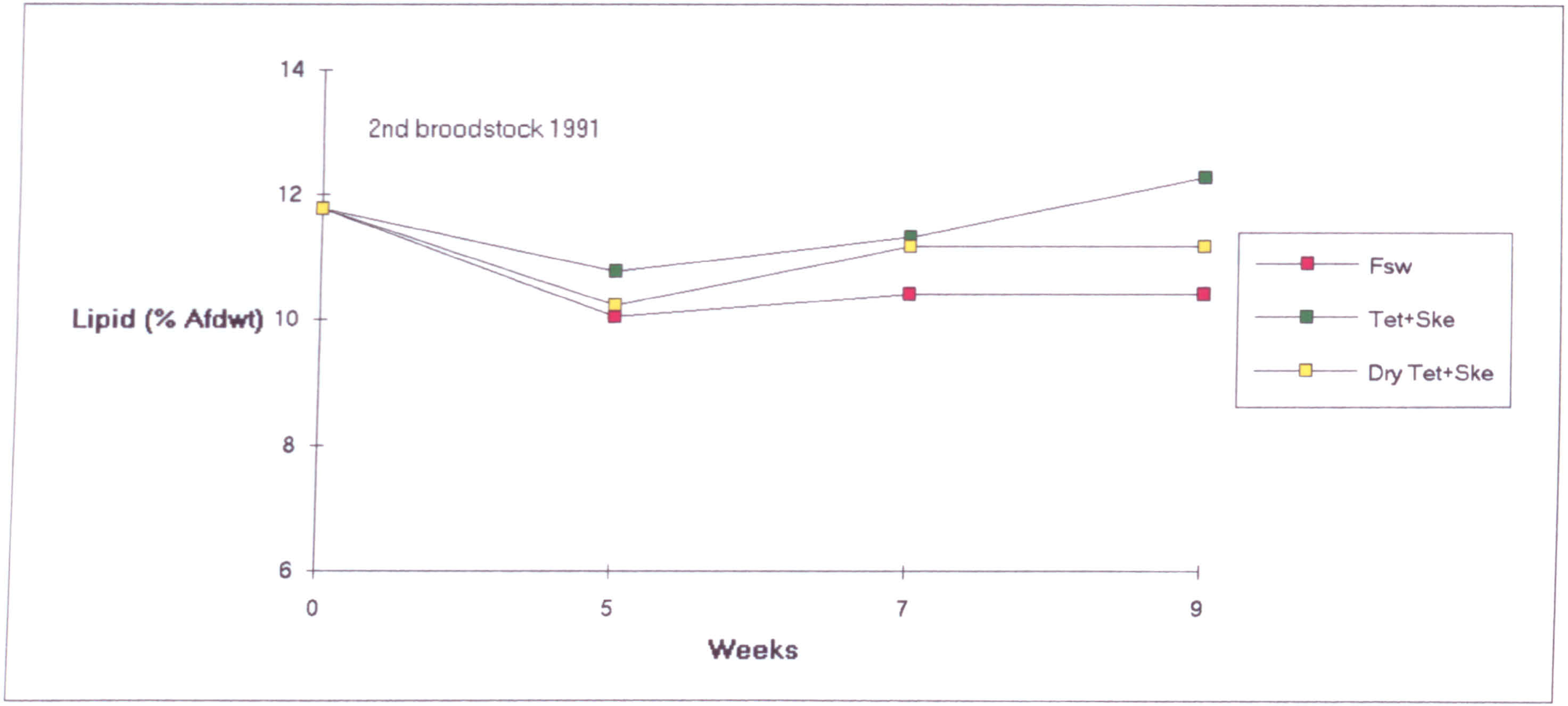


Figure 3.10. Mean lipid content of the initial T. philippinarum 2nd broodstock 1991, and conditioned for 5 (spawning I), 7 (spawning II) and 9 (spawning III) weeks with different diets. For clarity, standard errors (given in Table 3.24) are not included in this Figure. For abbreviations see Table 3.21.

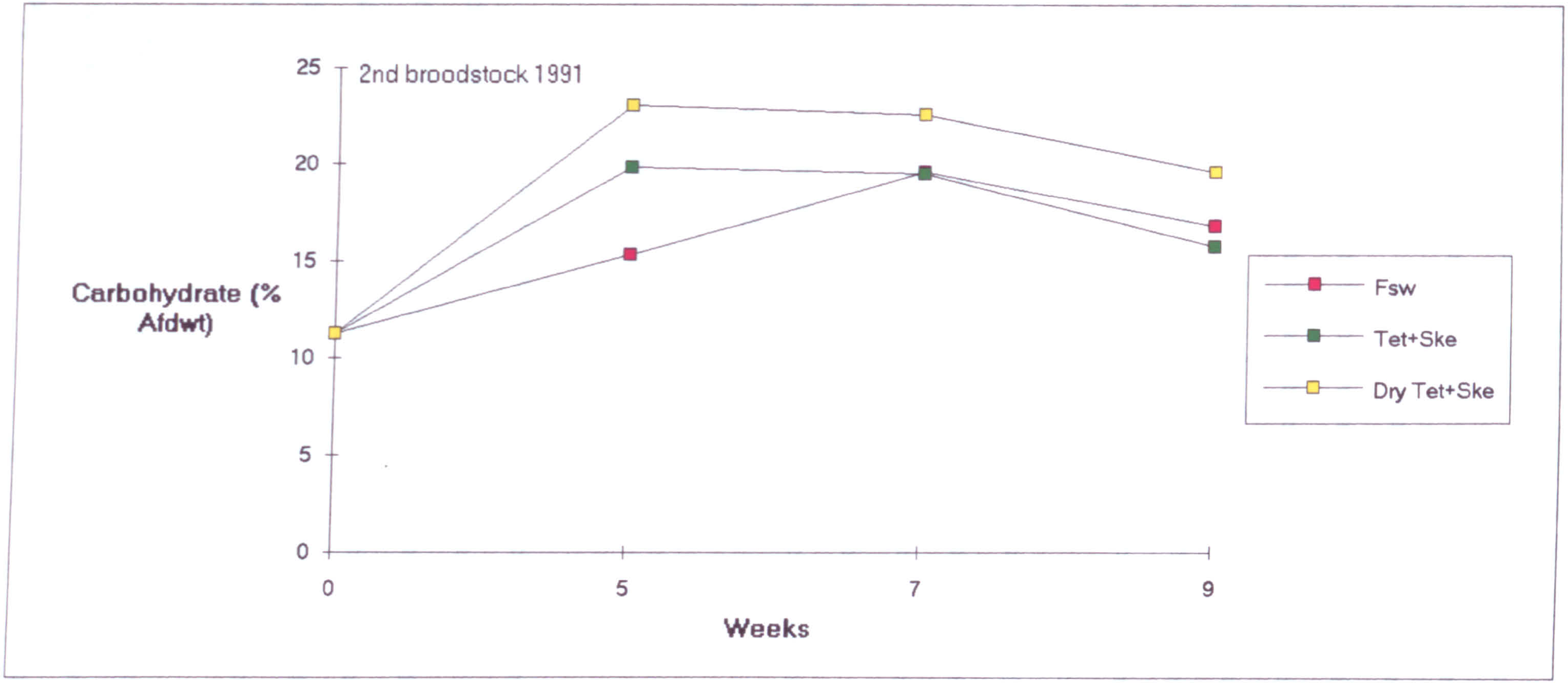


Figure 3.11. Mean carbohydrate content of the initial T. philippinarum 2nd broodstock 1991, and conditioned for 5 (spawning I), 7 (spawning II) and 9 (spawning III) weeks with different diets. For clarity, standard errors (given in Table 3.24) are not included in this Figure. For abbreviations see Table 3.21.

significant differences between replicates. From the results of ANOVA, there was no significant difference in length, breadth, ash-free dry weight and lipid among the different diet groups (Table 3.27). There were significant differences ($p < 0.05$) in total live weight between two of the diet treatments (T. *suecica+S. costatum* was higher than filtered sea water). There were highly significant differences ($p < 0.001$) between the diet treatments for meat dry weight and carbohydrate . The meat weight of clams in the live and dry *T. suecica+S. costatum* treatments were not significantly different and both were higher than the meat weight of broodstock in the unfed treatment (Table 3.27a). The condition index of clams in the unfed broodstock was significantly lower than the condition index of clams fed with live *T.suecica+S.costatum* but not significantly different from clams fed with dry *T.suecica+S.costatum*. There was no difference between the fed treatments. Also, lipid was not significantly different between treatments. The carbohydrate was significantly higher in clams fed dry *T.suecica+S.costatum*, reflecting the high percentage of carbohydrate in this dry alga. Carbohydrate in unfed broodstock was significantly lower than in the two other fed groups.

III. <u>Broodstock conditioning for 7 weeks</u>

The biochemical composition and condition of the broodstock after 7 weeks of conditioning are presented in Table 3.24 and Appendix 7b. From the results of ANOVA (Table 3.28), there was no significant difference in length and breadth among the different diet groups, but there were significant differences due to diet treatment in all the other parameters. The most significant differences ($p<0.001$) between the diet treatments were found for ash-free dry weight and carbohydrate content of clams.

The t-test showed where there were significant differences among the groups in the live weight, meat dry weight, condition index, ash-free dry weight, lipid and carbohydrate. (Table 3.28a). The live weight and meat dry weight of the live *T.*

Table 3.27. Analysis of variance for variation in condition indices and biochemical composition of T. philippinarum conditioned for five weeks with different nutritional diets in the 2nd broodstock 1991. For abbreviations and other details see Table 3.7.

2nd broodstock 1991

5 weeks conditioning

Source of variation	d.f.	Sum of squares	Mean square	F Value	P	S
Live weight						
Treatment	2	1.03640E+01	5.18198E+00	3.57	0.0323	*
Residual	87	1.26166E+02	1.45018E+00			
Total	89	1.36530E+02				
Length						
Treatment	2	9.86667E+00	4.93333E+00	1.71	0.1863	n.s.
Residual	87	2.50533E+02	2.87969E+00			
Total	89	2.60400E+02				
Breadth						
Treatment	2	6.20000E+00	3.10000E+00	2.14	0.1242	n.s.
Residual	87	1.26200E+02	1.45057E+00			
Total	89	1.32400E+02				
Meat dry weight						
Treatment	2	1.23636E-01	6.18178E-02	8.65	0.0004	***
Residual	87	6.21493E-01	7.14360E-03			
Total	89	7.45129E-01				

Table 3.27. (continued)

Condition index						
Treatment	2	2.02771E+03	1.01386E+03	5.55	0.0054	* *
Residual	87	1.58975E+04	1.82729E+02			
Total	89	1.79252E+04				
Ash free dry weight						
Treatment	2	5.76148E+00	2.88074E+00	0.57	0.5665	n.s.
Residual	87	4.38215E+02	5.03696E+00			
Total	89	4.43977E+02				
Lipid						
Treatment	2	8.30350E+00	4.15175E+00	2.49	0.0889	n.s.
Residual	87	1.45148E+02	1.66837E+00			
Total	89	1.53451E+02				
Carbohydrate						
Treatment	2	9.13344E+02	4.56672E+02	71.77	0.0001	* * *
Residual	87	5.53588E+02	6.36308E+00			
Total	89	1.46693E+03				

Table 3.27a. Results of the t-test (LSD) for condition indices and biochemical composition of T. philippinarum conditioned for 5 weeks with different nutritional diets in the 2nd broodstock 1991. For abbreviations and other details see Table 3.7 and 3.21

2nd broodstock 1991

5 weeks conditioning

	Tet+Ske	Dry Tet+ske
Live weight		
Dry Tet+Ske	n.s.	
Fsw	*	n.s.
Meat dry weight		
Dry Tet+Ske	n.s.	
Fsw	*	*
Condition index		
Dry Tet+Ske	n.s.	
Fsw	*	n.s.
Carbohydrate		
Dry Tet+Ske	*	
Fsw	*	*

Table 3.28. Analysis of variance for variation in condition indices and biochemical composition of T. philippinarum conditioned for seven weeks with different nutritional diets in the 2nd broodstock 1991. For abbreviations and other details see Table 3.7.

2nd broodstock 1991

7 weeks conditioning

Source of variation	d.f.	Sum of squares	Mean square	F Value	P	S
Live weight						
Treatment	2	2.53820E+01	1.26910E+01	5.56	0.0053	**
Residual	87	1.98559E+02	2.28229E+00			
Total	89	2.23941E+02				
Length						
Treatment	2	1.61556E+01	8.07778E+00	2.21	0.1157	n.s.
Residual	87	3.17800E+02	3.65287E+00			
Total	89	3.33956E+02				
Breadth						
Treatment	2	1.32667E+01	6.63333E+00	2.99	0.0556	n.s.
Residual	87	1.93133E+02	2.21992E+00			
Total	89	2.06400E+02				
Meat dry weight						
Treatment	2	1.96740E-01	9.83700E-02	6.91	0.0016	**
Residual	87	1.23815E+00	1.42316E-02			
Total	89	1.43489E+00				

Table 3.28. (continued)

Condition index						
Treatment	2	1.64874E+03	8.24370E+02	4.17	0.0187	*
Residual	87	1.72006E+04	1.97709E+02			
Total	89	1.88494E+04				
Ash free dry weight						
Treatment	2	5.26249E+01	2.63125E+01	9.68	0.0002	* * *
Residual	87	2.36527E+02	2.71870E+00			
Total	89	2.89152E+02				
Lipid						
Treatment	2	1.16467E+01	5.82337E+00	4.70	0.0115	*
Residual	87	1.07714E+02	1.23809E+00			
Total	89	1.19360E+02				
Carbohydrate						
Treatment	2	1.86274E+02	9.31371E+01	14.75	0.0001	* * *
Residual	87	5.49248E+02	6.31319E+00			
Total	89	7.35522E+02				

Table 3.28a. Results of the t-test (LSD) for condition indices and biochemical composition of T. philippinarum conditioned for 7 weeks with different nutritional diets in the 2nd broodstock 1991. For abbreviations and other details see Table 3.7 and 3.21.

2nd broodstock 1991

7 weeks conditioning

	Tet+Ske	Dry Tet+Ske
Live weight		
Dry Tet+Ske	*	
Fsw	*	n.s.
Meat dry weight		
Dry Tet+Ske	*	
Fsw	*	n.s.
Condition index		
Dry Tet+Ske	n.s.	
Fsw	*	n.s.
Ash-free dry weight		
Dry Tet+Ske	n.s.	
Fsw	*	*
Lipid		
Dry Tet+Ske	n.s,	
Fsw	*	*
Carbohydrate		
Dry Tet+Ske	*	
Fsw	n.s.	*

Table 3.28b. Analysis of variance for variation by sex (male and female) and non-spawners of T. philippinarum conditioned for seven weeks with different nutritional diets in the 2nd broodstock 1991. For abbreviations and other details see Table 3.7.

2nd broodstock 1991

7 weeks conditioning

Source of variation	d.f.	Sum of squares	Mean square	F Value	P	S
Live weight						
Treatment	2	1.94545E+01	9.72726E+00	4.14	0.0192	*
Residual	87	2.04486E+02	2.35042E+00			
Total	89	2.23941E+02				
Meat dry weight						
Treatment	2	9.66578E-02	4.83289E-02	3.14	0.0481	*
Residual	87	1.33823E+00	1.53820E-02			
Total	89	1.43489E+00				
Lipid						
Treatment	2	2.45647E+01	12.28235433	11.27	0.0001	***
Residual	87	9.47955E+01	1.08960392			
Total	89	1.19360E+02				

suecica+S.costatum fed broodstock had significantly higher values compared with dry *T. suecica+S.costatum* and the unfed. The condition index of clams in the dry *T. suecica+S.costatum* and the unfed treatments were not significantly different but clams fed live *T. suecica+S.costatum* had a significantly higher condition index than unfed clams.

All broodstock that were fed a supplement of live or dry algae had significantly higher lipid levels than the broodstock that were unfed. As had occurred at week 5, the clams fed dry *T. suecica+S. costatum* had significantly higher carbohydrate levels than live *T. suecica+S. costatum* fed broodstock, reflecting the high percentage of carbohydrate in this dry alga. The clams fed on dry *T. suecica+S. costatum* also had a significantly higher carbohydrate level than clams in the unfed treatment. The carbohydrate level in live *T. suecica+S. costatum* fed broodstock and the unfed broodstock were not significantly different. This may have been related to the quantity and quality of the suspended particulate material in the sand filtered sea water passing through the conditioning tanks. The percentage ash-free dry weight content of the suspended particulate material during the conditioning period was 34.64% and this was higher than at any other period during 1991 and 1992.

The ANOVA for variation by sex (males and females) and non-spawners is presented in Table 3.28b. Live weight of male clams (11.49±0.28 g) was significantly higher than live weight of the females (10.50±0.28 g). Non-spawners (11.42±0.27 g) were not significantly different from males. Also the meat dry weight of male clams (0.73±0.02 g) was significantly higher than that of the females (0.66±0.02 g). Non-spawners (0.73±0.02 g) were not significantly different from males or females. It is possible that females expended more reserves and energy to prepare for spawning than the males. Alternatively it could have been related to the fact that the males were not allowed to spawn completely compared to the females. They were only allowed to release a quantity of sperm that was sufficient to artificially fertilise the eggs. Female clams had significantly higher levels of lipid (11.71±0.17 %) than males (11.16±0.12 %). Non-spawners (10.36±0.31 %) were significantly lower than males or females. Although females may have expended more energy for spawning, lipid is an important energy source and the higher level in females could have been related to the fact that lipid is less dense (ie by weight) than other components of the meat.

The percentage fatty acid content of broodstock meats after spawning is shown in Table 3.26. There was a decrease in 20:5w3 and a increase in 22:6w3 and 18:4w3 from the initial broodstock in all treatments. Meats of live *T. suecica+S. costatum* fed broodstock had higher levels of 20:5w3 and 22:6w3 than meats from clams in the dry *T. suecica+S. costatum* treatment. Also the unfed broodstock meats contained slightly lower levels of these PUFAs. There was a difference between the replicates in the unfed broodstock in this treatment. It was not possible to do any ANOVA of these results because there was only one value per treatment. It was also interesting that overall, the percentage PUFAs in fed treatments increased from the initial broodstock and similar values were found for live and dry *T. suecica+S. costatum* fed broodstock.

IV. <u>Broodstock conditioning for 9 weeks</u>

The biochemical composition and condition of the broodstock conditioned for 9 weeks with different diets are presented in Table 3.24 and Appendix 7c. There were significant differences in all the variables measured (Table 3.29). For ANOVA the data sets for the replicates within diet treatments were combined because there were no significant differences between replicates.

The live weight and the condition index were significantly lower in the unfed broodstock compared with the live and dry *T. suecica+S. costatum* treatments. This showed a loss of overall condition in the adults. The shell length and breadth were significantly higher with the live *T. suecica+S. costatum* than the other treatments

Table 3.29. Analysis of variance for variation in condition indices and biochemical composition of T. philippinarum conditioned for nine weeks with different nutritiona diets in the 2nd broodstock 1991. For abbreviations and other details see Table 3.7.

2nd broodstock 1991

9 weeks conditioning

Source of variation	d.f.	Sum of squares	Mean square	F Value	P	S
Live weight						
Treatment	2	1.79549E+01	8.97744E+00	5.13	0.0079	* *
Residual	87	1.52331E+02	1.75093E+00			
Total	89	1.70286E+02				
Length						
Treatment	2	4.16889E+01	2.08444E+01	6.71	0.0019	* *
Residual	87	2.70100E+02	3.10460E+00			
Total	89	3.11789E+02				
Breadth						
Treatment	2	3.44000E+01	1.72000E+01	10.65	0.0001	* * *
Residual	87	1.40500E+02	1.61494E+00			
Total	89	1.74900E+02				
Meat dry weight						
Treatment	2	1.17457E+00	5.87284E-01	26.54	0.0001	* * *
Residual	87	1.92483E+00	2.21244E-02			
Total	89	3.09940E+00				

Table 3.29. (continued)

Condition index						
Treatment	2	1.79783E+04	8.98915E+03	13.53	0.0001	***
Residual	87	5.78173E+04	6.64566E+02			
Total	89	7.57956E+04				
Ash free dry weight						
Treatment	2	2.83892E+02	1.41946E+02	52.42	0.0001	***
Residual	87	2.35562E+02	2.70761E+00			
Total	89	5.19454E+02				
Lipid						
Treatment	2	5.39552E+01	2.69776E+01	8.44	0.0004	***
Residual	87	2.78083E+02	3.19636E+00			
Total	89	3.32038E+02				
Carbohydrate						
Treatment	2	2.39844E+02	1.19922E+02	17.42	0.0001	***
Residual	87	5.99031E+02	6.88542E+00			
Total	89	8.38875E+02				

Table 3.29a. Results of the t-tests (LSD) for condition indices and biochemical composition of T. philippinarum conditioned for 9 weeks with different nutritional diets in the 2nd broodstock 1991 For abbreviations see Table 3.7 and 3.21.

2nd broodstock 1991

9 weeks conditioning

	Tet+Ske	Dry Tet+Ske
Live weight		
Dry Tet+Ske	n.s.	
Fsw	*	*
Length		
Dry Tet+Ske	*	
Fsw	*	n.s.
Breadth		
Dry Tet+Ske	*	
Fsw	*	n.s.
Meat dry weight		
Dry Tet+Ske	*	
Fsw	*	*
Condition index		
Dry Tet+Ske	n.s.	
Fsw	*	*
Ash-free dry weight		
Dry Tet+Ske	*	
Fsw	*	*
Lipid		
Dry Tet+Ske	*	
Fsw	*	n.s.
Carbohydrate		
Dry Tet+Ske	*	
Fsw	n.s.	*

Table 3.29b. Analysis of variance for variation by sex (male and female) and non-spawners of T. philippinarum conditioned for nine weeks with different nutritional diets in the 2nd broodstock 1991. For abbreviations and other details see Table 3.7.

2nd broodstock 1991

9 weeks conditioning

Source of variation	d.f.	Sum of squares	Mean square	F Value	P	S
Condition index						
Treatment	2	7.03944E+03	3.51972E+03	4.45	0.0144	*
Residual	87	6.87561E+04	7.90300E+02			
Total	89	7.57956E+04				
Lipid						
Treatment	2	2.25381E+01	11.26904534	3.17	0.047	*
Residual	87	3.09500E+02	3.55747436			
Total	89	3.32038E+02				

which indicated growth in this broodstock treatment. The meat dry weight and ash-free dry weight in the starved broodstock were significantly lower than the other diet groups. Clams fed live *T. suecica+S. costatum* had significantly higher meat dry weight and ash-free dry weight than clams fed dry *T. suecica+S. costatum* (Table 3.29a). The broodstock fed live *T. suecica+S.costatum* had significantly higher lipid levels than dry *T. suecica+S.costatum* reflecting in part the composition of the live algae. The carbohydrate content of clams fed dry *T. suecica+S. costatum* was significantly higher than the live diet which reflected the composition of this dry alga.

There were significant differences between female and male clams and non-spawners for condition index [$F_{2,87} = 4.45$, $p < 0.05$] and lipid content [$F_{2,87} = 3.17$, $p < 0.05$]. The condition index in the males (141.69±5.56) was significantly higher than in the females (120.52±4.53). Non-spawners (130.34±4.83) were not significantly different from males and females. As in week 7, it is possible that females expended more reserves and energy to prepare for spawning than males (Table 3.29b). As an alternative it could also be related to the fact that the males were not allowed to spawn completely compared to females. Males were only allowed to release sufficient sperm for artificially fertilising eggs. Female clams had significantly higher levels of lipid (12.00±0.38%) than males (10.81±0.15%), possibly for the same reasons given above. Non-spawners (11.14±0.50%) were not significantly different from males and females.

3.2.3. Fecundity

Attempts to spawn the clams were made 3 times (spawning I, II and III) after 5 (385 D^o), 7 (540 D^o) and 9 (707 D^o) weeks of conditioning in the 2nd broodstock 1991. Spawning condition was reached within 540 to 707 D^o of conditioning. The results are given in Table 3.30 and Figure 3.12. Spawning was started after 5 weeks (385 D^o) of conditioning but only one male and two females produced some sperm and

Table 3.30. Fecundity of T. philippinarum after being conditioned for 5 (Spawning I), 7 (Spawning II) and 9 (Spawning III) weeks on mixed diets of 70% T. suecica and 30% S. costatum and 70% dry T. suecica and 30% S. costatum. For abbreviations and other details see Table 3. 21.

2nd Broodstock conditioning - 1991

Spawning	Treatment	Nº of clams induced	Total nº spawned	Female/male spawning	Total nº of eggs produced (in millions)	Eggs mean per female (in millions)
I	Tet + Ske	15	1	1/0	0.890	0.890
	Tet + Ske - R	15	0	0/0	0	0
	Dry Tet + Ske	15	2	1/1	0.056	0.056
	Dry Tet + Ske - R	15	0	0/0	0	0
	Fsw	15	1	0/1	0	0
	Fsw - R	15	0	0/0	0	0
II	Tet + Ske	15	9	2/7	4.858	2.429 (± 0.54)
	Tet + Ske - R	15	13	4/9	10.273	2.568 (± 1.19)
	Dry Tet + Ske	15	13	10/3	21.090	2.109 (± 0.56)
	Dry Tet + Ske - R	15	12	8/4	14.950	1.869 (± 0.35)
	Fsw	15	10	2/8	2.901	1.451 (± 1.22)
	Fsw - R	15	10	7/3	5.679	0.811 (± 0.15)
III	Tet + Ske	15	14	6/8	15.272	2.545 (± 0.79)
	Tet + Ske - R	15	12	4/8	22.970	5.743 (± 1.68)
	Dry Tet + Ske	15	12	8/4	43.625	5.453 (± 0.59)
	Dry Tet + Ske - R	15	10	5/5	24.330	4.866 (± 1.23)
	Fsw	15	4	2/2	2.746	1.373 (± 0.40)
	Fsw - R	15	11	3/8	7.193	2.398 (± 1.14)

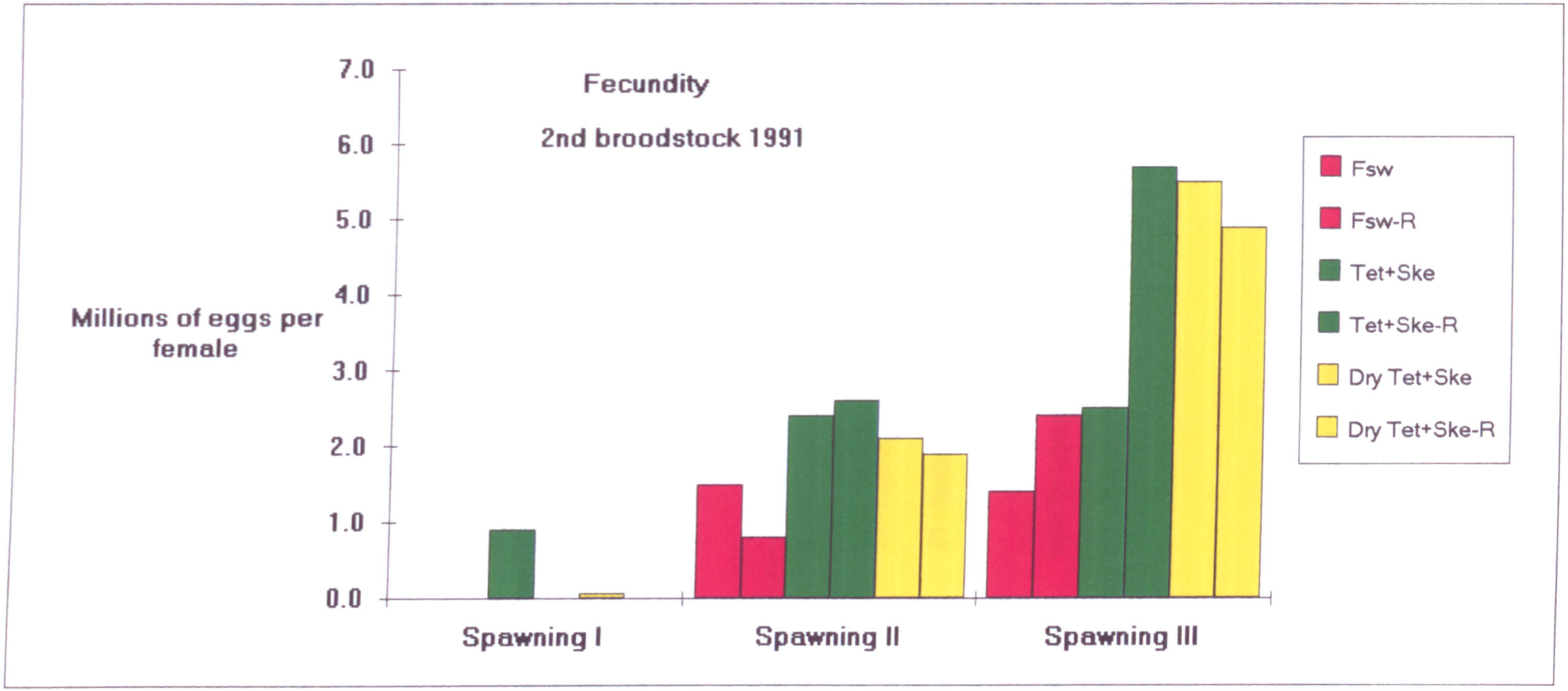


Figure 3.12. Mean fecundity of <u>T. philippinarum</u> in relation to diet after being conditioned for 5 (spawning I), 7 (spawning II) and 9 (spawning III) weeks (2nd broodstock 1991). For clarity, standard errors (given in Table 3.30) are not included in this Figure. For abbreviations see Table 3.21.

Table 3.31. Analysis of variance for variation in number of eggs per female of T. philippinarum conditioned with different nutritional diets from the 2 nd broodstock 1991. For abbreviations and other details see Table 3.7.

2nd broodstock 1991

7 weeks conditioning

Source of variation	d.f.	Sum of squares	Mean square	F Value	P	S
Treatment	5	1.12297E+13	2.24594E+12	1.07	0.3972	n.s.
Residual	27	5.65123E+13	2.09305E+12			
Total	32	6.77420E+13				

9 weeks conditioning

Source of variation	d.f.	Sum of squares	Mean square	F Value	P	S
Treatment	5	6.63807E+13	1.32761E+13	2.64	0.0511	n.s.
Residual	22	1.10459E+14	5.02087E+12			
Total	27	1.76840E+14				

eggs . From the histology data (Appendix 3a) clams after 4 weeks of conditioning were not ready to spawn, the majority were in stage III (late gametogenesis).

The fecundity of the females was very variable within and between treatments (Appendix 8). The mean number of eggs per female ranged from 811,000 to 2.6 million in spawning II and from 1.4 million to 5.8 million in spawning III. Within the spawning period (which took approximately 7 hrs), the percentage of the clams which spawned in the *T. suecica+S. costatum* and dry *T. suecica+S. costatum* treatments ranged from 60 to 87% (spawning II) and from 67 to 93% (spawning III) respectively. In the filtered sea water treatment the percentage of the clams which spawned was 67% (spawning II) and from 27 to 73% (spawning III).

ANOVA was carried out on data for fecundity, and each replicate was used as a separate treatment due to the possibility that there might be significant differences between some of them. After 7 weeks there was no significant difference between the mean numbers of eggs produced per female in the live *T. suecica+S. costatum*, dry *T. suecica+S. costatum* treatments and in the unfed broodstock [$F_{5,27} = 1.07$, $p = 0.40$] (Table 3.31). There was no significant difference between replicates. There were many more females that spawned in the dry *T. suecica* treatment therefore more eggs were produced from this treatment.

After 9 weeks there was also no significant difference between the mean numbers of eggs produced per female in the live *T. suecica+S. costatum*, dry *T. suecica+S. costatum* treatments and in the unfed broodstock (Table 3.31). There was no significant difference between replicates.

It was not expected that the unfed broodstock in the sand-filtered sea water treatment would spawn. The fact that they did, indicated that the quantity of particles in the water were probably of nutritional value or it was a indication of the utilisation of body reserves.

3.2.4. Egg and larvae quality

3.2.4.1. Survival of D-larvae

The percentage survival of D-larvae after 24 h of incubation in natural sea water in the 2nd broodstock 1991 is shown in Table 3.32. The mean percentage survival of D-larvae after 7 (540 D^{o}) weeks conditioning ranged from 16.03 - 36.82% and increased from week 7 to week 9 (707 D^{o}) in all treatments. After 9 weeks the mean survival in live *T. suecica+S. costatum*, dry *T. suecica+S.costatum* and from the unfed broodstock treatment was 68.08%, 60.61% and 79.04% respectively (Table 3.32). It was not possible to carry out any meaningful statistical analysis because there was not enough data.

The quality of the egg, expressed as percentage survival of D-larvae in artificial sea water, tended to increase from week 7 to week 9 in all fed treatments (Table 3.33). After 7 [$F_{5,27} = 1.21$, $p = 0.33$] and 9 weeks [$F_{5,22} = 1.06$, $p = 0.41$] there was no significant difference in the percentage survival of D-larvae from the diet treatments. When data from each diet treatment were pooled there was still no significant difference in week 7 [$F_{2,30} = 0.71$, $p = 0.50$] and week 9 [$F_{2,25} = 1.48$, $p = 0.25$] in the percentage survival of D-larvae. Eggs from the same diet treatment which developed well in natural sea water also developed well in artificial sea water.

3.2.4.2. Lipid and fatty acid analysis

The ash-free dry weight and the lipid content of *T. philippinarum* eggs and D-larvae which developed from them, after spawnings II and III in the 2nd broodstock

Table 3.32. Percentage survival of T. philippinarum D-larvae after 24 h of incubation from the 2nd broodstock 1991. Clams were conditioned for 7 (Spawning II) and 9 (Spawning III) weeks with a mixed diet of live T. suecica+S. costatum and dry T. suecica+S. costatum. For abbreviations see Table 3.21.

Spawning	Treatment	No. embryos incubated (in millions)	No. D-larvae recovered (in millions)	% survival from D-larvae
2nd broodstock 1991				
II	Tet+Ske	3.970	1.166	29.36
	Tet+Ske-R	8.302	3.675	44.27
	Dry Tet+Ske	15.000	2.376	15.84
	Dry Tet+Ske-R	11.942	1.936	16.21
	Fsw	2.921	1.410	48.29
	Fsw-R	3.443	0.386	11.22
III	Tet+Ske	7.500	5.369	71.59
	Tet+Ske-R	7.500	4.842	64.56
	Dry Tet+Ske	7.500	4.601	61.35
	Dry Tet+Ske-R	7.500	4.490	59.86
	Fsw	1.858	1.823	98.12
	Fsw-R	2.250	1.349	59.96

Table 3.33. Percentage survival of T. philippinarum D-larvae after 24 h of incubation in artificial sea water in the 2nd broodstock 1991. Clams were conditioned for 5 (Spawning I), 7 (Spawning II) and 9 (Spawning III) weeks with a mixed diet of T. suecica+S. costatum and dry T. suecica+S. costatum. For abbreviations see Table 3.21.

		Females nº											
Spawning	Treatment	1	2	3	4	5	6	7	8	9	10	Mean	SE
2nd broodstock 1991													
I	Tet+Ske	1.4	_	_	_	_	_	_	_	_	_	1.4	_
	Tet+Ske-R	_	_	_	_	_	_	_	_	_	_	_	_
	DryTet+Ske	8.4	_	_	_	_	_	_	_	_	_	8.4	_
	DryTet+Ske-R	_	_	_	_	_	_	_	_	_	_	_	_
	Fsw	^	^	^	^	^	^	^	^	^	^	^	^
	Fsw-R	_	_	_	_	_	_	_	_	_	_	_	_
II	Tet+Ske	22.0	13.8	_	_	_	_	_	_	_	_	17.90	4.10
	Tet+Ske-R	8.3	22.3	21.5	13.3	_	_	_	_	_	_	16.35	3.37
	DryTet+Ske	8.2	4.4	6.1	9.4	29.4	15.2	28.8	28.2	13.2	8.5	15.14	3.14
	DryTet+Ske-R	10.5	5.5	5.8	4.8	4.5	4.3	28.3	10.4	_	_	9.26	2.86
	Fsw	38.3	4.5	_	_	_	_	_	_	_	_	21.40	16.90
	Fsw-R	4.0	4.4	3.6	2.9	1.2	32.0	2.0	_	_	_	7.16	4.16
III	Tet+Ske	32.3	31.4	30.3	27.3	21.8	8.6	_	_	_	_	25.28	3.68
	Tet+Ske-R	33.3	17.2	19.8	7.7	_	_	_	_	_	_	19.50	5.28
	DryTet+Ske	27.0	12.3	14.2	4.5	17.7	53.0	33.5	43.7	_	_	25.74	5.91
	DryTet+Ske-R	8.4	6.5	29.7	34.8	16.7	_	_	_	_	_	19.22	5.65
	Fsw	32.7	7.2	_	_	_	_	_	_	_	_	19.95	12.75
	Fsw-R	1.3	17.3	1.9	_	_	_	_	_	_	_	6.83	5.24

_ clams did not spawn
^ only males

1991 is shown together with the mean shell length of D-larvae from the different diet treatments in Table 3.34. These larvae were used to start semi-commercial scale trials.

The lipid content of eggs spawned by *T. philippinarum* from the different broodstock diets was between 7.2 and 9.1 ng egg^{-1} (24 to 35% of Afdwt) from the two spawnings. In spawning II lipid ranged from 7.2 to 8.8 ng egg^{-1} (25 to 35% of Afdwt). In spawning III, lipid ranged from 8.2 to 9.1 ng egg^{-1} (24 to 27% of Afdwt). However, the highest and lowest values for lipid as ng egg^{-1} did not always correspond with the highest and lowest values for lipid as % Afdwt. The lipid content of D-larvae was between 5.1 and 7.7 ng $larvae^{-1}$ from the two spawnings. In spawning II [$F_{5,26} = 0.88$, $p = 0.51$] and III [$F_{5,20} = 0.24$, $p = 0.94$], lipid as ng per egg was not significantly different in live *T. suecica+S. costatum*, dry *T. suecica+S. costatum* and in filtered sea water treatments. Because there were no significant differences between replicates, data was pooled and after 7 weeks [$F_{2,29} = 1.32$, $p = 0.28$] or 9 weeks [$F_{2,23} = 0.04$, $p = 0.96$] there were no significant differences between all treatments.

The lipid energy reserves utilized during development from the egg to the D-larva stage, in the first 24h before feeding, was determined (Table 3.34). In spawning II and III, the percentage utilization of lipid was not significantly different [$F_{1,1} = 37.82$, $p = 0.10$] in *T. suecica+S. costatum* compared with dry *T. suecica+S. costatum* within treatments and was not significantly different between spawnings [$F_{1,1} = 1.71$, $p = 0.42$].

Changes in the lipid content of *T. philippinarum* larvae (ng per larva) during development are presented in Table 3.35, from day 5 to 14 (spawning II) and from day 5 to 10 (spawning III). Changes in the quantity of lipid (ng $larva^{-1}$) during development are shown in Figure 3.13. Larvae from the two broodstock treatments were grown in natural sea water and measurements were made on the same days. In

Table 3.34. Ash-free dry weight (Afdwt) and lipid content of eggs and D-larvae of T. philippinarum in the 2nd broodstock 1991. Clams were conditioned for 7 (Spawning II) and 9 (Spawning III) weeks with a mixed diet of T. suecica+S. costatum and dry T. suecica+S. costatum. For abbreviations see Table 3.21.

Broodstock diet	Egg			D-larvae				% utilization of lipid during egg development
	Afdwt (ng egg)	Lipid (ng egg)	Lipid (% Afdwt)	Mean larval size (µm)	Afdwt (ng D-larva)	Lipid (ng D-larva)	Lipid (% Afdwt)	
Spawning II (7 weeks)								
Tet + Ske	25.99	8.84	34.01	96.70	42.96	7.16	16.67	19.00
Tet + Ske - R	28.03	8.10	28.90	96.80	38.48	5.37	13.96	33.70
Mean	27.01	8.47	31.46	96.75	40.72	6.27	15.31	26.35
Dry Tet + Ske	24.49	8.45	34.50	96.90	43.73	7.58	17.33	10.30
Dry Tet + Ske - R	28.49	7.89	27.69	95.20	39.18	6.35	16.21	19.52
Mean	26.49	8.17	31.10	96.05	41.46	6.97	16.77	14.91
Fsw	24.94	8.14	32.64	95.80	_	_	_	_
Fsw - R	29.10	7.15	24.57	95.40	_	_	_	_
Mean	27.02	7.65	28.60	95.60	_	_	_	_
Spawning III (9 weeks)								
Tet + Ske	33.72	8.21	24.35	99.90	50.89	6.72	13.20	18.15
Tet + Ske - R	33.63	9.13	27.15	100.10	55.22	6.65	12.04	27.16
Mean	33.68	8.67	25.75	100.00	53.06	6.69	12.62	22.66
Dry Tet + Ske	32.88	8.30	25.24	98.40	49.74	6.89	13.85	16.99
Dry Tet + Ske - R	32.84	8.69	26.46	98.40	46.77	7.66	16.38	11.85
Mean	32.86	8.50	25.85	98.40	48.26	7.28	15.12	14.42
Fsw	34.44	9.10	26.42	99.70	50.31	6.92	13.75	23.96
Fsw - R	32.18	8.16	25.36	98.20	47.86	5.08	10.61	37.75
Mean	33.31	8.63	25.89	98.95	49.09	6.00	12.18	30.85

_ lost sample

Table 3.35. Ash-free dry weight (Afdwt) and lipid content of larvae of T. philippinarum in the 2nd broodstock 1991. Clams were conditioned for 7 (Spawning II) and 9 (Spawning III) weeks with a mixed diet of T. suecica+S. costatum and dry T. suecica+S. costatum. For abbreviations see Table 3.21.

Broodstock diet	Day 5			Day 10			Day 14		
	Afdwt (ng D-larva)	Lipid (ng D-larva)	Lipid (% Afdwt)	Afdwt (ng D-larva)	Lipid (ng D-larva)	Lipid (% Afdwt)	Afdwt (ng D-larva)	Lipid (ng D-larva)	Lipid (% Afdwt)
Spawning II (7 weeks)									
Tet + Ske	_	40.75	_	476.80	81.52	17.10	603.50	61.79	10.24
Tet + Ske - R	_	20.98	_	489.10	79.83	16.32	660.02	72.24	10.95
Mean	_	30.87	_	482.95	80.68	16.71	631.76	67.02	10.60
Dry Tet + Ske	_	22.63	_	501.80	94.14	18.76	625.04	69.17	11.07
Dry Tet + Ske - R	_	29.96	_	463.00	56.97	12.30	675.42	70.67	10.46
Mean	_	26.30	_	482.40	75.56	15.53	631.76	69.92	10.77
Fsw	_	25.01	_	371.21	57.24	15.42	740.55	83.47	11.27
Fsw - R	_	16.62	_	370.00	55.77	15.07	721.12	79.64	11.04
Mean	_	20.82	_	370.61	56.51	15.25	730.84	81.56	11.16
Spawning III (9 weeks)									
Tet + Ske	230.14	24.92	10.83	601.23	93.53	15.56	_	_	_
Tet + Ske - R	255.36	25.56	10.01	687.14	103.63	15.08	_	_	_
Mean	242.75	25.24	10.42	644.19	98.58	15.32	_	_	_
Dry Tet + Ske	215.98	21.36	9.89	707.32	98.22	13.89	_	_	_
Dry Tet + Ske - R	209.58	20.14	9.61	512.36	76.71	14.97	_	_	_
Mean	212.78	20.75	9.75	609.84	87.47	14.43	_	_	_
Fsw	245.47	25.30	10.31	487.51	76.29	15.65	_	_	_
Fsw - R	195.00	16.12	8.27	699.25	94.18	13.47	_	_	_
Mean	220.24	20.71	9.29	593.38	85.24	14.56	_	_	_

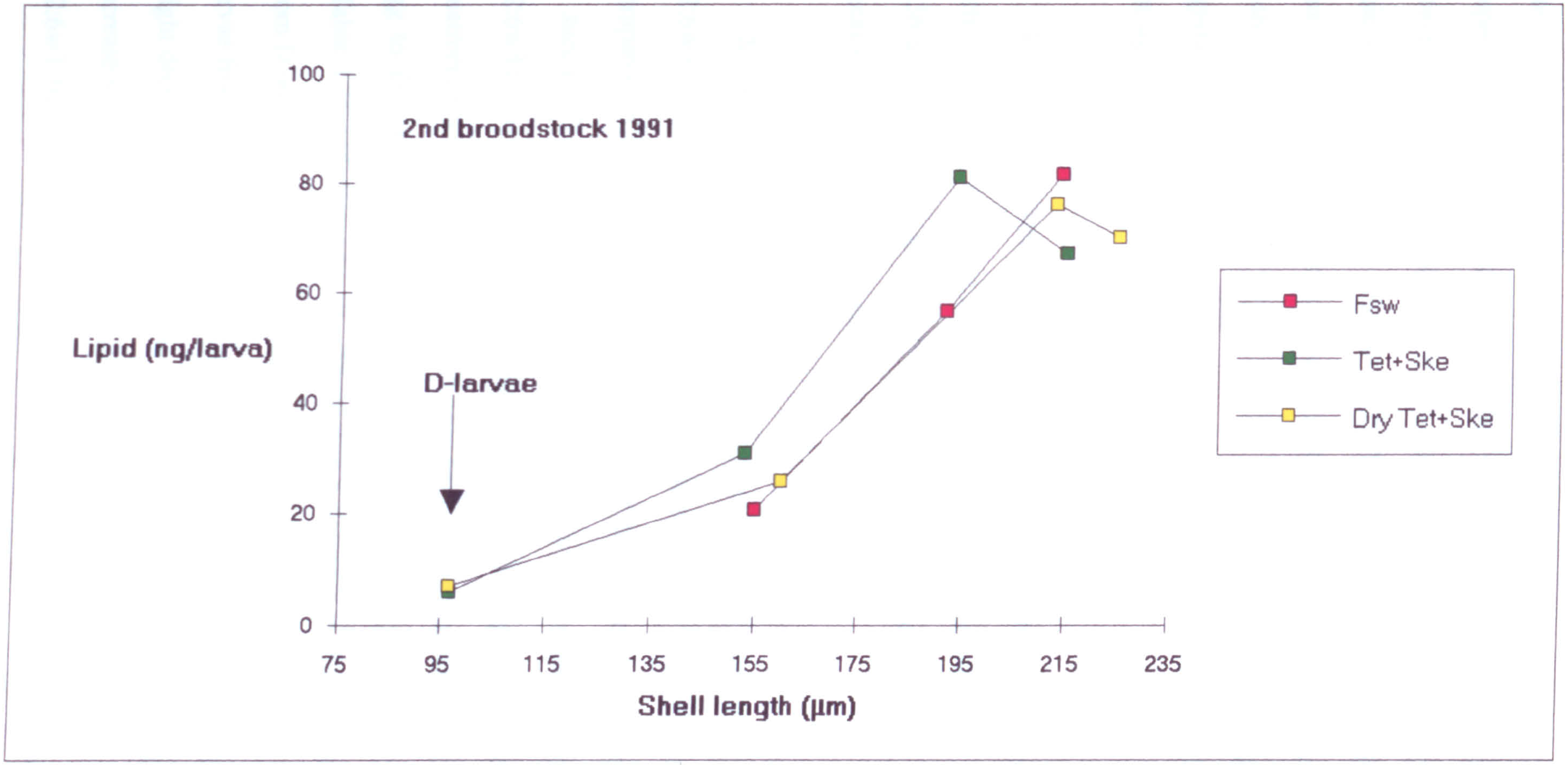


Figure 3.13. Lipid content of T. philippinarum larvae from the 2nd broodstock 1991 (spawning II) conditioned on live T. suecica+S. costatum (Tet+Ske) and dry T. suecica+S. costatum (Dry Tet+Ske) diets. Points on each line represent, from left to right, day 0, 5, 10 and 14.

larvae from *T. suecica+S. costatum* and dry *T. suecica+S. costatum* treatments, there was an increase in the quantity of lipid, until larvae reached 193 μm and 207 μm respectively (from day 5 to day 10). This was followed by a decrease. The decrease in lipid probably indicated that larvae had reached the stage of metamorphosis and utilised lipid as an energy source (Figure 3.13). The lipid data were combined because there was no significant difference between replicates. At day 10, in spawning II [$F_{2,13}$ = 3.46, p = 0.06] and III [$F_{2,15}$ = 1.54, p = 0.25] lipid as ng per larva was not significantly different between diet treatments. Between spawnings lipid per larva was not significantly different [$F_{1,2}$ = 15.72, p = 0.06].

The fatty acid content of eggs, D-larvae and 5 to 14 day-old larvae (as % of total fatty acids) from spawning II and III in the 2nd broodstock 1991 is shown in Table 3.36 and Appendix 9 and 9a. It was not possible to carry out any statistical analysis because there were not enough data.

In spawning II, eggs from all treatments had a high level of 18:4w3, 20:5w3 and 22:6w3 (Table 3.36 and Appendix 9). These PUFAs were higher in this spawning compared with spawning III. The 20:5w3 and 22:6w3 levels in eggs from live and dry *T. suecica+S. costatum* were generally similar in both spawnings (the exception was 22:6w3 in spawning III). In both spawnings 22:6w3 in eggs from the filtered sea water treatment was always higher than in eggs from other treatments. In spawning II, from egg to D-larvae there was decrease in the levels of 18:4w3, 20:5w3 and 22:6w3 (Table 3.36 and Appendix 9). There was a decrease in 22:6w3 during development from D-larvae onwards in all treatments. With 20:5w3, there was a general increase in larvae from the *T. suecica+S. costatum* treatment from D-larvae till day 5-10 and a slight decrease by day 14. In the dry *T. suecica+S. costatum* treatment, there was a increase in 20:5w3 from D-larvae till day 14. In spawning III, there was an increase in 22:6w3 from egg to D-larvae in all treatments. There was an increase in 20:5w3

Table 3.36. Fatty acid composition of T. philippinarum eggs and larvae fed on different diets. Values are percentages of total fatty acid. For abbreviations see Table 3.21.

2nd broodstock 1991			Age of larvae		
	Eggs	D-larvae	5	10	14
Tet+Ske					
Spawning II					
18:3w3	3.02	5.45	1.00	1.05	1.31
20:5w3	9.45	6.23	21.76	21.67	19.58
22:6w3	12.55	8.26	6.34	3.85	2.35
Polyenoic	39.44	32.83	35.41	31.48	28.09
Spawning III					
18:3w3	1.72	1.77	-	-	-
20:5w3	7.26	6.12	-	-	-
22:6w3	2.94	6.91	-	-	-
Polyenoic	21.06	20.46	-	-	-
Dry Tet+Ske					
Spawning II					
18:3w3	2.71	2.34	1.10	1.03	1.24
20:5w3	8.76	6.75	22.27	22.36	23.56
22:6w3	12.73	11.31	7.09	3.05	2.62
Polyenoic	38.17	32.12	37.36	30.72	31.06
Spawning III					
18:3w3	1.85	1.24	-	-	-
20:5w3	6.21	7.03	-	-	-
22:6w3	6.52	7.73	-	-	-
Polyenoic	23.51	21.25	-	-	-
Fsw					
Spawning II					
18:3w3	3.26	3.31	0.76	1.08	1.31
20:5w3	5.43	4.04	20.51	15.17	22.84
22:6w3	14.27	12.07	6.08	3.97	2.09
Polyenoic	38.91	34.34	33.18	29.08	30.28
Spawning III					
18:3w3	2.21	2.53	-	-	-
20:5w3	4.92	7.30	-	-	-
22:6w3	9.30	9.55	-	-	-
Polyenoic	22.53	27.32	-	-	-

during development from egg to D-larvae in dry *T. suecica+S. costatum* and filtered sea water treatment (Table 3.36).

Overall the polyenoic fatty acids generally decreased from egg to D-larvae (this is most evident in the 2nd broodstock 1991, spawning II). Differences in total polyenoic fatty acids in eggs were found between spawnings II and III. Values were significantly higher in spawning II [$F_{1,2} = 234.88$, $p < 0.01$]. There was a general increase in the percentage of the polyenoic fatty acids during larval development, from D-larvae to day 5, in *T. suecica+S. costatum* and dry *T. suecica+S. costatum* which corresponded to the increasing level of lipid in larvae (when expressed as ng larva^{-1}). This was followed by a decrease in these fatty acids during development from day 5 to day 10.

3.2.4.3. Growth of larvae

Results for the initial shell length of the *T. philippinarum* D-larvae, larval shell length on day 5 through to day 16 and the percentage survival of larvae from the 2nd broodstock 1991 are shown in Table 3.37. The growth coefficients of the larvae at day 10 and 14 in this broodstock are shown are Table 3.38.

The shell length of the D-larvae in spawning II [$F_{2,3} = 1.32$, $p = 0.39$] and III [$F_{2,3} = 3.46$, $p = 0.17$] was not significantly different between treatments. The shell length of D-larvae was significantly greater in spawning III than in spawning II [$F_{1,2} = 88.02$, $p < 0.01$].

In spawning II [$F_{2,3} = 8.03$, $p = 0.06$] and III [$F_{2,3} = 1.65$, $p = 0.33$], the shell lengths of larvae at day 14, in the live *T. suecica+S. costatum*, dry *T. suecica+S. costatum* and in the unfed treatments were not significantly different. There were no significant difference between replicates, so the data were pooled. Although D-larvae in spawning II were significantly smaller than D-larvae in spawning III, they grew to

Table 3.37. Larval performance in natural sea water in relation to broodstock diet quality . Shell length (μm) and percentage survival of T. philippinarum larvae in the 2nd broodstock 1991 (Spawning II and III) are presented. Standard error in parentheses. For abbreviations see Table 3.21.

Initial D-larvae size in Spawning II: Tet+Ske - 96.7 ; 96.8
Dry Tet+Ske - 96.9 ; 95.2
Fsw - 95.8 ; 95.4

Spawning III: Tet+Ske - 99.9 ; 100.1
Dry Tet+Ske - 98.4 ; 98.4
Fsw - 99.7 ; 98.2

		Tet + Ske		Dry Tet + Ske		Fsw	
Spawning	Days of growth	% survival	Larvae shell length (μm)	% survival	Larvae shell length (μm)	% survival	Larvae shell length (μm)
2nd broodstock 1991							
	5	61.2	153.2 (±0.73)	63.7	159.7 (±0.83)	64.3	155.1 (±0.72)
Spawning II	10	41.7	194.3 (±1.07)	49.0	212.8 (±1.30)	49.0	191.7 (±1.14)
(7 weeks)	14	26.5	215.4 (±1.31)	28.8	220.2 (±1.44)	31.2	214.1 (±1.30)
	16	22.3	224.9 (±1.73)	21.1	230.3 (±1.72)	18.3	216.8 (±1.37)
	5	62.8	152.8 (±0.70)	64.9	156.9 (±0.79)	46.5	158.6 (±0.76)
(replicate)	10	37.8	192.4 (±1.01)	39.3	202.0 (±1.17)	16.1	200.5 (±1.18)
	14	25.4	218.9 (±1.30)	24.6	222.6 (±1.37)	8.9	210.5 (±1.28)
	16	20.2	221.4 (±1.46)	19.3	228.5 (±1.59)	-	-
Spawning III	5	66.0	161.7 (±0.90)	68.7	158.0 (±0.79)	54.1	166.2 (±0.86)
(9 weeks)	10	42.3	206.3 (±1.24)	52.0	210.7 (±1.32)	38.9	190.6 (±1.11)
	14	31.2	215.3 (±1.30)	38.4	220.4 (±1.46)	28.1	211.2 (±1.39)
	16	24.8	221.3 (±1.61)	-	-	23.1	219.1 (±1.54)
(replicate)	5	60.6	156.4 (±0.75)	79.7	158.8(±0.76)	73.8	158.4 (±0.73)
	10	49.1	202.8 (±1.14)	58.7	217.7 (±1.39)	42.7	206.1 (±1.20)
	14	29.9	219.3 (±1.39)	39.8	223.9 (±1.61)	23.7	219.2 (±1.48)
	16	23.8	224.3 (±1.65)	-	-	-	-

Table 3.38. Growth coefficient (G) of T. philippinarum larvae in natural sea water in relation to broodstock diet quality in the 2nd broodstock 1991. For abbreviations see Table 3.21.

Spawning	Conditioning diet	Initial Shell length (μm)	Growth coefficient (G)	
			Larvae (at day 10)	Larvae (at day 14)
2nd broodstock 1991				
I	*	*	*	*
II	Tet+Ske	96.7	0.070	0.057
	Tet+Ske-R	96.8	0.069	0.058
	Dry Tet+Ske	96.9	0.079	0.057
	Dry Tet+Ske-R	95.2	0.075	0.057
	Fsw	95.8	0.069	0.057
	Fsw-R	95.4	0.074	0.057
III	Tet+Ske	99.9	0.073	0.057
	Tet+Ske-R	100.1	0.071	0.057
	Dry Tet+Ske	98.4	0.076	0.057
	Dry Tet+Ske-R	98.4	0.079	0.057
	Fsw	99.7	0.065	0.058
	Fsw-R	98.2	0.074	0.057

* not enough embryos to be incubate

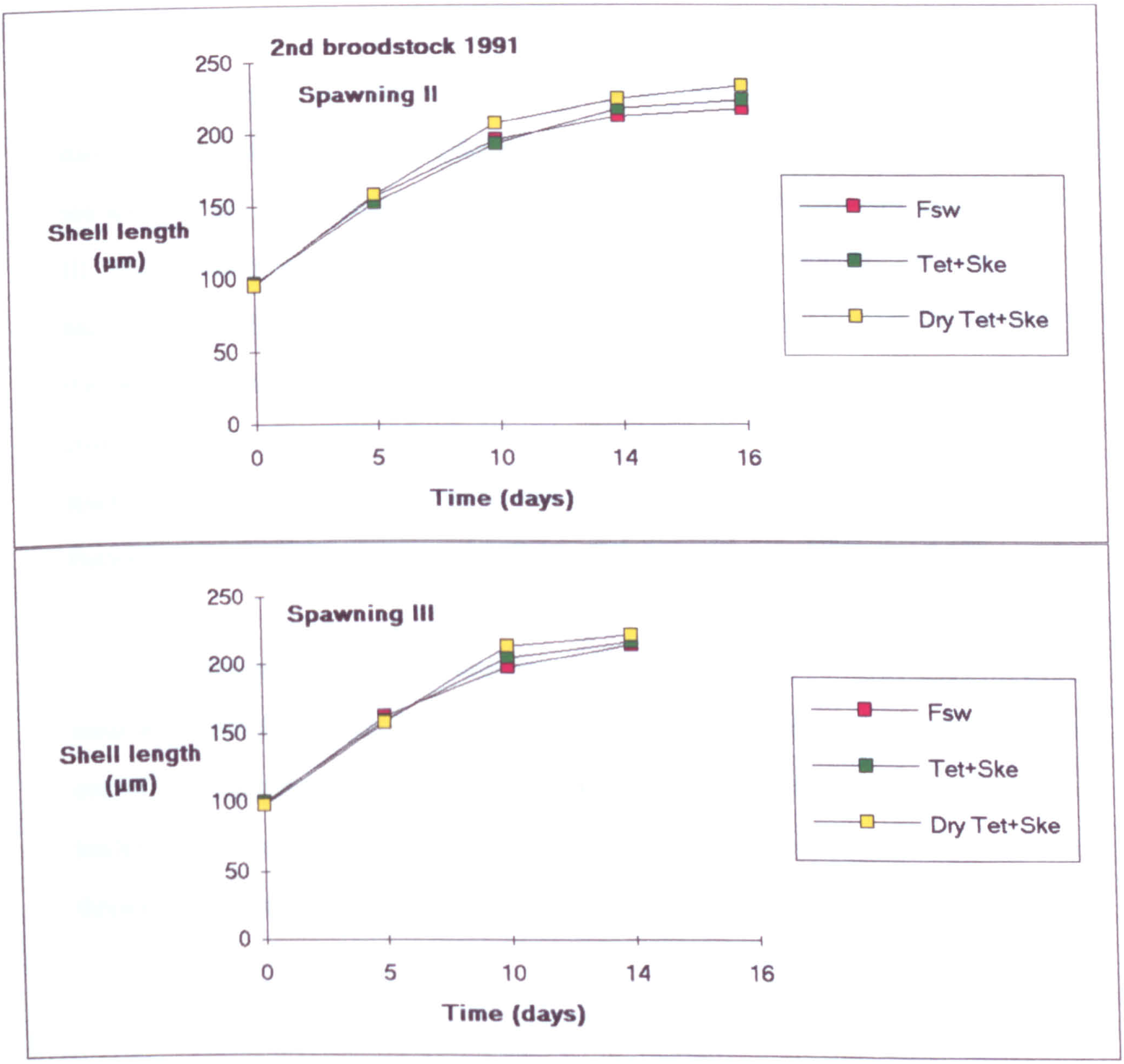


Figure 3.14. Growth of T. philippinarum larvae in natural sea water from the 2nd broodstock 1991, conditioned on live (Tet+Ske) and dry T. suecica+S. costatum (Dry Tet+Ske) and on a filtered sea water control. For clarity, standard errors (given in Table 3.37) are not included in this Figure.

a similar size by day 14 or 16. The growth of larvae from all broodstock treatments was not significantly different [$F_{1,2}$ = 2.30, p = 0.27] between spawnings. (Figure 3.14). The growth coefficient of larvae to day 14 in spawning II and III was 0.06 for all the broodstock treatments.

In spawning II, the survival of larvae at day 14 was 26% (from live *T. suecica+S. costatum*), 27% (from dry *T. suecica+S. costatum*) and 20% (from filtered sea water) of the initial number (0.841, 1 and 1.085 million respectively). In spawning III, for the same number of days, the survival of larvae was respectively 31%, 39% and 26% of the initial number (1.085 million). There was no significant difference in the percentage survival of larvae whether from broodstock fed live *T. suecica+S. costatum* or dry *T. suecica+S. costatum* or from the filtered sea water treatment in spawning II [$F_{2,3}$ = 0.31, p = 0.76] and in spawning III [$F_{2,3}$ = 0.40, p = 0.70]. Between spawnings there was no significant difference [$F_{1,2}$ = 4.60, p = 0.17].

In assessing survival, the numbers of larvae removed from each treatment for measuring shell length and percentage survival were taken into consideration. At the end of all experiments many of the larvae had developed into pediveligers which tended to crawl on the bottom of the culture bins and attached to surfaces with byssus threads, but this fact did not affect the survival estimations.

3.2.5. Sea water quality

3.2.5.1. Suspended particulate material

(i) Size composition and number of particles in suspension

The size distribution of suspended particulate material between 2.5 and 10 μm diameter, in sand-filtered sea water in the 2nd broodstock conditioning trial carried out in 1991 is shown in Appendix 6. The mean total number of particles (2.5 - 10 μm diameter) in the sand-filtered sea water during the experiment was 58.67 per μl (Table 3.39). Particles of 2.5 - 5.0 μm diameter accounted for 78.5% of the total, while particles between 5.0 - 10 μm diameter represented 21.5%. The mean total values (± standard error) of three size groups 0.22 - 1, 2.5 - 5.0 and 5.0 - 10 μm diameter in the water are given in Table 3.39.

The amount of suspended particulate material, between 2.5 - 5.0 and 5.0 - 10 μm in size, varied between weeks and there were more particles in this 2nd broodstock 1991 experiment compared with the 1st experiment 1991 (Appendix 6).

(ii) Dry weight, ash-free dry weight and lipid content of particles

The percentage ash-free dry weight content of the suspended particulate material was 34.64%. The dry weights and ash-free dry weights of particles in suspension are shown in Appendix 6. The lipid content of suspended particulate material in the sea water expressed as a percentage of the ash-free dry weight is shown in Appendix 6. Lipid content was usually around 6% of the ash-free dry weight. Lipid content of sea water in the hatchery was variable and 2% higher, than in the 1st broodstock 1991 experiment.

Table 3.39. Mean number, weight, lipid content and chlorophyll a of particles in sand-filtered sea water flowing through broodstock tanks in the hatchery. Standard error are in parentheses.

	Sand-filtered sea water
2nd broodstock 1991	
Number of particles (/µl)	
2.5 - 5.0 µm	46.08 (± 10.58)
5.0 - 10 µm	12.59 (± 4.02)
Total dry weight (mg/l)	
0.22 - 1 µm	30.07 (± 10.28)
> 1 µm	14.44 (± 2.47)
Ash-free dry weight (mg/l)*	5.01 (± 0.94)
% ash-free dry weight*	34.64 (± 4.59)
Lipid (% ash-free dry weight)	6.10 (± 1.26)
Chlorophyll a (µg/l)	2.77 (± 0.29)

* 2.5 - 5.0 µm diameter

3.2.5.2. Chlorophyll a determination

The occurrence of chlorophyll a in the sea water is shown in Figure 3.7 and Appendix 6. Chorophyll a was higher than in the 1st broodstock because it was later in the year when there was more phytoplankton in sea water taken from the Conwy estuary.

3.2.6. Summary of results

In this study live *T. suecica* and dry *T. suecica* were compared in mixtures consisting of 70% *T. suecica* and 30% *S. costatum*. In the 2nd broodstock 1991 with 6% food ration, spawning started after 5 (385 D^{o}) weeks with only some eggs produced. Spawning condition was reached within 540 (7 weeks) to 707 (9 weeks) day degrees of conditioning. After 7 and 9 weeks there was no significant difference between the mean numbers of eggs produced per female in the live *T. suecica+S. costatum*, dry *T.suecica+S. costatum* and in the the unfed broodstock. There was no difference between replicates. The number of eggs spawned after 9 weeks was higher than after 7 weeks. It was not expected that the unfed broodstock in the sand-filtered sea water treatment would spawn.

There were significant differences in live weight, meat dry weight, condition index, ash-free dry weight, lipid and carbohydrate of the broodstock from the different diet treatments after 5 (exception for ash-free dry weight and lipid) and 7 weeks of conditioning. After 9 weeks there were significant differences in all the parameters studied of the broodstock. In spawning II (7 weeks) and III (9 weeks), there was a significant difference between male, female and non-spawners. Male clams tended to have significantly higher values than females for live weight, meat dry weight and condition index. Females clams had significantly higher levels of lipid than males.

The quality of the egg, expressed as percentage survival of D-larvae in artificial sea water, increased from week 7 to week 9 in all treatments. In boths weeks there were no significant difference in the percentage survival of D-larvae from the treatments.

The lipid content of eggs spawned by *T. philippinarum* from the different broodstock diets was between 7.2 and 9.1 ng egg^{-1}. Lipid as ng per egg was not significantly different between treatments and spawnings. Lipid content of larvae at day 10 was not significantly different between treatments and spawnings.

The fatty acid composition of the lipid reserves in eggs from all treatments in (spawning II) had a high level of 18:4w3, 20:5w3 and 22:6w3. From egg to D-larvae there was a decrease in the levels of these PUFAs and they had higher values in this spawning compared with spawning III. Overall the polyenoic fatty acids generally decreased from egg to D-larvae. Values were significantly higher in spawning II. There was a general increase in the percentage of the polyenoic fatty acids during larval development, from D-larvae to day 5, in *T. suecica+S. costatum* and dry *T. suecica+S. costatum* which corresponded to the increasing level of lipid in larvae.

The growth of larvae from all broodstock treatments was not significantly different between treatments and between spawnings. The percentage survival of D-larvae at day 14 was not significantly different between treatments and between spawnings.

3.3. BROODSTOCK FED ON DRY *T. SUECICA* AND THREE SPECIES OF LIVE ALGAE (6% DIET, 1st AND 2nd BROODSTOCK 1992).

3.3.1 Biochemical analysis of the cultured algae

The gross biochemical composition of the cultured algae fed to both 1992 broodstocks is presented in Table 3.40. There was good agreement in the values between the two broodstocks. Lipid was higher in *I. galbana* than the other diets. The lipid content of dry *T. suecica* was similar to that of live *T. suecica* (see section 3.2.1., Table 3.19). The dry *T. suecica* was the same as that used in the 2nd broodstock 1991. It was very low in lipid and PUFAs (see section 3.2.1., Table 3.20). The polyenoic fatty acid composition, as a percentage of the total fatty acids, was an average of 39% for *D. tertiolecta*, 34% for *S. costatum* and 47% for *I. galbana*. The qualitative fatty acid composition of the live algae (1st and 2nd broodstock 1992) is presented in Table 3.41. *D. tertiolecta*, had high levels of 18C fatty acids, particulary 18:3w3, negligible quantities of 20C PUFAs and no 22C PUFAs. *S. costatum* and *I. galbana* had a higher levels of 20C and 22C PUFAs. *I. galbana* had significant quantities of 18:4w3 and 22:6w3 while *S. costatum* was higher in 20:5w3.

3.3.2. Broodstock conditioning

3.3.2.1. Temperature and salinity

Sea water temperature during the 1st broodstock conditioning ranged from 19.8 to 23.1 ^{o}C (spawning I, II and III). In the 2nd broodstock the sea water temperature during conditioning ranged from 22.1 to 24.0 ^{o}C (spawning I, II and III). Mean temperature values for each experiment in 1992 are shown in Table 3.42. In the 1st [$F_{4,270} = 0.54$, $p > 0.71$] and 2nd [$F_{4,245} = 1.07$, $p > 0.37$] broodstock 1992, temperatures between broodstock tanks were not significantly different. The

Table 3.40. Ash free dry weight (Afdwt, mg per million cells and as a % of the dry weight) and biochemical composition of diets in the1st and 2nd broodstock 1992. Protein values were obtained by subtraction of lipid and carbohydrate percentages. Standard error in parentheses.

Diet species	Afdwt (mg) / million cells	% Afdwt	Lipid (% Afdwt)	Carbohydrate (% Afdwt)	Protein (% Afdwt)
1st broodstock 1992					
Dry T. suecica *	–	91.06 (±0.02)	3.00 (±0.17)	57.46 (±0.08)	39.54
D. tertiolecta	0.15 (±0.05)	48.93 (±3.03)	12.26 (±1.96)	«	«
S. costatum	0.03 (±0.004)	54.77 (±3.14)	15.46 (±1.33)	4.67 (±1.04)	79.87
I. galbana	0.02 (±0.004)	79.90 (±1.96)	23.46 (±1.78)	23.11 (±1.81)	53.43
2nd broodstock 1992					
Dry T. suecica	–	90.32 (±0.01)	3.50 (±0.21)	56.86 (±0.06)	39.64
D. tertiolecta	0.13 (±0.01)	50.29 (±2.00)	13.00 (±1.68)	«	«
S. costatum	0.03 (±0.004)	57.33 (±2.57)	13.94 (±1.13)	4.41 (±1.08)	81.65
I. galbana	0.02 (±0.007)	77.00 (±1.71)	23.85 (±1.68)	23.42 (±1.71)	52.73

* the same dry algae used in 1991
« contaminated sample

Table 3.41. Fatty acid composition of the live algae used on both broodstocks 1992. Values are percentages of total fatty acids.

	1st broodstock 1992			2nd broodstock 1992		
Algae	*D.tertiolecta*	*S. costatum*	*I. galbana*	*D. tertiolecta*	*S. costatum*	*I. galbana*
Fatty acid						
14:0	17.85	26.24	16.66	14.18	21.65	15.71
16:0	19.63	10.86	13.07	24.21	11.77	13.57
18:0	2.70	1.69	1.70	3.71	3.36	2.55
16:1w7/9	5.32	20.66	6.31	7.52	22.57	5.57
18:1w9	12.16	5.82	14.31	12.04	5.21	13.04
18:1w7	1.09	0.55	1.28	1.24	0.81	1.25
20:1w9	0.00				0.23	0.19
18:2w6	6.63	1.11	5.14	8.76	1.16	5.69
18:3w3	30.61	0.00	4.54	25.62	0.32	4.46
18:4w3	0.00	4.93	25.03	1.13	4.64	24.47
20:2w6	4.01	2.43	1.80	1.59	0.56	0.66
20:3w3	0.00	0.00	0.00	0.00	0.00	0.00
20:4w6	0.00	1.61	0.00	0.00	1.12	1.02
20:5w3	0.00	22.45	0.63	0.00	22.57	0.68
22:5w3	0.00	0.06	0.04	0.00	0.63	0.55
22:6w3	0.00	1.58	9.48	0.00	3.40	10.58
Saturated	40.18	38.79	31.43	42.10	36.78	31.83
Monoenoic	18.57	27.03	21.90	20.80	28.82	20.05
Polyenoic	41.25	34.17	46.66	37.10	34.40	48.11

Table 3.42. Mean temperature and day-degrees in T. philippinarum broodstock tanks (1st and 2nd broodstock 1992). Standard error in parentheses. Dun=D.tertiolecta; Ske=S.costatum; Iso=I.galbana; Dry Tet=Dry T. suecica and Fsw=Filtered sea water.

Spawning	Week	Broodstock treatment				
		Fsw	Dry Tet	Dry Tet + Dun	Dry Tet + Ske	Dry Tet + Iso
1st broodstock 1992						
I	6	21.71 (±0.14) 504	21.77 (±0.14) 506	21.87 (±0.14) 510	21.88 (±0.14) 511	21.85 (±0.14) 510
II	7	21.70 (±0.13) 562	21.78 (±0.13) 565	21.86 (±0.13) 569	21.88 (±0.13) 570	21.84 (±0.13) 568
III	8	21.77 (±0.12) 647	21.88 (±0.12) 653	21.97 (±0.12) 658	21.99 (±0.12) 659	21.94 (±0.12) 657
2nd broodstock 1992						
I	5	22.76 (±0.10) 459	22.84 (±0.10) 462	22.89 (±0.10) 464	22.85 (±0.10) 463	22.83 (±0.09) 462
II	6	22.75 (±0.08) 548	22.86 (±0.09) 553	22.94 (±0.08) 556	22.91 (±0.08) 555	22.89 (±0.08) 554
III	7	22.73 (±0.08) 637	22.86 (±0.07) 642	22.93 (±0.07) 646	22.90 (±0.07) 645	22.89 (±0.07) 644

Table 3.43. Mean salinity in T. philippinarum broodstock tanks (1st and 2nd broodstock 1992). Standard error in parentheses. For abbreviations see Table 3.42.

Spawning	Week	Broodstock treatment				
		Fsw	Dry Tet	Dry Tet + Dun	Dry Tet + Ske	Dry Tet + Iso
1st broodstock 1992						
I	6	31.14 (±0.07)	31.14 (±0.08)	31.16 (±0.07)	31.15 (±0.08)	31.15 (±0.07)
II	7	31.13 (±0.08)	31.13 (±0.08)	31.15 (±0.08)	31.16 (±0.08)	31.16 (±0.08)
III	8	30.85 (±0.22)	30.85 (±0.22)	30.88 (±0.21)	30.89 (±0.22)	30.87 (±0.22)
2nd broodstock 1992						
I	5	30.57 (±0.36)	30.56 (±0.36)	30.60 (±0.36)	30.64 (±0.37)	30.63 (±0.37)
II	6	30.35 (±0.33)	30.34 (±0.33)	30.38 (±0.32)	30.42 (±0.33)	30.41 (±0.33)
III	7	30.10 (±0.30)	30.09 (±0.30)	30.14 (±0.30)	30.17 (±0.30)	30.15 (±0.30)

Table 3.44. Percentage filtration and filtration rate (particles between 2.5 - 10 μm) of T. philippinarum broodstocks in 1992. For abbreviations see Table 3.42.

Date	Percentage filtration					Filtration rate				
	Dry Tet+Dun	Dry Tet+Ske	Dry Tet+Iso	Dry Tet	Fsw	Dry Tet+Dun	Dry Tet+Ske	Dry Tet+Iso	Dry Tet	Fsw
1st broodstock 1992										
(22.01.92)	93.87	91.66	93.54	92.22	70.89	563.24	549.98	561.25	553.35	425.36
(27.01.92)	91.52	89.04	90.10	88.92	69.55	549.14	534.25	540.63	533.54	417.29
(3.02.92)	92.11	93.18	93.27	91.97	73.09	552.69	559.07	559.63	551.82	438.53
(10.02.92)	88.76	96.28	93.00	93.14	85.09	532.56	577.70	558.00	558.85	510.56
(17.02.92)	90.26	95.94	91.15	71.73	64.86	541.55	575.67	546.89	430.36	389.14
(24.02.92)	95.79	91.72	87.82	88.08	78.99	574.77	550.34	526.93	528.48	473.94
(2.03.92)	93.42	87.83	93.92	91.05	79.10	560.49	526.99	563.53	546.28	474.59
Mean	92.25	92.24	91.83	88.16	74.51	553.49	553.43	550.98	528.95	447.06
SE	0.89	1.21	0.85	2.82	2.60	5.33	7.23	5.08	16.91	15.63

Table 3.44 (continued)

2nd broodstock 1992										
(16.03.92)	93.87	91.66	93.54	92.22	70.89	563.24	549.98	561.25	553.35	425.36
(23.03.92)	91.71	94.48	92.47	72.66	56.82	550.29	566.87	554.81	435.98	340.91
(1.04.92)	90.96	94.61	92.10	89.11	60.09	545.77	567.66	552.66	534.64	360.52
(6.04.92)	88.92	94.57	88.52	87.27	89.25	533.52	567.40	531.12	523.64	535.50
(13.04.92)	87.44	90.11	90.22	91.36	83.26	524.62	540.64	541.29	548.16	499.59
(20.04.92)	88.89	90.47	90.15	91.45	84.07	533.32	542.83	540.87	548.71	504.42
(27.04.92)	89.96	97.53	92.46	91.40	38.00	539.74	585.16	554.73	548.43	227.97
Mean	90.25	93.35	91.35	87.92	68.91	541.50	560.08	548.10	527.56	413.47
SE	0.81	1.02	0.66	2.62	6.95	4.84	6.09	3.99	15.73	41.68

temperature in the 2nd broodstock was significantly higher than in the 1st broodstock [$F_{1,523} = 221.63$, $p < 0.001$].

The salinity ranged from 25 to 32 psu during the course of the three spawnings in both 1992 broodstocks. In the 1st [$F_{4,265} = 0.006$, $p > 0.99$] and 2nd [$F_{4,245} = 0.01$, $p > 0.99$] broodstock, salinity between tanks was not significantly different (Table 3.43).

3.3.2.2. Filtration rate of clams

The percentage filtration (P) and the filtration rate (F) of the clams from the 1st and 2nd broodstock 1992 are shown in Table 3.44. From the results of ANOVA and t-test, in the 1st [$F_{4,30} = 16.37$, $p < 0.001$] and 2nd [$F_{4,30} = 8.62$, $p < 0.001$] broodstocks the percentage filtration for *T. philippinarum* was significantly higher with clams fed dry *T. suecica* mixed with live algae than the unfed broodstock. The filtration rate gave the same result. In general, the 1992 broodstocks showed higher filtration than the 1991 broodstocks, but clams were larger in 1992 and the volume of water flowing through the tanks was the same.

3.3.2.3. 1st broodstock conditioning 1992

I. <u>Initial broodstock</u>

The gross biochemical composition and condition indices of the initial broodstock are shown in Table 3.45 and Appendix 10. There were significant correlations between many of the measured variables. Values for the regression equations which described the relationships between the different parameters of the initial broodstock are given in Table 3.46. The percentage fatty acid content of broodstock meats is shown in Table

Table 3.45. Mean values for condition indices and biochemical composition of _T. philippinarum_. 1st broodstock 1992 conditioned from 22.01.92 to 17.03.92. Values are given for initial and 6, 7 and 8 weeks of conditioning. Protein values were calculated by difference. Standard error in parentheses. For abbreviations see Table 3.42. Afdwt = ash-free dry weight.

Broodstock diet	Live weight g	Length mm	Breadth mm	Meat dry weight, g	Condition index	% Afdwt	Lipid (% Afdwt)	Carbohydrate (% Afdwt)	Protein (% Afdwt)
Initial									
N=30	20.85 (± 0.67)	44.27 (± 0.44)	32.43 (±0.32)	0.95 (± 0.03)	99.72 (± 2.31)	79.77 (± 0.43)	9.76 (± 0.16)	17.11 (± 0.69)	73.13 (± 0.67)
6 weeks									
N=15									
Fsw	23.01 (± 1.11)	45.53 (± 0.69)	34.60 (± 0.65)	0.85 (± 0.05)	75.94 (± 3.58)	76.88 (± 0.81)	9.29 (±0.22)	10.97 (± 1.01)	79.75 (± 1.07)
Dry Tet	22.05 (± 0.74)	45.27 (± 0.41)	33.80 (± 0.43)	1.07 (± 0.04)	96.50 (± 3.19)	82.35 (± 0.43)	10.25 (± 0.24)	20.73 (± 1.14)	69.02 (± 1.06)
Dry Tet+Dun	21.37 (± 1.47)	44.60 (± 1.08)	33.00 (± 0.85)	0.84 (± 0.06)	83.36 (± 2.99)	85.67 (± 0.52)	9.60 (± 0.21)	16.29 (± 0.67)	74.11 (± 0.72)
Dry Tet+Ske	21.48 (± 0.82)	44.73 (± 0.37)	32.93 (± 0.34)	1.02 (± 0.03)	101.81 (± 3.23)	82.23 (± 0.68)	11.78 (± 0.32)	14.93 (± 0.30)	73.29 (± 0.45)
Dry Tet+Iso	22.11 (± 0.83)	45.27 (± 0.38)	34.13 (± 0.50)	1.16 (± 0.06)	105.52 (± 4.32)	82.99 (± 0.53)	10.63 (± 0.22)	23.23 (± 0.85)	66.15 (± 0.86)

(continued)

Table 3.45. (continued)

7 weeks									
N=15									
Fsw	21.26 (± 0.89)	44.67 (± 0.52)	32.60 (± 0.46)	0.74 (± 0.03)	66.99 (± 2.88)	76.10 (± 0.54)	8.01 (± 0.13)	6.52 (± 0.58)	85.47 (± 0.50)
Dry Tet	19.57 (± 0.78)	43.87 (± 0.58)	32.67 (± 0.44)	1.01 (± 0.04)	101.35 (± 3.02)	81.58 (± 0.57)	8.97 (± 0.41)	19.89 (± 0.92)	71.14 (± 0.69)
Dry Tet+Dun	21.19 (± 0.63)	45.07 (± 0.54)	33.00 (± 0.51)	0.98 (± 0.04)	93.09 (± 3.48)	81.48 (± 0.43)	12.10 (± 0.28)	16.17 (± 1.05)	71.73 (± 0.95)
Dry Tet+Ske	22.61 (± 0.72)	45.20 (± 0.43)	33.80 (± 0.40)	1.07 (± 0.04)	96.58 (± 3.81)	82.21 (± 0.45)	10.50 (± 0.24)	17.22 (± 0.70)	72.29 (± 0.57)
Dry Tet+Iso	23.86 (± 4.42)	46.60 (± 3.20)	34.33 (± 2.72)	1.37 (± 0.32)	116.16 (± 20.78)	85.08 (± 1.87)	10.04 (± 0.97)	20.95 (± 3.83)	69.01 (± 3.67)
8 weeks									
N=15									
Fsw	19.52 (± 0.92)	43.87 (± 0.55)	32.00 (±0.67)	0.69 (± 0.04)	69.04 (± 1.59)	78.26 (± 0.82)	9.20 (± 0.29)	8.32 (± 1.18)	82.48 (± 1.05)
Dry Tet	18.26 (± 0.46)	46.07 (± 0.55)	34.20 (± 0.44)	0.96 (± 0.04)	101.22 (±5.66)	87.56 (± 0.61)	9.87 (± 0.22)	19.43 (± 0.81)	70.70 (± 0.82)
Dry Tet+Dun	21.55 (± 0.90)	44.40 (± 0.69)	33.53 (± 0.62)	0.96 (± 0.05)	89.76 (± 2.54)	82.03 (± 0.79)	8.20 (± 0.36)	10.40 (± 0.81)	81.39 (± 0.83)
Dry Tet+Ske	21.08 (± 1.20)	43.87 (± 0.61)	32.20 (± 0.66)	1.05 (± 0.05)	104.83 (± 4.11)	83.49 (± 0.79)	11.34 (± 0.40)	15.60 (± 0.77)	73.06 (± 0.53)
Dry Tet+Iso	19.35 (± 0.92)	43.67 (± 0.76)	32.93 (± 0.64)	1.13 (± 0.06)	116.12 (± 3.26)	88.14 (± 0.39)	11.51 (± 0.47)	16.40 (± 0.76)	72.09 (± 0.70)

Table 3.46. Linear regression equations of the initial 1st broodstock conditioning 1992. For abbreviations and other details see Table 3.7.

1st broodstock 1992 (initial)

Parameters estimated (y x)	Intercept (A)	Slope (B)	r	d.f.	S
Live weight x length	-37.246	1.312	0.868	29	* * *
Live weight x breadth	-35.599	1.740	0.839	29	* * *
Live weight x meat dry weight	4.721	16.889	0.873	29	* * *
Length x breadth	6.449	1.166	0.850	29	* * *
Length x meat dry weight	34.944	9.762	0.763	29	* * *
Breadth x meat dry weight	25.834	6.910	0.741	29	* * *
Meat dry weight x condition inde:	0.128	0.008	0.551	29	* *
Meat dry weight x carbohydrate	0.416	0.032	0.629	29	* * *
Condition index x carbohydrate	67.561	1.879	0.566	29	* *

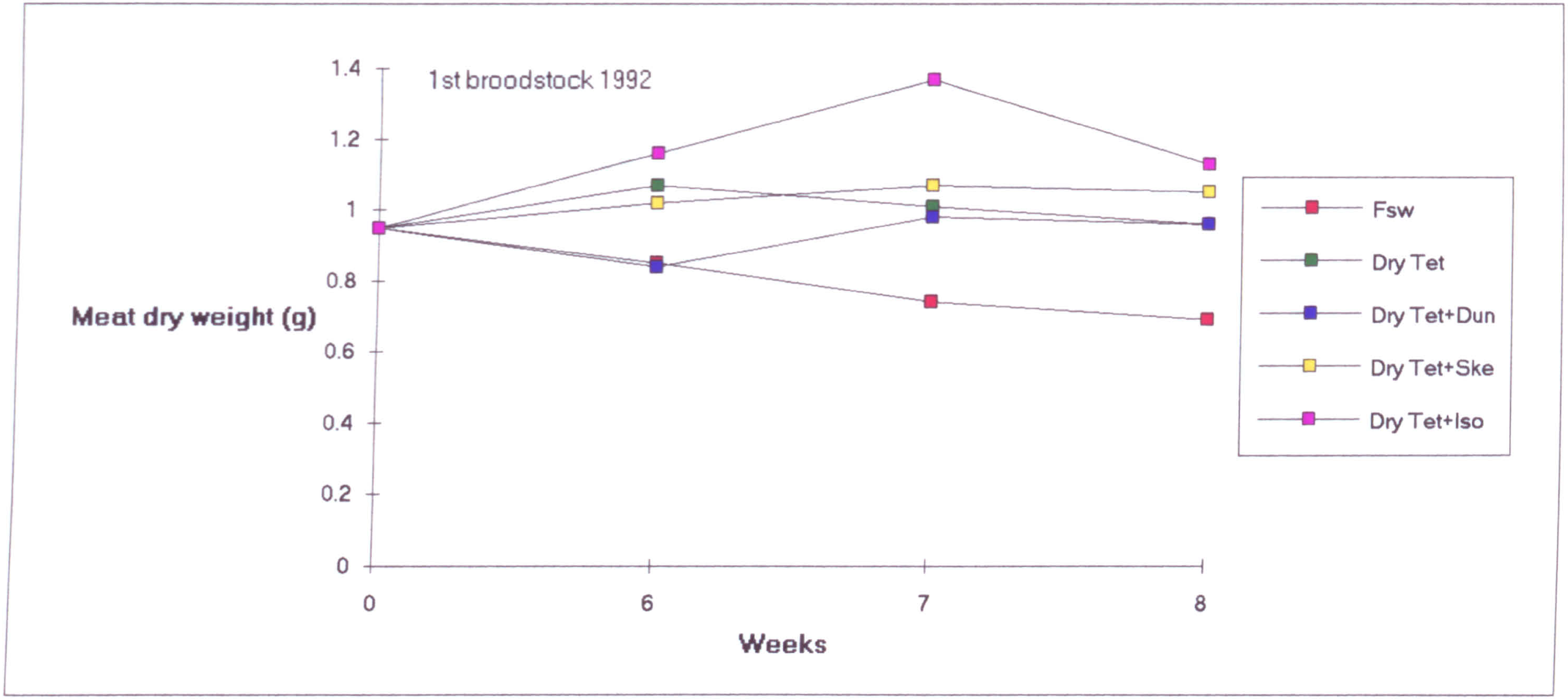


Figure 3.15. Mean meat dry weight of the initial T. philippinarum 1st broodstock 1992, and conditioned for 6 (spawning I), 7 (spawning II) and 8 (spawning III) weeks with different diets. For clarity, standard errors (given in Table 3.45) are not included in this Figure. For abbreviations see Table 3.42.

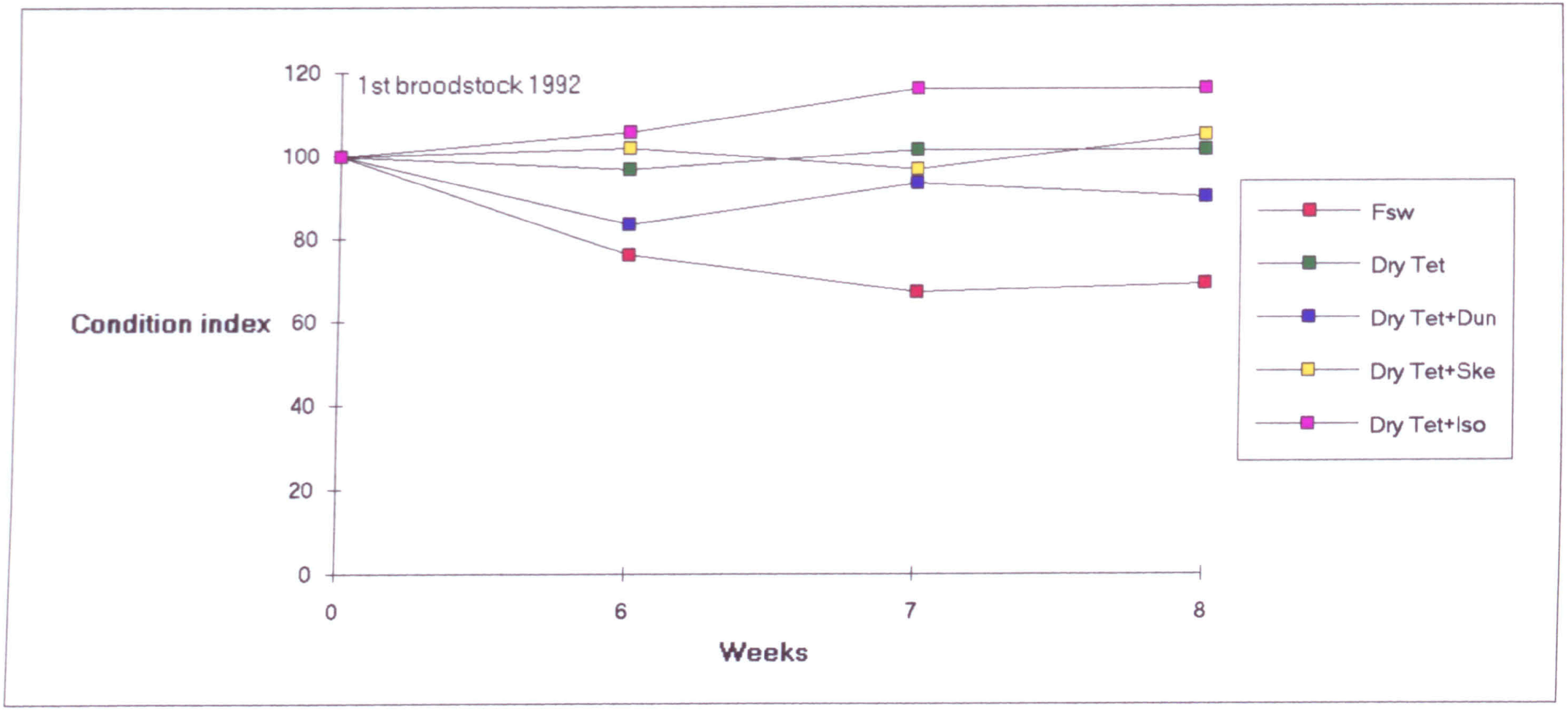


Figure 3.16. Mean condition index of the initial <u>T. philippinarum</u> 1st broodstock 1992, and conditioned for 6 (spawning I), 7 (spawning II) and 8 (spawning III) weeks with different diets. For clarity, standard errors (given in Table 3.45) are not included in this Figure. For abbreviations see Table 3.42.

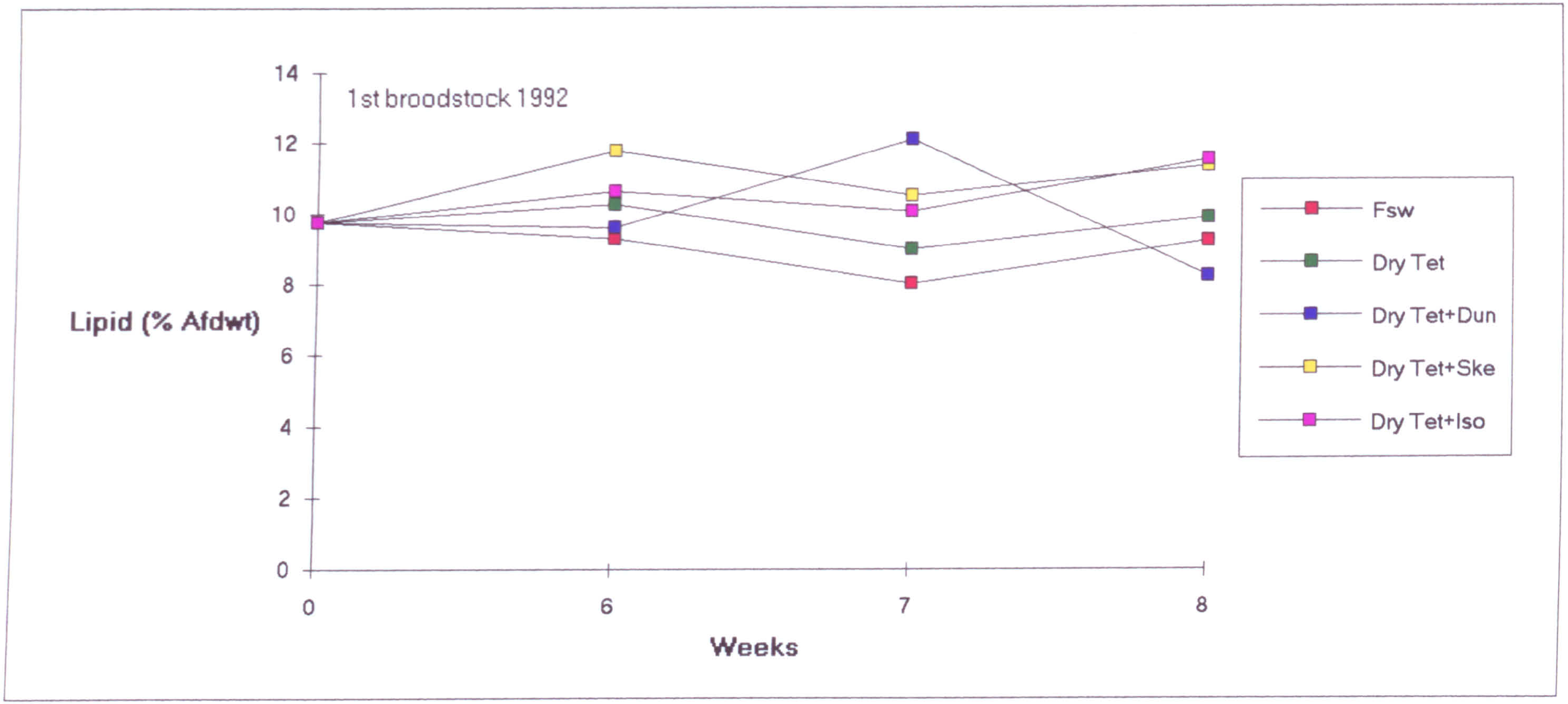


Figure 3.17. Mean lipid content of the initial T. philippinarum 1st broodstock 1992, and conditioned for 6 (spawning I), 7 (spawning II) and 8 (spawning III) weeks with different diets. For clarity, standard errors (given in Table 3.45) are not included in this Figure. For abbreviations see Table 3.42.

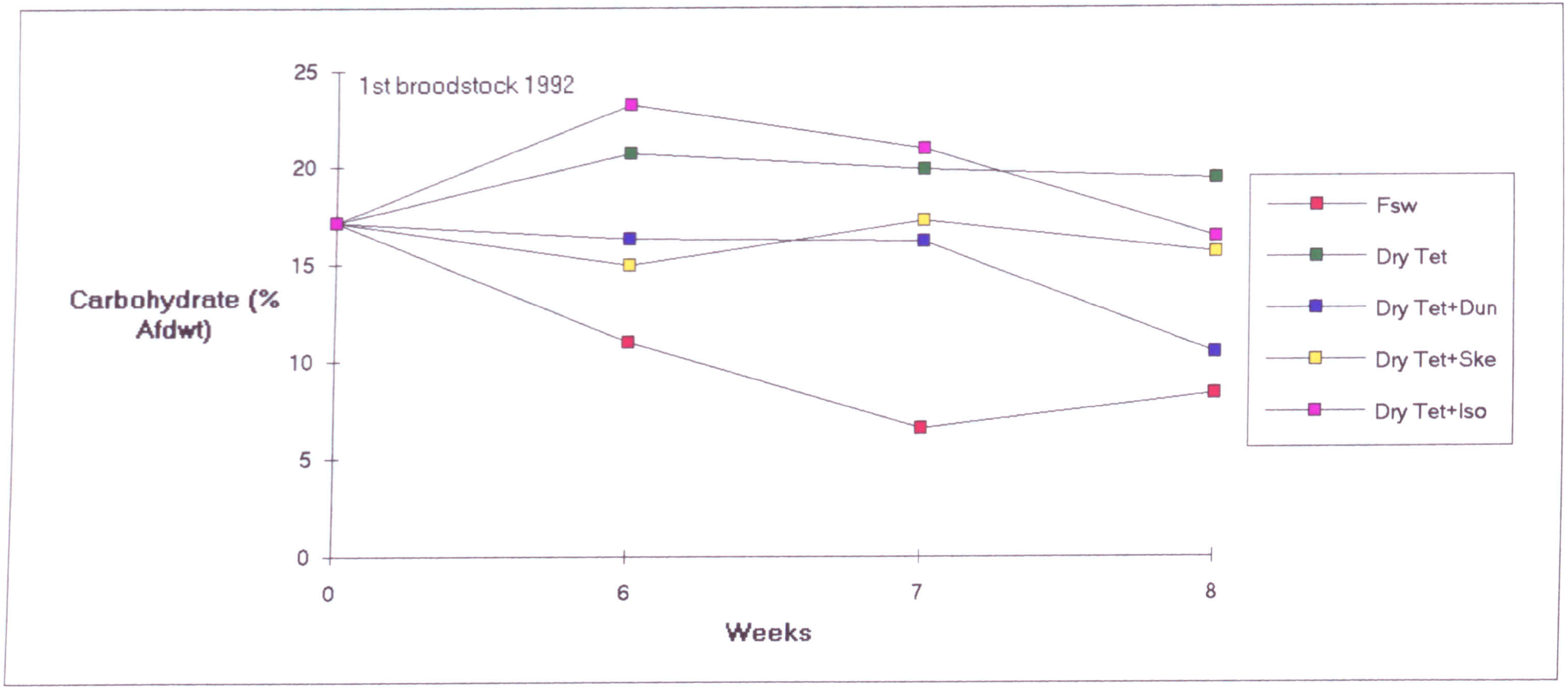


Figure 3.18. Mean carbohydrate content of the initial T. philippinarum 1st broodstock 1992, and conditioned for 6 (spawning I), 7 (spawning II) and 8 (spawning III) weeks with different diets. For clarity, standard errors (given in Table 3.45) are not included in this Figure. For abbreviations see Table 3.42.

Table 3.47. Fatty acid composition of T. philippinarum meats from the 1st broodstock 1992. Values are given for initial and broodstock fed on different diets. Values are percentages of total fatty acids. For abbreviations see Table 3.42.

1st broodstock 1992	**Initial**	**Spawning II**				
Diet		Dry Tet	Dry Tet+Dun	Dry Tet+Ske	Dry Tet+Iso	Fsw
Fatty acid						
14:0	9.92	6.50	6.58	8.02	5.63	10.09
16:0	27.66	27.84	26.43	27.89	25.27	24.82
18:0	9.97	8.53	9.31	8.93	7.09	13.21
16:1w7/9	5.57	8.91	7.71	10.32	5.15	8.38
18:1w9	11.43	15.39	10.32	8.89	13.50	10.14
18:1w7	4.03	5.24	5.55	7.06	5.74	4.34
20:1w9	6.28	9.80	7.30	7.40	7.94	9.92
18:2w6	0.76	1.09	1.98	0.87	2.52	0.77
18:3w3	1.07	0.98	4.67	0.88	2.01	0.62
18:4w3	3.00	1.92	1.66	1.99	5.78	1.08
20:2w6	2.15	2.05	3.75	2.13	2.83	3.05
20:3w3	0.15	0.24	1.51	0.32	0.47	0.20
20:4w6	2.16	1.58	2.19	2.09	1.72	2.67
20:5w3	4.97	3.29	3.85	7.35	3.77	2.99
22:5w3	2.00	1.12	1.20	1.21	0.91	1.63
22:6w3	8.88	5.52	6.00	4.65	9.67	6.08
Saturated	47.55	42.87	42.32	44.84	37.99	48.12
Monoenoic	27.31	39.34	30.88	33.67	32.33	32.78
Polyenoic	25.14	17.79	26.81	21.49	29.68	19.09

(continued)

Table 3.47 - (continued)

Spawning III

Dry Tet	Dry Tet+Dun	Dry Tet+Ske	Dry Tet+Iso	Fsw
6.34	11.20	8.92	6.83	7.28
31.31	24.86	34.36	33.37	28.42
8.32	13.63	8.12	7.09	11.66
10.73	8.63	13.41	7.23	10.30
14.42	9.37	8.69	12.40	12.60
5.53	4.26	7.23	5.68	5.02
9.73	9.19	7.33	8.24	10.27
1.13	0.63	0.68	2.00	0.71
0.96	0.36	0.57	1.52	0.71
1.60	1.06	1.11	3.21	0.81
2.06	3.17	1.61	2.72	2.18
0.28	0.00	0.16	0.51	0.15
1.17	2.79	1.20	1.40	1.69
2.11	2.91	3.97	1.80	2.50
0.63	1.61	0.59	0.60	1.08
3.69	6.33	2.07	5.41	4.62
45.97	49.69	51.40	47.29	47.36
40.41	31.45	36.66	33.55	38.19
13.63	18.86	11.96	19.17	14.45

3.47. The long chain polyunsaturated fatty acids in the initial meats, the highest values were found for 20:5w3 and 22:6w3.

As a summary of some general trends, all the meat dry weight, condition index, lipid and carbohydrate results, from the initial broodstock and after conditioning for 6, 7 and 8 weeks, are summarised in Figures 3.15 - 3.18.

II. Broodstock conditioning for 6 weeks

The gross biochemical composition and condition of the broodstock after 6 weeks of conditioning is presented in Table 3.45 and Appendix 10a. From the results of ANOVA, there was no significant difference in total live weight, length and breadth among the different diet groups (Table 3.48). However, there were highly significant differences $p < 0.001$ between the diet treatments for meat dry weight, condition index, ash-free dry weight, lipid and carbohydrate (Table 3.48a).

The meat weight and the condition index of clams in the dry *T. suecica+D. tertiolecta* and Fsw treatments were significantly lower than in the dry *T. suecica*, dry *T. suecica+S. costatum* and dry *T. suecica+I. galbana* treatments. This showed a loss of condition in the adults in the former treatments (Table 3.48a). Lipid levels were significantly higher in the clams fed dry *T. suecica+S. costatum*. The carbohydrates were significanty higher in clams fed dry *T. suecica* and dry *T. suecica+I. galbana*, reflecting the high percentage of carbohydrates in these algae. The unfed broodstock were significantly lower in carbohydrates than all the other fed groups.

There were some significant differences between male, female and non-spawning clams (Table 3.48b). The condition index in the males (99.98 ±2.96) was significantly higher than the females (89.94 ±2.68) or non-spawners (81.71 ±4.03). The dry meat

Table 3.48. Analysis of variance for variation in condition indices and biochemical composition of T. philippinarum conditioned for six week with different nutritional diets in the 1st broodstock 1992. For abbreviations and other details see Table 3.7.

1st broodstock 1992

6 weeks conditioning

Source of variation	d.f.	Sum of squares	Mean square	F Value	P	S
Live weight						
Treatment	4	2.55605E+01	6.39013E+00	0.40	0.8072	n.s.
Residual	70	1.11506E+03	1.59294E+01			
Total	74	1.14062E+03				
Length						
Treatment	4	9.38667E+00	2.34667E+00	0.37	0.8258	n.s.
Residual	70	4.38133E+02	6.25905E+00			
Total	74	4.47520E+02				
Breadth						
Treatment	4	3.12800E+01	7.82000E+00	1.55	0.1967	n.s.
Residual	70	3.52667E+02	5.03810E+00			
Total	74	3.83947E+02				
Meat dry weight						
Treatment	4	1.15809E+00	2.89523E-01	7.51	0.0001	***
Residual	70	2.69757E+00	3.85368E-02			
Total	74	3.85567E+00				

Table 3.48. (continued)

Condition index						
Treatment	4	9.44926E+03	2.36231E+03	12.92	0.0001	***
Residual	70	1.28017E+04	1.82881E+02			
Total	74	2.22510E+04				
Ash free dry weight						
Treatment	4	6.11940E+02	1.52985E+02	27.44	0.0001	***
Residual	70	3.90254E+02	5.57505E+00			
Total	74	1.00219E+03				
Lipid						
Treatment	4	5.72594E+01	1.43148E+01	16.22	0.0001	***
Residual	70	6.17722E+01	8.82460E-01			
Total	74	1.19032E+02				
Carbohydrate						
Treatment	4	1.40329E+03	3.50823E+02	32.58	0.0001	***
Residual	70	7.53667E+02	1.07667E+01			
Total	74	2.15696E+03				

Table 3.48a. Results of the t-test (LSD) for condition indices and biochemical composition of T. philippinarum conditioned for six weeks with different nutritional diets in the 1st broodstock 1992. For abbreviations see Table 3.7 and 3.42.

1st broodstock 1992

6 weeks conditioning

	Dry Tet	Dry Tet+Dun	Dry Tet+Ske	Dry Tet+Iso
Meat dry weight				
Dry Tet+Dun	*			
Dry Tet+Ske	n.s.	*		
Dry Tet+Iso	n.s.	*	n.s.	
Fsw	*	n.s.	*	*
Condition index				
Dry Tet+Dun	*			
Dry Tet+Ske	n.s.	*		
Dry Tet+Iso	n.s.	*	n.s.	
Fsw	*	n.s.	*	*
Ash free dry weight				
Dry Tet+Dun	*			
Dry Tet+Ske	n.s.	*		
Dry Tet+Iso	n.s.	*	n.s.	
Fsw	*	*	*	*
Lipid				
Dry Tet+Dun	n.s.			
Dry Tet+Ske	*	*		
Dry Tet+Iso	n.s.	*	*	
Fsw	*	n.s.	*	*
Carbohydrate				
Dry Tet+Dun	*			
Dry Tet+Ske	*	n.s.		
Dry Tet+Iso	*	*	*	
Fsw	*	*	*	*

Table 3.48b. Analysis of variance for variation by sex (male and female) and non-spawners of T. philippinarum conditioned for six weeks with different nutritional diets in the 1st broodstock 1992. For abbreviations and other details see Table 3.7.

1st broodstock 1992

6 weeks conditioning

Source of variation	d.f.	Sum of squares	Mean square	F Value	P	S
Condition index						
Treatment	2	4.03858E+03	2.01929E+03	7.98	0.0007	***
Residual	72	1.82124E+04	2.52950E+02			
Total	74	2.22510E+04				
Meat dry weight						
Treatment	2	3.94237E-01	1.97118E-01	4.10	0.0206	*
Residual	72	3.46143E+00	4.80754E-02			
Total	74	3.85567E+00				

weight in the males (1.07 ±0.04g) was also significantly higher than in females (0.93 ±0.04g). Non-spawners (0.92 ±0.06g) were not significantly different from females.

III. Broodstock conditioning for 7 weeks

The biochemical composition and condition of the broodstock after 7 weeks of conditioning are presented in Table 3.45 and Appendix 10b. From the results of ANOVA (Table 3.49), there were significant differences among the groups in total live weight ($p = 0.0097$) and, more unexpectedly, length ($P = 0.0323$). This may have been related to some accidental bias in the selection of clams from the tanks before spawning. There were significant differences due to diet treatment in all the other parameters with the exception of shell breadth.

Least Significant Difference ("t"-tests) showed where there were significant differences among the groups in the meat dry weight, condition index and ash-free dry weight (organic weight) (Table 3.49a). Generally dry *T. suecica+I. galbana* fed broodstock had significantly higher values and the unfed broodstock (Fsw) had significantly lower values for those parameters compared with the other diets. Broodstock fed on dry *T. suecica*, dry *T. suecica+D. tertiolecta* or *D. tertiolecta+S. costatum* were similar in composition.

All broodstock that were fed a supplement of live algae had significantly higher lipid levels than the broodstock that were given only dry *T. suecica* or that were unfed. The clams that were fed with dry *T. suecica* contained significantly less lipid than dry *T.suecica+S. costatum* and dry *T. suecica+I. galbana* and this was probably a reflection of the low level of lipid (3.0% ash-free dry weight) in the dry alga. The unfed broodstock had a significantly lower lipid level compared with the other diet treatments.

Table 3.49. Analysis of variance for variation in condition indices and biochemical composition of T. philippinarum conditioned for 7 weeks with different nutritional diets in the 1st broodstock 1992. For abbreviations and other details see Table 3.7.

1st broodstock 1992

7 weeks conditioning

Source of variation	d.f.	Sum of squares	Mean square	F Value	P	S
Live weight						
Treatment	4	1.57437E+02	3.93592E+01	3.62	0.0097	**
Residual	70	7.61092E+02	1.08727E+01			
Total	74	9.18529E+02				
Length						
Treatment	4	5.95200E+01	1.48800E+01	2.80	0.0323	*
Residual	70	3.72000E+02	5.31429E+00			
Total	74	4.31520E+02				
Breadth						
Treatment	4	3.44533E+01	8.61333E+00	2.18	0.0802	n.s.
Residual	70	2.76667E+02	3.95238E+00			
Total	74	3.11120E+02				
Meat dry weight						
Treatment	4	3.01429E+00	7.53571E-01	18.70	0.0001	***
Residual	70	2.82079E+00	4.02970E-02			
Total	74	5.83507E+00				

Table 3.49. (continued)

Condition index						
Treatment	4	1.91771E+04	4.79428E+03	21.96	0.0001	* * *
Residual	70	1.52858E+04	2.18368E+02			
Total	74	3.44629E+04				
Ash free dry weight						
Treatment	4	6.34225E+02	1.58556E+02	42.39	0.0001	* * *
Residual	70	2.61837E+02	3.74054E+00			
Total	74	8.96062E+02				
Lipid						
Treatment	4	1.44786E+02	3.61965E+01	31.42	0.0001	* * *
Residual	70	8.06289E+01	1.15184E+00			
Total	74	2.25415E+02				
Carbohydrate						
Treatment	4	1.96321E+03	4.90803E+02	43.41	0.0001	* * *
Residual	70	7.91411E+02	1.13059E+01			
Total	74	2.75462E+03				

Table 3.49a. Results of the t-test (LSD) for condition indices and biochemical composition of T. philippinarum conditioned for 7 weeks with different nutritional diets in the 1st broodstock 1992.
For abbreviations see Table 3.7 and 3.42.

1st broodstock 1992

7 weeks conditioning

	Dry Tet	Dry Tet+Dun	Dry Tet+Ske	Dry Tet+Iso
Live weight				
Dry Tet+Dun	n.s.			
Dry Tet+Ske	*	n.s.		
Dry Tet+Iso	*	*	n.s.	
Fsw	n.s.	n.s.	n.s.	
Length				
Dry Tet+Dun	n.s.			
Dry Tet+Ske	n.s.	n.s.		
Dry Tet+Iso	*	n.s.	n.s.	
Fsw	n.s.	n.s.	n.s.	*
Meat dry weight				
Dry Tet+Dun	n.s.			
Dry Tet+Ske	n.s.	n.s.		
Dry Tet+Iso	*	*	*	
Fsw	*	*	*	*
Condition index				
Dry Tet+Dun	n.s.			
Dry Tet+Ske	n.s.	n.s.		
Dry Tet+Iso	*	*	*	
Fsw	*	*	*	*
Ash free dry weight				
Dry Tet+Dun	n.s.			
Dry Tet+Ske	n.s.	n.s.		
Dry Tet+Iso	*	*	*	
Fsw	*	*	*	*

Table 3.49a (continued)

Lipid				
Dry Tet+Dun	*			
Dry Tet+Ske	*	*		
Dry Tet+Iso	*	*	n.s.	
Fsw	*	*	*	*
Carbohydrate				
Dry Tet+Dun	*			
Dry Tet+Ske	*	n.s.		
Dry Tet+Iso	n.s.	*	*	
Fsw	*	*	*	*

Table 3.49b. Analysis of variance for variation by sex (male and female) and non-spawners of T. philippinarum conditioned for 7 weeks with different nutritional diets in the 1st broodstock 1992. For abbreviations and other details see Table 3.7.

1st broodstock 1992

7 weeks conditioning

Source of variation	d.f.	Sum of squares	Mean square	F Value	P	S
Meat dry weight						
Treatment	2	1.10493E+00	5.52465E-01	8.41	0.0005	**
Residual	72	4.73014E+00	6.56964E-02			
Total	74	5.83507E+00				
Condition index						
Treatment	2	5.22875E+03	2.61437E+03	6.44	0.0027	**
Residual	72	2.92341E+04	4.06030E+02			
Total	74	3.44629E+04				

As had occurred at week 6, the clams fed dry diets, with or without live algae supplements, had significantly higher carbohydrate levels than the unfed broodstock. In the unfed treatment, carbohydrate decreased from 11.0% to 6.5% between weeks 6 and week 7. As with the previous (6 weeks) sample, clams fed on dry *T. suecica* and dry *T. suecica+I. galbana* had significantly higher carbohydrate levels than treatments where supplements of live *D. tertiolecta* or *S. costatum* were provided.

The ANOVA for variation by males, females and non-spawners is given in Table 3.49b. Male clams had significantly higher values than females for dry meat weight [$F_{2,72} = 8.41$, $p < 0.001$] and condition index [$F_{2,72} = 6.44$, $p < 0.01$] for the same reasons given in section 3.2.2.3. Non-spawners were not significantly different from females.

The percentage fatty acid content of broodstock meats after spawning is shown in Table 3.47. Meats of dry *T. suecica+D. tertiolecta* fed broodstock were high in 20:5w3 and 22:6w3. This result is interesting since this live alga had negligible levels of 20C and no 22C (Table 3.2 and 3.41). Also in the Fsw treatment broodstock meats contained similar levels of 22:6w3 too and there was no 20:5w3 or 22:6w3 in the particles (Table 3.71).

IV. <u>Broodstock conditioning for 8 weeks</u>

The biochemical composition and condition of the broodstock conditioned for 8 weeks with different diets are presented in Table 3.45 and Appendix 10c. As with week 6, there were no significant differences in total live weight or length and breadth among the treatments. In all the other parameters, there were significant differences among the diet treatments (Table 3.50). Meat dry weight, condition index (with the exception of dry *T. suecica+D. tertiolecta)* and ash free dry weight were higher than in the initial broodstock. The exception was the unfed group where these variables were much

Table 3.50. Analysis of variance for variation in condition indices and biochemical composition of T. philippinarum conditioned for 8 weeks with different nutritional diets in the 1st broodstock 1992. For abbreviations and other details see Table 3.7.

1st broodstock 1992

8 weeks conditioning

Source of variation	d.f.	Sum of squares	Mean square	F Value	P	S
Live weight						
Treatment	4	1.08349E+02	2.70871E+01	2.17	0.0807	n.s.
Residual	70	8.71919E+02	1.24560E+01			
Total	74	9.80267E+02				
Length						
Treatment	4	5.82133E+01	1.45533E+01	2.40	0.0585	n.s.
Residual	70	4.25333E+02	6.07619E+00			
Total	74	4.83547E+02				
Breadth						
Treatment	4	5.04800E+01	1.26200E+01	2.27	0.0704	n.s.
Residual	70	3.89467E+02	5.56381E+00			
Total	74	4.39947E+02				
Meat dry weight						
Treatment	4	1.64810E+00	4.12025E-01	11.43	0.0001	***
Residual	70	2.52391E+00	3.60558E-02			
Total	74	4.17201E+00				

Table 3.50. (continued)

Condition index						
Treatment	4	1.91316E+04	4.78291E+03	23.25	0.0001	* * *
Residual	70	1.43983E+04	2.05690E+02			
Total	74	3.35299E+04				
Ash free dry weight						
Treatment	4	1.00227E+03	2.50567E+02	34.21	0.0001	* * *
Residual	70	5.12744E+02	7.32492E+00			
Total	74	1.51501E+03				
Lipid						
Treatment	4	1.19519E+02	2.98796E+01	15.41	0.0001	* * *
Residual	70	1.35719E+02	1.93885E+00			
Total	74	2.55238E+02				
Carbohydrate						
Treatment	4	1.24536E+03	3.11339E+02	26.87	0.0001	* * *
Residual	70	8.11008E+02	1.15858E+01			
Total	74	2.05637E+03				

Table 3.50a. Results of the t-test (LSD) for condition indices and biochemical composition of T. philippinarum conditioned for 8 weeks with different nutritional diets in the 1st broodstock 1992.
For abbreviations see Table 3.7 and 3.42.

1st broodstock 1992

8 weeks conditioning

	Dry Tet	Dry Tet+Dun	Dry Tet+Ske	Dry Tet+Iso
Meat dry weight				
Dry Tet+Dun	n.s.			
Dry Tet+Ske	n.s.	n.s.		
Dry Tet+Iso	*	*	n.s.	
Fsw	*	*	*	*
Condition index				
Dry Tet+Dun	*			
Dry Tet+Ske	n.s.	*		
Dry Tet+Iso	*	*	*	
Fsw	*	*	*	*
Ash free dry weight				
Dry Tet+Dun	*			
Dry Tet+Ske	*	n.s.		
Dry Tet+Iso	n.s.	*	*	
Fsw	*	*	*	*
Lipid				
Dry Tet+Dun	*			
Dry Tet+Ske	*	*		
Dry Tet+Iso	*	*	n.s.	
Fsw	n.s.	n.s.	*	*
Carbohydrate				
Dry Tet+Dun	*			
Dry Tet+Ske	*	*		
Dry Tet+Iso	*	*	n.s.	
Fsw	*	n.s.	*	*

Table 3.50b. Analysis of variance for variation by sex (male and female) and non-spawners of T. philippinarum conditioned for 8 weeks with different nutritional diets in the 1st broodstock 1992. For abbreviations and other details see Table 3.7.

1st broodstock 1992

8 weeks conditioning

Source of variation	d.f.	Sum of squares	Mean square	F Value	P	S
Ash free dry weight						
Treatment	2	3.381115E+02	1.69058E+02	10.34	0.0001	* * *
Residual	72	1.17690E+03	1.63458E+01			
Total	74	1.51501E+03				

lower. The condition index in the clams fed dry *T. suecica+I. galbana* was significantly higher than the other diets (Table 3.50a).

The broodstock fed with a mixed diet of dry *T. suecica* plus *S. costatum* or *I. galbana* had significantly higher lipid levels than the other groups reflecting in part the composition of the live algae. The carbohydrate content of clams fed dry *T. suecica* was significantly higher compared with clams on the other diets and remained more or less constant during weeks 6, 7 and 8 of conditioning. In week 8, clams fed a dry and mixed diet of live algae had significantly lower quantities of carbohydrate than they had in weeks 6 and 7.

The ash free dry weight of male clams was significantly higher than the ash-free dry weight of the females [$F_{2,72} = 10.34$, $p < 0.001$] and for the same reasons given already in section 3.2.2.3. Non-spawners were not significantly different from female clams. (Table 3.50b).

The percentage fatty acid content of broodstock meats after spawning is shown in Table 3.47. As in week 7, the long chain polyunsaturated fatty acids in meats in all treatment are high in 20:5w3 and 22:6w3. Generally there was a decrease in 20:5w3 and 22:6w3 from weeks 7 (spawning II) to 8 (spawning III).

3.3.2.4. 2nd broodstock conditioning 1992

I. Initial broodstock

The gross biochemical composition and condition indices of the initial broodstock are shown in Table 3.51 and Appendix 11. There were significant relationships between many of the measured variables eg the live weight and the shell length, breadth and meat dry weight of the clams. Values for the regression equations which described the

Table 3.51. Mean values for condition indices and biochemical composition of T. philippinarum. 2nd broodstock 1992 conditioned from 16.03.92 to 05.05.92. Values are given for initial and 5, 6 and 7 weeks of conditioned. Protein values were calculated by difference. Standard error in parentheses. For abbreviations see Table 3.42. Afdwt = ash-free dry weight

Broodstock diet	Live weight g	Length mm	Breadth mm	Meat dry weight, g	Condition index	% Afdwt	Lipid (% Afdwt)	Carbohydrate (% Afdwt)	Protein (% Afdwt)
Initial									
N=30	15.81 (± 0.46)	40.30 (± 0.37)	29.90 (± 0.34)	0.74 (± 0.03)	98.12 (± 2.13)	82.02 (± 0.68)	11.01(± 0.43)	15.97 (± 0.93)	73.01 (± 1.02)
5 weeks									
N=15									
Fsw	18.36 (± 0.53)	42.87 (± 0.49)	32.13 (± 0.40)	0.70 (± 0.03)	76.79 (± 2.59)	78.56 (± 0.60)	9.73 (± 0.19)	9.26 (± 0.83)	81.01 (± 0.75)
Dry Tet	18.77 (± 0.66)	42.67 (± 0.36)	31.87 (± 0.40)	0.88 (± 0.04)	92.79 (± 4.52)	81.14 (± 0.82)	8.12 (± 0.25)	18.92 (± 0.80)	72.96 (± 0.73)
Dry Tet+Dun	16.41 (± 0.61)	40.73 (± 0.62)	30.53 (± 0.42)	0.75 (± 0.03)	102.04 (± 6.78)	81.19 (± 0.74)	8.74 (± 0.28)	17.03 (± 0.68)	74.23 (± 0.69)
Dry Tet+Ske	17.69 (± 0.66)	41.93 (± 0.56)	31.13 (± 0.35)	0.86 (± 0.04)	101.08 (± 4.27)	82.57 (± 1.01)	9.20 (± 0.23)	17.09 (± 0.69)	73.71 (± 0.60)
Dry Tet+Iso	18.11 (± 0.75)	41.93 (± 0.49)	31.13 (± 0.29)	1.03 (± 0.04)	118.09 (± 2.83)	83.99 (± 0.48)	8.91 (± 0.26)	21.78 (± 0.75)	69.31 (± 0.66)

(continued)

Table 3.51. (continued)

6 weeks									
N=15									
Fsw	16.87 (± 0.68)	41.00 (± 0.59)	30.27 (± 0.55)	0.60 (± 0.03)	72.83 (± 3.96)	77.79 (± 0.68)	8.89 (± 0.29)	9.50 (± 0.90)	70.41 (± 0.81)
Dry Tet	16.61 (± 0.56)	40.53 (± 0.54)	29.93 (± 0.46)	0.75 (± 0.03)	92.40 (± 3.93)	82.37 (± 1.09)	9.24 (± 0.30)	16.19 (± 0.97)	74.58 (± 0.97)
Dry Tet+Dun	16.03 (± 0.65)	40.80 (± 0.45)	30.53 (± 0.41)	0.79 (± 0.04)	103.47 (± 3.02)	83.48 (± 0.80)	9.97 (± 0.49)	16.39 (± 0.72)	73.64 (± 0.79)
Dry Tet+Ske	16.57 (± 0.67)	40.73 (± 0.65)	30.27 (± 0.49)	0.81 (± 0.04)	107.09 (± 6.28)	82.11 (± 0.50)	10.01 (± 0.34)	16.63 (± 0.85)	73.36 (± 0.90)
Dry Tet+Iso	17.13 (± 0.88)	40.27 (± 0.71)	29.93 (± 0.59)	0.92 (± 0.05)	112.73 (± 3.08)	82.32 (± 1.19)	10.54 (± 0.37)	21.19 (± 0.73)	68.27 (± 0.79)
7 weeks									
N=15									
Fsw	15.81 (± 0.64)	39.60 (± 0.55)	29.27 (± 0.45)	0.54 (± 0.02)	71.39 (± 2.06)	75.26 (± 1.04)	10.07 (± 0.22)	8.55 (± 0.85)	81.38 (± 0.75)
Dry Tet	15.93 (± 0.69)	41.07 (± 0.57)	30.27 (± 0.36)	0.73 (± 0.03)	94.56 (± 5.00)	81.78 (± 0.82)	9.86 (± 0.39)	20.12 (± 0.83)	70.49 (± 1.22)
Dry Tet+Dun	17.11 (± 0.92)	41.33 (± 0.75)	31.07 (± 0.54)	0.74 (± 0.03)	87.73 (± 3.03)	79.85 (± 0.86)	9.22 (± 0.18)	14.51 (± 0.99)	77.37 (± 1.62)
Dry Tet+Ske	16.42 (± 0.47)	40.80 (± 0.56)	30.93 (± 0.38)	0.83 (± 0.03)	99.01 (± 3.04)	81.54 (± 0.54)	9.59 (± 0.25)	16.39 (± 0.63)	74.01 (± 0.71)
Dry Tet+Iso	16.01 (± 0.64)	40.73 (± 0.54)	29.93 (± 0.36)	0.86 (± 0.04)	115.30 (± 5.03)	83.55 (± 0.67)	10.09 (± 0.28)	20.04 (± 0.80)	71.90 (± 1.64)

Table 3.52. Linear regression equations of the initial 2nd broodstock conditioning 1992. For abbreviation and other details see Table 3.7.

2nd broodstock 1992 (initial)

Parameters estimated (y x)	Intercept (A)	Slope (B)	r	d.f.	S
Live weight x length	-23.642	0.979	0.778	29	* * *
Live weight x breadth	-15.198	1.037	0.76	29	* * *
Live weight x meat dry weight	4.731	14.905	0.821	29	* * *
Live weight x carbohydrate	12.349	0.217	0.432	29	*
Length x breadth	13.904	0.883	0.814	29	* * *
Length x meat dry weight	32.348	10.698	0.742	29	* * *
Length x carbohydrate	37.550	0.172	0.432	29	*
Breadth x meat dry weight	23.384	8.766	0.659	29	* * *
Meat dry weight x condition inde.	0.289	0.005	0.386	29	*
Meat dry weight x carbohydrate	0.508	0.015	0.532	29	*
Condition index x carbohydrate	84.404	0.859	0.373	29	*

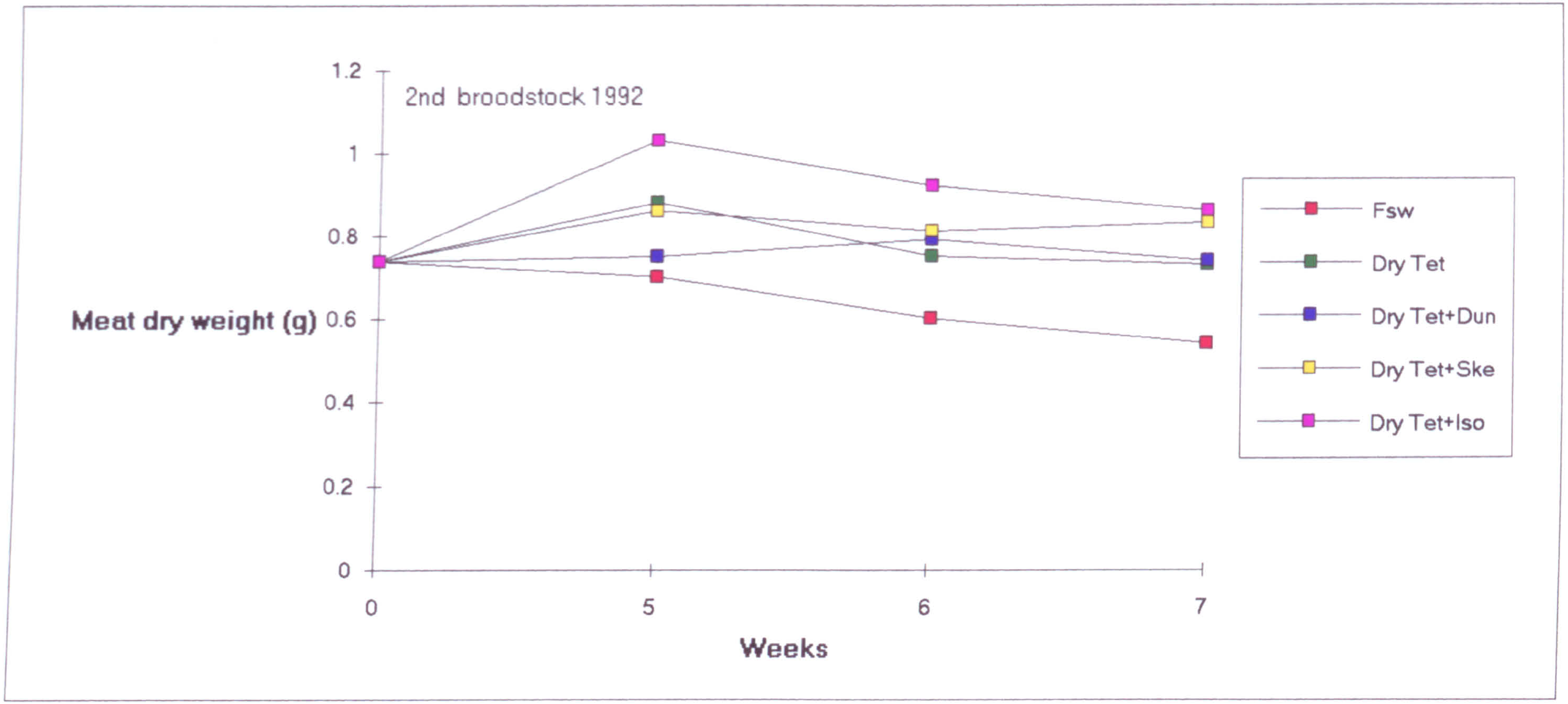


Figure 3.19. Mean meat dry weight of the initial <u>T. philippinarum</u> 2nd broodstock 1992, and conditioned for 5 (spawning I), 6 (spawning II) and 7 (spawning III) weeks with different diets. For clarity, standard errors (given in Table 3.51) are not included in this Figure. For abbreviations see Table 3.42.

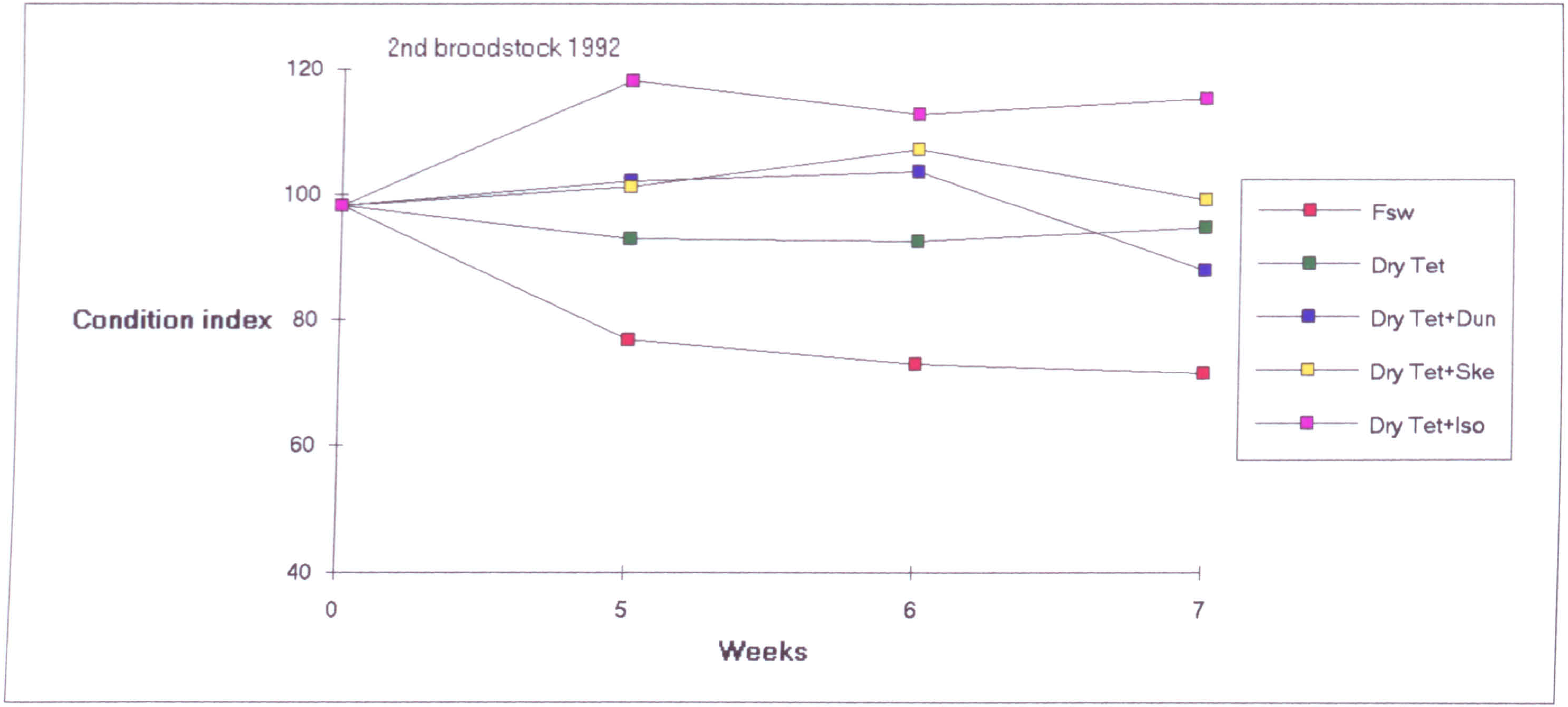


Figure 3.20. Mean condition index of the initial T. philippinarum 2nd broodstock 1992, and conditioned for 5 (spawning I), 6 (spawning II) and 7 (spawning III) weeks with different diets. For clarity, standard errors (given in Table 3.51) are not included in this Figure. For abbreviations see Table 3.42.

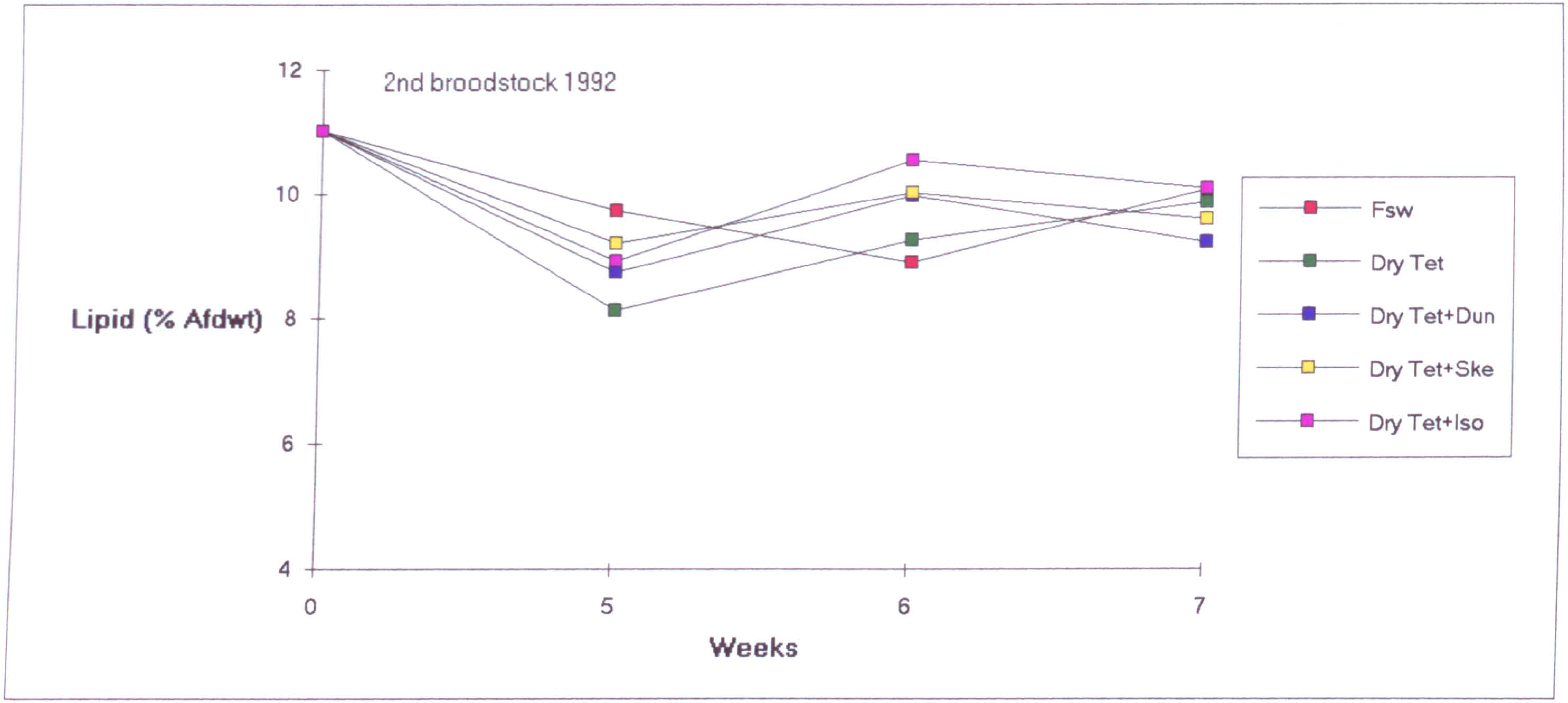


Figure 3.21. Mean lipid content of the initial T. philippinarum 2nd broodstock 1992, and conditioned for 5 (spawning I), 6 (spawning II) and 7 (spawning III) weeks with different diets. For clarity, standard errors (given in Table 3.51) are not included in this Figure. For abbreviations see Table 3.42.

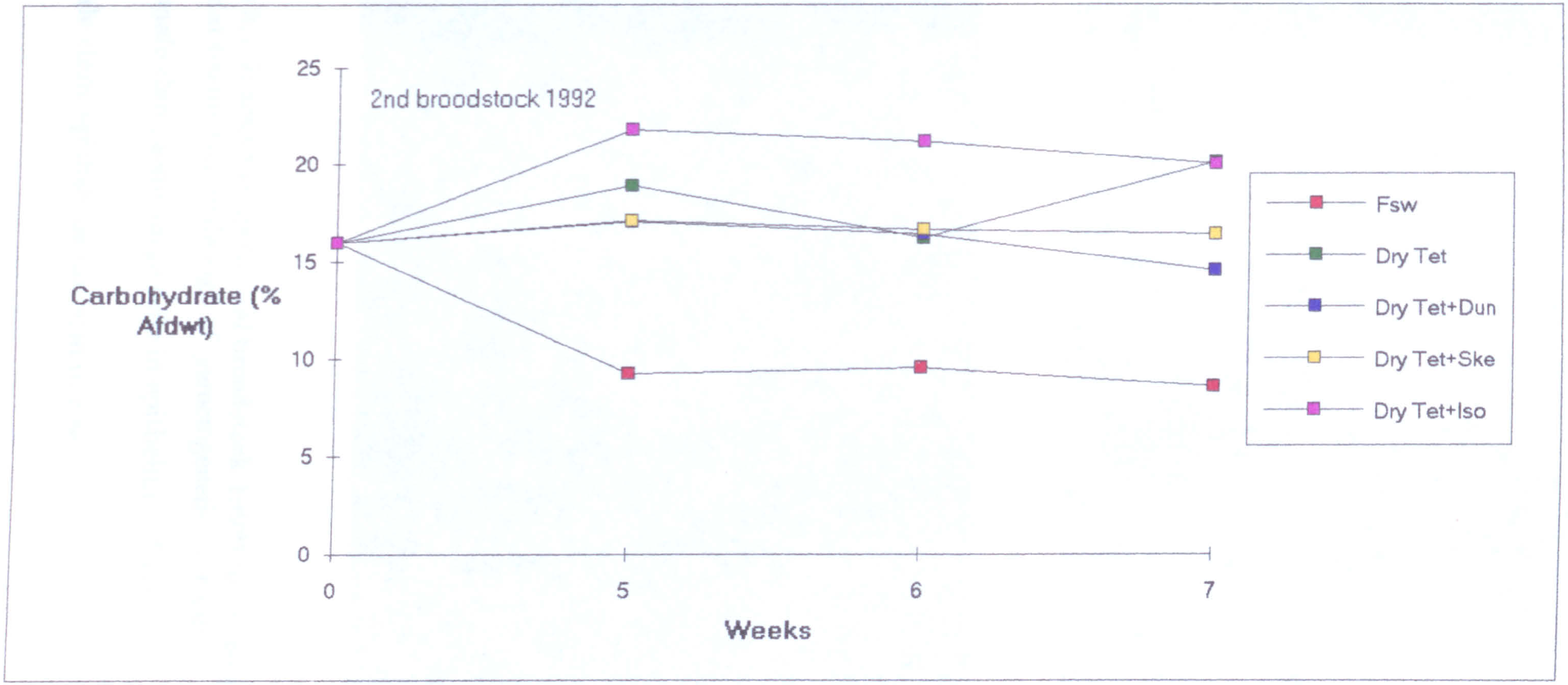
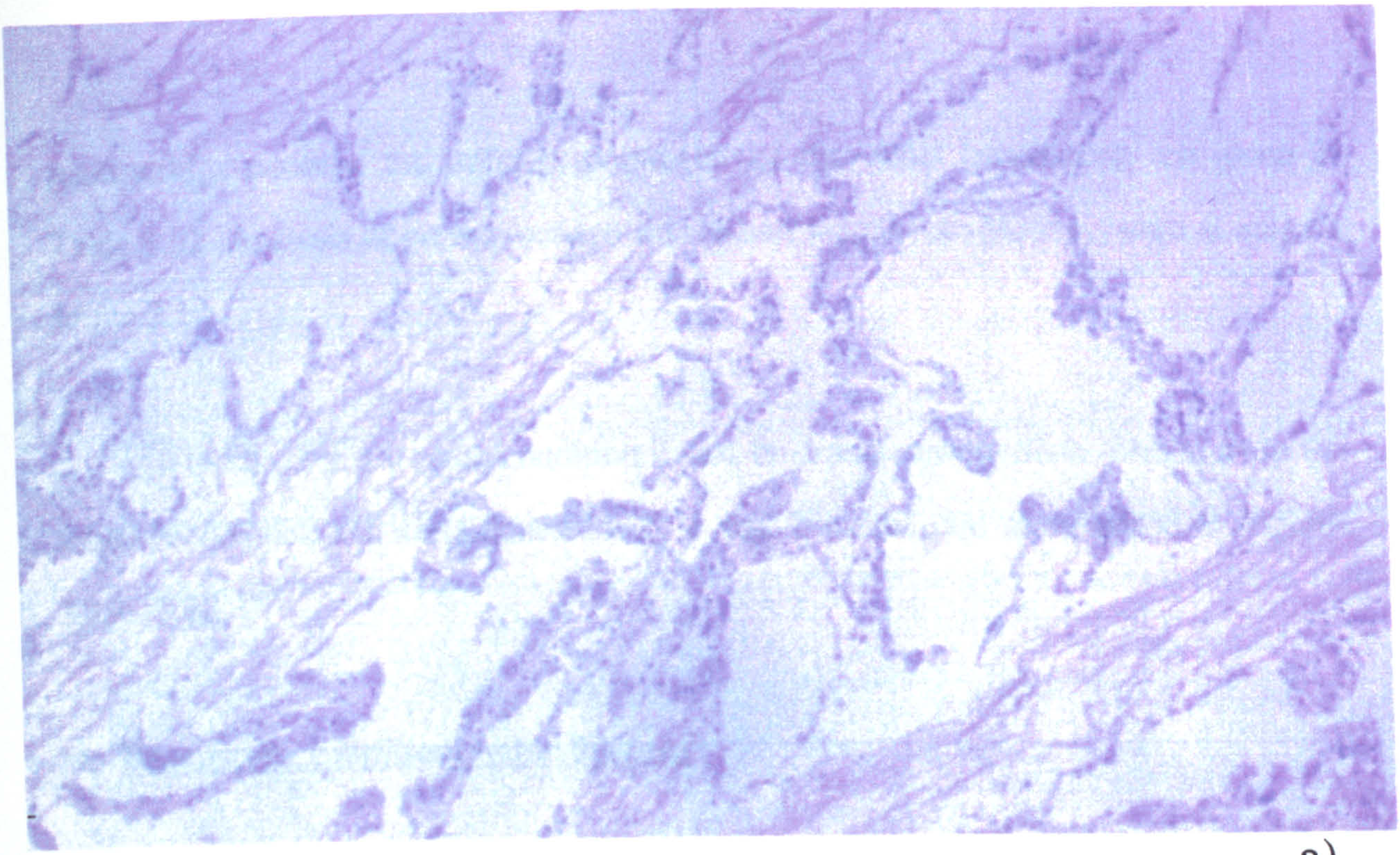
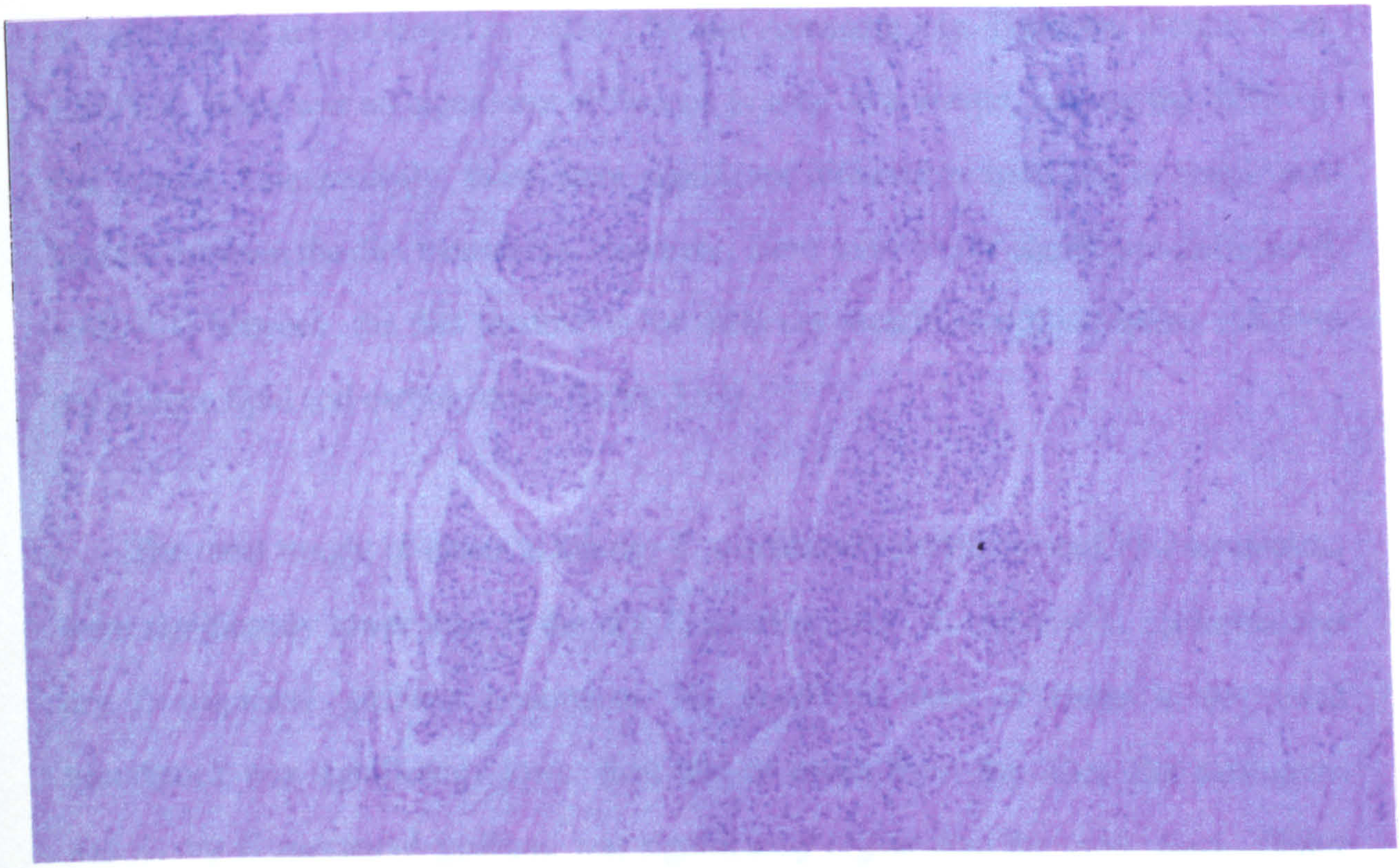


Figure 3.22. Mean carbohydrate content of the initial T. philippinarum 2nd broodstock 1992, and conditioned for 5 (spawning I), 6 (spawning II) and 7 (spawning III) weeks with different diets. For clarity, standard errors (given in Table 3.51) are not included in this Figure. For abbreviations see Table 3.42.



a)



b)

Plate 3.1. Inactive stage (initial broodstock 1992) clam showing reduced gonadal tissue with little sign of gametogenetic activity.

a) Female clam, some large cells in epithelium of ovary.

b) Male clam, epithelium uniform in size.

relationships between the different parameter of the initial broodstock are given in Table 3.52. Analysis of gonad sections showed an inactive stage, they were at stage I (Plate 3.1).

All the meat dry weight, condition index, lipid and carbohydrate results, from the initial broodstock and conditioned for 5, 6 and 7 weeks, are summarised in Figures 3.19 - 3.22.

II. Broodstock conditioning for 5 weeks

The gross biochemical composition and condition of the broodstock after 5 weeks of conditioning are presented in Table 3.51 and Appendix 11a. From the results of an ANOVA, there was no significant difference in total live weight, among the different diet groups. Unexpectedly, there were significant differences ($p<0.05$) in length and breadth between the diet treatments. However, there were highly significant differences ($p<0.001$) between the diet treatments for meat dry weight, condition index, ash-free dry weight, lipid and carbohydrate (Table 3.53).

The meat weight of clams in the dry *T. suecica+D. tertiolecta* and Fsw treatments were significantly lower than in the dry *T. suecica*, dry *T. suecica+S. costatum* and dry *T. suecica+I. galbana* treatments. The condition index of clams in the unfed broodstock was significantly lower than that of clams from the other diet treatments and in dry *T. suecica+I. galbana* was significantly higher than the other diets. (Table 3.53a). Lipid was significantly higher in the clams fed dry *T. suecica+S. costatum* or maintained in the filtered sea water. The carbohydrates were significanty higher in clams fed dry*T. suecica+I. galbana*, reflecting the high percentage of carbohydrates in these algae. The unfed broodstock had significantly less carbohydrate than all the fed groups.

Table 3.53. Analysis of variance for variation in condition indices and biochemical composition of T. philippinarum conditioned for five weeks with different nutritional diets in the 2nd broodstock 1992. For abbreviations and other details see Table 3.7.

2nd broodstock 1992

5 weeks conditioning

Source of variation	d.f.	Sum of squares	Mean square	F Value	P	S
Live weight						
Treatment	4	4.89781E+01	1.22445E+01	1.96	0.1102	n.s.
Residual	70	4.37321E+02	6.24745E+00			
Total	74	4.86299E+02				
Length						
Treatment	4	4.20800E+01	1.05200E+01	2.67	0.0392	*
Residual	70	2.75867E+02	3.94095E+00			
Total	74	3.17947E+02				
Breadth						
Treatment	4	2.46133E+01	6.15333E+00	2.90	0.028	*
Residual	70	1.48667E+02	2.12381E+00			
Total	74	1.73280E+02				
Meat dry weight						
Treatment	4	9.65368E-01	2.41342E-01	11.89	0.0001	***
Residual	70	1.42116E+00	2.03023E-02			
Total	74	2.38653E+00				

Table 3.53. (continued)

Condition index						
Treatment	4	1.35928E+04	3.39819E+03	11.40	0.0001	* * *
Residual	70	2.08720E+04	2.98171E+02			
Total	74	3.44648E+04				
Ash free dry weight						
Treatment	4	2.42666E+02	6.06664E+01	7.12	0.0001	* * *
Residual	70	5.96160E+02	8.51658E+00			
Total	74	8.38826E+02				
Lipid						
Treatment	4	2.12069E+01	5.30172E+00	6.00	0.0003	* * *?
Residual	70	6.18655E+01	8.83793E-01			
Total	74	8.30724E+01				
Carbohydrate						
Treatment	4	1.29488E+03	3.23721E+02	38.23	0.0001	* * *
Residual	70	5.92730E+02	8.46757E+00			
Total	74	1.88761E+03				

Table 3.53a. Results of the t-test (LSD) for condition indices and biochemical composition of T. philippinarum conditioned for 5 weeks with different nutritional diets in the 2nd broodstock 1992. For abbreviations see Table 3.7 and 3.42.

2nd broodstock 1992

5 weeks conditioning

	Dry Tet	Dry Tet+Dun	Dry Tet+Ske	Dry Tet+Iso
Length				
Dry Tet+Dun	*			
Dry Tet+Ske	n.s.	n.s.		
Dry Tet+Iso	n.s.	n.s.	n.s.	
Fsw	n.s.	*	n.s.	n.s.
Breadth				
Dry Tet+Dun	*			
Dry Tet+Ske	n.s.	n.s.		
Dry Tet+Iso	n.s.	n.s.	n.s.	
Fsw	n.s.	*	n.s.	n.s.
Meat dry weight				
Dry Tet+Dun	*			
Dry Tet+Ske	n.s.	*		
Dry Tet+Iso	*	*	*	
Fsw	*	n.s.	*	*
Condition index				
Dry Tet+Dun	n.s.			
Dry Tet+Ske	n.s.	n.s.		
Dry Tet+Iso	*	*	*	
Fsw	*	*	*	*
Ash free dry weight				
Dry Tet+Dun	n.s.			
Dry Tet+Ske	n.s.	n.s.		
Dry Tet+Iso	*	*	n.s.	
Fsw	*	*	*	*

Table 3.53a (continued)

Lipid				
Dry Tet+Dun	n.s.			
Dry Tet+Ske	*	n.s.		
Dry Tet+Iso	*	n.s.	n.s.	
Fsw	*	*	n.s.	*
Carbohydrate				
Dry Tet+Dun	n.s.			
Dry Tet+Ske	n.s.	n.s.		
Dry Tet+Iso	*	*	*	
Fsw	*	*	*	*

III. Broodstock conditioning for 6 weeks

The biochemical composition and condition of the broodstock after 6 weeks of conditioning are presented in Table 3.51 and Appendix 11b. From the results of ANOVA (Table 3.54), there was no significant difference in total live weight, length and breadth among the different diet groups. There were significant differences due to diet treatment in all the other parameters particularly ($p<0.001$) for meat dry weight, condition index and carbohydrate.

Least Significant Difference ("t"-tests) showed where there were significant differences among the groups in the meat dry weight (Table 3.54a). Dry *T. suecica+I. galbana* fed broodstock had significantly higher values and the unfed broodstock (Fsw) had significantly lower values compared with the other diets. Broodstock fed on dry *T. suecica*, dry *T. suecica+D. tertiolecta* or *D. tertiolecta+ S. costatum* were similar in composition. The condition index of clams in the Fsw treatments was significantly lower than the other treatments.

All broodstock that were fed a supplement of live algae had significantly higher lipid levels than the broodstocks that were unfed. As had occurred at week 5, the clams fed dry diets, with and without live algae supplements, had significantly higher carbohydrate levels than the unfed broodstock. As with the previous (5 weeks) sample, clams fed on dry *T. suecica+I. galbana* had a significantly higher carbohydrate level than treatments where supplements of live *D. tertiolecta* or *S. costatum* were provided.

IV. Broodstock conditioning for 7 weeks

The biochemical composition and condition of the broodstock conditioned for 7 weeks with different diets are presented in Table 3.51 and Appendix 11c. As with week

Table 3.54. Analysis of variance for variation in condition indices and biochemical composition of T. philippinarum conditioned for six weeks with different nutritional diets in the 2nd broodstock 1992. For abbreviations and other details see Table 3.7.

2nd broodstock 1992

6 weeks conditioning

Source of variation	d.f.	Sum of squares	Mean square	F Value	P	S
Live weight						
Treatment	4	1.01613E+01	2.54033E+00	0.35	0.8426	n.s.
Residual	70	5.06839E+02	7.24055E+00			
Total	74	5.17000E+02				
Length						
Treatment	4	4.66667E+00	1.16667E+00	0.22	0.9267	n.s.
Residual	70	3.72000E+02	5.31429E+00			
Total	74	3.76667E+02				
Breadth						
Treatment	4	3.92000E+00	9.80000E-01	0.26	0.9048	n.s.
Residual	70	2.67467E+02	3.82095E+00			
Total	74	2.71387E+02				
Meat dry weight						
Treatment	4	8.09525E-01	2.02381E-01	10.42	0.0001	***
Residual	70	1.35989E+00	1.94271E-02			
Total	74	2.16942E+00				

Table 3.54. (continued)

<u>Condition index</u>						
Treatment	4	1.49108E+04	3.72770E+03	13.93	0.0001	***
Residual	70	1.87302E+04	2.67574E+02			
Total	74	3.36410E+04				
<u>Ash free dry weight</u>						
Treatment	4	2.91780E+02	7.29451E+01	6.13	0.0003	**
Residual	70	8.32701E+02	1.18957E+01			
Total	74	1.12448E+03				
<u>Lipid</u>						
Treatment	4	2.60024E+01	6.50059E+00	3.28	0.016	*
Residual	70	1.38792E+02	1.98274E+00			
Total	74	1.64794E+02				
<u>Carbohydrate</u>						
Treatment	4	1.04645E+03	2.61612E+02	24.71	0.0001	***
Residual	70	7.41051E+02	1.05864E+01			
Total	74	1.78750E+03				

Table 3.54a. Results of the t-tests (LSD) for condition indices and biochemical composition of T. philippinarum conditioned for 6 weeks with different nutritional diets in the 2nd broodstock 1992. For abbreviations see Table 3.7 and 3.42.

2nd broodstock 1992

6 weeks conditioning

	Dry Tet	Dry Tet+Dun	Dry Tet+Ske	Dry Tet+Iso
Meat dry weight				
Dry Tet+Dun	n.s.			
Dry Tet+Ske	n.s.	n.s.		
Dry Tet+Iso	*	*	*	
Fsw	*	*	*	*
Condition index				
Dry Tet+Dun	n.s.			
Dry Tet+Ske	*	n.s.		
Dry Tet+Iso	*	n.s.	n.s.	
Fsw	*	*	*	*
Ash free dry weight				
Dry Tet+Dun	n.s.			
Dry Tet+Ske	n.s.	n.s.		
Dry Tet+Iso	n.s.	n.s.	n.s.	
Fsw	*	*	*	*
Lipid				
Dry Tet+Dun	n.s.			
Dry Tet+Ske	n.s.	n.s.		
Dry Tet+Iso	*	n.s.	n.s.	
Fsw	n.s.	*	*	*
Carbohydrate				
Dry Tet+Dun	n.s.			
Dry Tet+Ske	n.s.	n.s.		
Dry Tet+Iso	*	*	*	
Fsw	*	*	*	*

Table 3.55. Analysis of variance for variation in condition indices and biochemical composition of T. philippinarum conditioned for seven weeks with different nutritional diets in the 2nd broodstock 1992. For abbreviations and other details see Table 3.7.

2nd broodstock 1992

7 weeks conditioning

Source of variation	d.f.	Sum of squares	Mean square	F Value	P	S
Live weight						
Treatment	4	1.66915E+01	4.17287E+00	0.59	0.6719	n.s.
Residual	70	4.96173E+02	7.08819E+00			
Total	74	5.12865E+02				
Length						
Treatment	4	2.63467E+01	6.58667E+00	1.22	0.3092	n.s.
Residual	70	3.77200E+02	5.38857E+00			
Total	74	4.03547E+02				
Breadth						
Treatment	4	3.28800E+01	8.22000E+00	3.05	0.0224	*
Residual	70	1.88667E+02	2.69524E+00			
Total	74	2.21547E+02				
Meat dry weight						
Treatment	4	9.69432E-01	2.42358E-01	15.42	0.0001	***
Residual	70	1.10015E+00	1.57164E-02			
Total	74	2.06958E+00				

Table 3.55. (continued)

Condition index						
Treatment	4	1.54295E+04	3.85737E+03	17.63	0.0001	* * *
Residual	70	1.53149E+04	2.18784E+02			
Total	74	3.07444E+04				
Ash free dry weight						
Treatment	4	5.97907E+02	1.49477E+02	15.37	0.0001	* * *
Residual	70	6.80644E+02	9.72349E+00			
Total	74	1.27855E+03				
Lipid						
Treatment	4	7.98270E+00	1.99567E+00	1.78	0.1427	n.s.
Residual	70	7.851116E+01	1.12159E+00			
Total	74	8.64943E+01				
Carbohydrate						
Treatment	4	1.36775E+03	3.41938E+02	33.11	0.0001	* * *
Residual	70	7.22844E+02	1.03263E+01			
Total	74	2.09060E+03				

Table 3.55a. Results of the t-test (LSD) for condition indices and biochemical composition of T. philippinarum conditioned for 7 weeks with different nutritional diets in the 2nd broodstock 1992. For abbreviations see Table 3.7 and 3.42.

2nd broodstock 1992

7 weeks conditioning

	Dry Tet	Dry Tet+Dun	Dry Tet+Ske	Dry Tet+Iso
Breadth				
Dry Tet+Dun	n.s.			
Dry Tet+Ske	n.s.	n.s.		
Dry Tet+Iso	n.s.	n.s.	n.s.	
Fsw	n.s.	*	*	n.s.
Meat dry weight				
Dry Tet+Dun	n.s.			
Dry Tet+Ske	*	*		
Dry Tet+Iso	*	*	n.s.	
Fsw	*	*	*	*
Condition index				
Dry Tet+Dun	n.s.			
Dry Tet+Ske	n.s.	*		
Dry Tet+Iso	*	*	*	
Fsw	*	*	*	*
Ash free dry weight				
Dry Tet+Dun	n.s.			
Dry Tet+Ske	n.s.	n.s.		
Dry Tet+Iso	n.s.	*	n.s.	
Fsw	*	*	*	*
Carbohydrate				
Dry Tet+Dun	*			
Dry Tet+Ske	*	n.s.		
Dry Tet+Iso	n.s.	*	*	
Fsw	*	*	*	*

Table 3.55b. Analysis of variance for variation by sex (male and female) and non-spawners of T. philippinarum conditioned for seven weeks with different nutritional diets in the 2nd broodstock 1992. For abbreviations and other details see Table 3.7.

2nd broodstock 1992

7 weeks conditioning

Source of variation	d.f.	Sum of squares	Mean square	F Value	P	S
Meat dry weight						
Treatment	2	5.95438E-01	2.97719E-01	14.54	0.0001	* * *
Residual	72	1.47414E+00	2.04742E-02			
Total	74	2.06958E+00				
Lipid						
Treatment	2	1.14428E+01	5.72140053	5.49	0.006	* *
Residual	72	7.50515E+01	1.04238247			
Total	74	8.64943E+01				

6, there were no significant differences in total live weight and length of clams among the treatments. In all of the other parameters except lipid (not significant) and breadth ($p<0.05$), there were highly significant differences ($p<0.001$) among the diet treatments (Table 3.55).

The meat dry weight and ash-free dry weight in the unfed broodstock were significantly lower than the other diet groups. The condition index in the clams fed dry *T. suecica+I. galbana* was significantly higher than the other diet treatments. The unfed treatment had significantly lower values compared with the other diets (Table 3.55a). The carbohydrate content of clams fed dry *T. suecica* and dry *T. suecica+I. galbana* was significantly higher than clams on the other diets. The carbohydrate content of clams on this last diet remained more or less constant during weeks 5, 6 and 7 of conditioning.

There were some significant differences in dry meat weight [$F_{2,72}$ =14.54, $p<0.001$] and lipid content [$F_{2,72}$ = 5.49, $p<0.01$] between male, female and non-spawning clams (Table 3.55b). The dry meat weight in the males (0.88±0.05g) was significantly higher than in the females (0.76±0.02g) and non-spawners (0.64±0.03g). Female (10.21±0.21%) clams had significantly higher levels of lipid than males (9.50±0.19%). Non-spawners were not significantly different from males (9.33±0.20%).

3.3.3. Fecundity

1st broodstock 1992

Attempts to spawn the clams were made 3 times (spawning I, II, III) after 6 (508 D^{O}), 7 (567 D^{O}) and 8 (655 D^{O}) weeks of conditioning in the 1st broodstock (Table

Table 3.56. Fecundity of T. philippinarum after being conditioned for 6 (Spawning I), 7 (Spawning II) and 8 (Spawning III) weeks on a mixed diet of 70% dry T. suecica and 30% live diet. Standard error in parentheses. For abbreviations see Table 3.42.

1st Broodstock conditioning - 1992

Spawning	Treatment	Nº of clams induced	Total nº spawned	Female/male spawning	Total nº of eggs produced (in millions)	Eggs mean per female (in millions)
I	Fsw	15	2	1/1	0.295	0.295
	Dry Tet	15	14	5/9	15.312	3.062 (± 0.56)
	Dry Tet + Dun	15	15	7/8	8.717	1.245 (± 0.24)
	Dry Tet + Ske	15	14	6/8	10.678	1.780 (± 0.46)
	Dry Tet + Iso	15	13	5/8	12.694	2.539 (± 0.45)
II	Fsw	15	0	0/0	0	0
	Dry Tet	15	9	5/4	6.956	1.391 (± 0.35)
	Dry Tet + Dun	15	11	7/4	17.596	2.514 (± 0.49)
	Dry Tet + Ske	15	7	4/3	18.219	4.555 (± 0.54)
	Dry Tet + Iso	15	9	2/7	12.995	6.498 (± 0.82)
III	Fsw	15	0	0/0	0	0
	Dry Tet	15	8	1/7	1.283	1.283
	Dry Tet + Dun	15	7	3/4	10.526	3.509 (± 0.65)
	Dry Tet + Ske	15	4	1/3	5.453	5.453
	Dry Tet + Iso	15	7	1/6	3.148	3.148

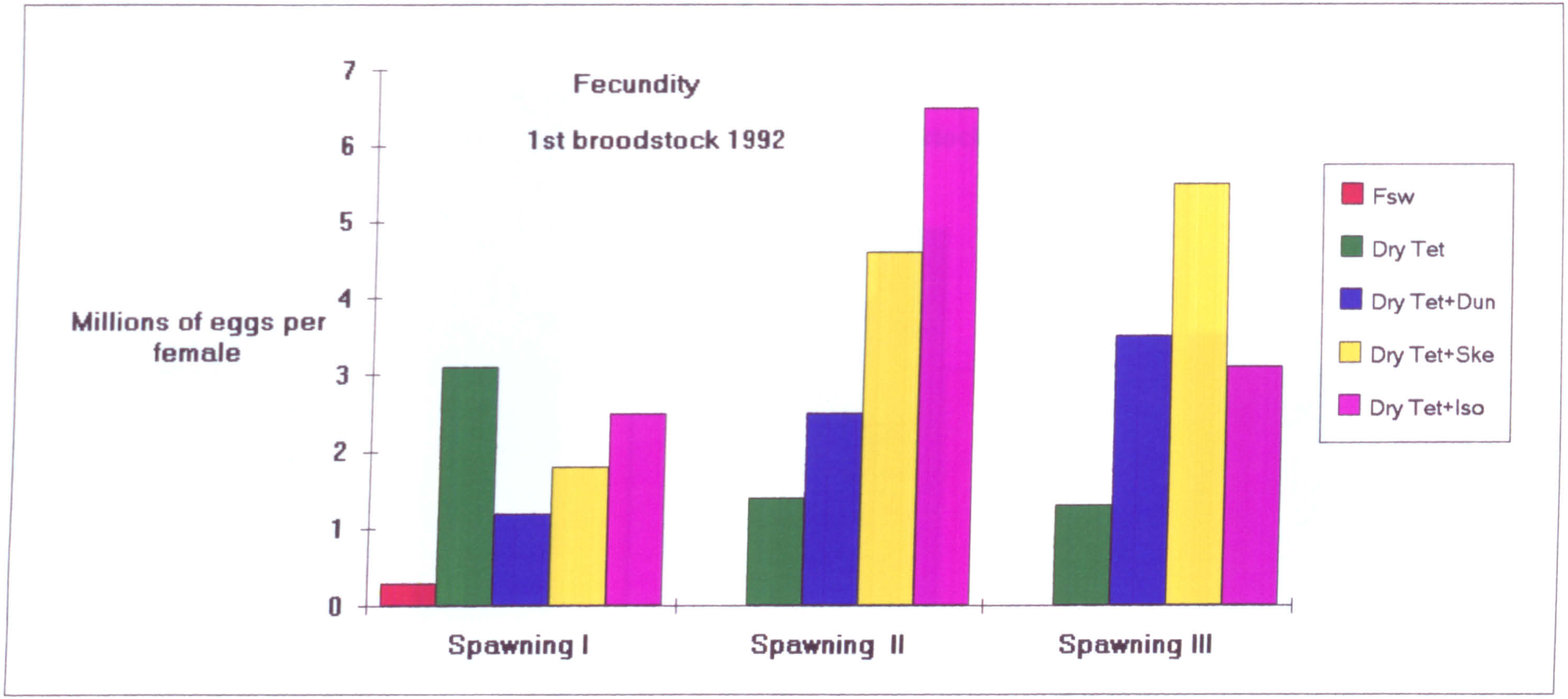


Figure 3.23. Mean fecundity of T. philippinarum in relation to diet after being conditioned for 6 (spawning I), 7(spawning II) and 8 (spawning III) weeks (1st broodstock 1992). For clarity, standard errors (given in Table 3.56) are not included in this Figure. For abbreviations see Table 3.42.

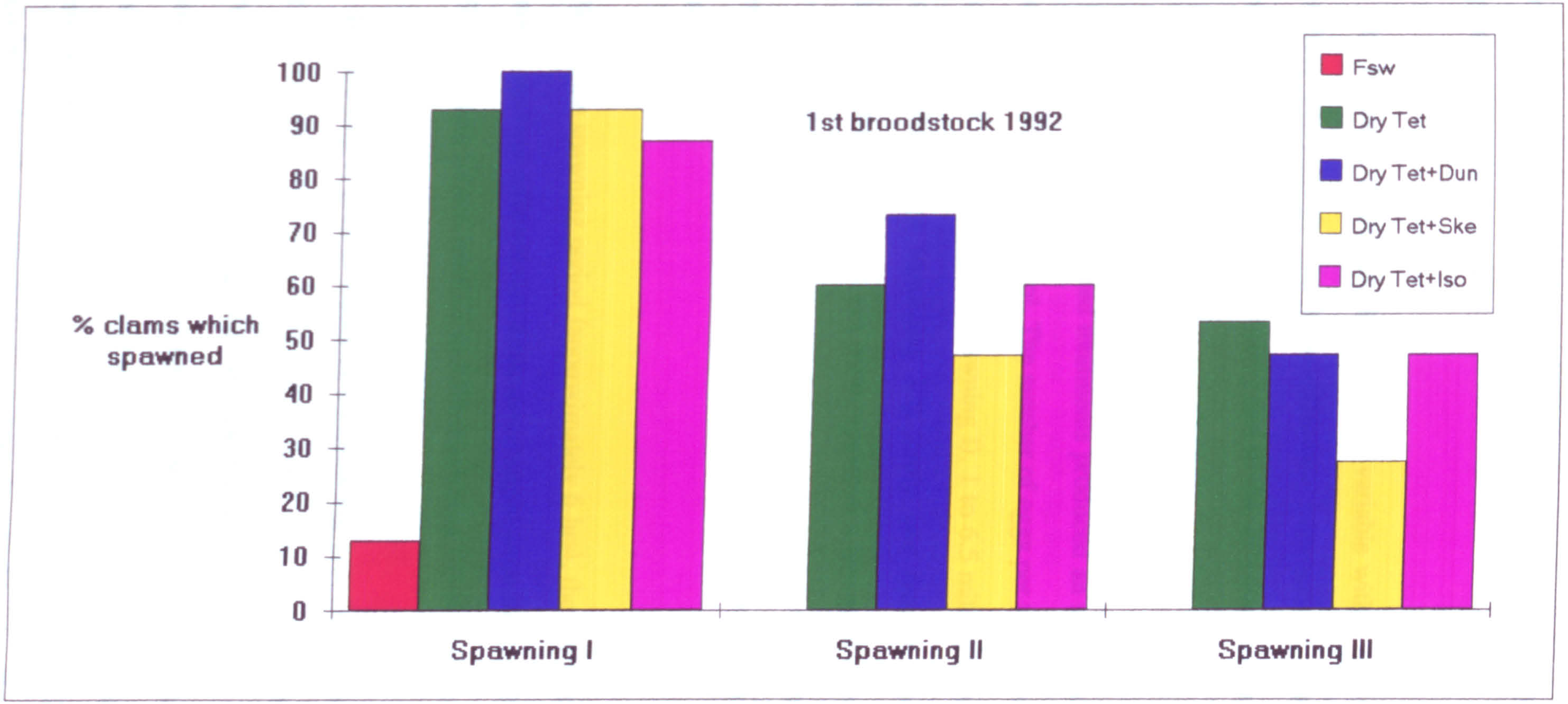


Figure 3.24. Percentage of T. philippinarum which spawned in relation to diet after being conditioned for 6 (spawning I), 7 (spawning II) and 8 (spawning III) weeks (1st broodstock 1992). For abbreviations see Table 3.42.

3.56). Spawning condition was reached within 508 to 655 degree days of conditioning (Figure 3.23). Successful spawning was dependent on the quality of the algae diet during gametogenesis.

The fecundity of the females was very variable within and between treatments (Appendices 12,12a,12b). The large difference between the total number of eggs produced by this broodstock was partly due to the difference in the number of females in the sample and to the different fecundities of the females.

In all spawnings, all the fed treatments produced an appreciable number of eggs compared with the unfed control. The number of eggs per female in the fed treatments ranged from 1.3 to 3.1 million (spawning I), 1 to 6.5 million (spawning II) and from 1.3 to 5.5 million eggs (spawning III). Only one female clam from the unfed broodstock in the sand-filtered sea water spawned a few eggs after 6 weeks of warm water conditioning (Figure 3.23), which indicated some, but minimal, nutritional value of particles in the water. This was also shown by a loss of condition in the adults in this treatment.

Within the spawning period (approximately 6 hrs), the percentage of clams which spawned in the four different diet treatments ranged from 87 to 100% (spawning I), 47 to 73% (spawning II) and from 27 to 53% (spawning III) (Figure 3.24).

In spawning I, after 6 weeks of conditioning broodstock diet had a significant effect on fecundity [$F_{3,19} = 3.73$, $p < 0.05$]. The highest number of eggs was observed in the dry *T. suecica* treatment and the dry *T. suecica+I. galbana* treatment. It would appear that broodstock conditioned with dry *T. suecica* for a short period, 6 weeks, can produce high numbers of eggs compared with week 7 or week 8. Statistical analysis of the number of eggs per female (Appendices 12 - 12b) showed dry *T. suecica* brooodstock produced more eggs per female than dry *T. suecica+S.costatum*

Table 3.57. Analysis of variance for variation in number of eggs per female of T. philippinarum conditioned with different nutritional diets from the 1st broodstock 1992. For abbreviations and other details see Table 3.7.

1st broodstock 1992

6 weeks conditioning

Source of variation	d.f.	Sum of squares	Mean square	F Value	P	S
Treatment	3	1.12899E+13	3.76332E+12	3.73	0.0290	*
Residual	19	1.91588E+13	1.00836E+12			
Total	22	3.04487E+13				

7 weeks conditioning

Source of variation	DF	Sum of squares	Mean square	F Value	P	S
Treatment	3	4.85605E+13	1.61868E+13	13.16	0.0002	***
Residual	14	1.72186E+13	1.22990E+12			
Total	17	6.57792E+13				

Table 3.57a. Results of the t-test (LSD) for the number of eggs per female of T. philippinarum conditioned with different nutritional diets from the 1st broodstock 1992. For abbreviations and other details see Table 3.7 and 3.42.

1st broodstock 1992

6 weeks conditioning

	Dry Tet	Dry Tet+Dun	Dry Tet+Ske
Dry Tet+Dun	*		
Dry Tet+Ske	*	n.s.	
Dry Tet+Iso	n.s.	*	n.s.

7 weeks conditioning

	Dry Tet	Dry Tet+Dun	Dry Tet+Ske
Dry Tet+Dun	n.s.		
Dry Tet+Ske	*	*	
Dry Tet+Iso	*	*	n.s.

and dry *T. suecica+D. tertiolecta* broodstocks (Table 3.57, 3.57a). There was no significant difference between dry *T. suecica* and the dry *T. suecica+I. galbana* treatment. Also there was a significant difference between dry *T. suecica+I. galbana* and dry *T. suecica+D. tertiolecta.*

After 7 weeks the effect of the diet was even more significant [$F_{3,14} = 13.16$, $p < 0.001$]. The numbers of eggs released from clams given the dry *T. suecica* and dry *T. suecica+D. tertiolecta* treatments were significantly lower than the other two treatments.

It was not possible to carry out ANOVA on the number of eggs after 8 weeks because only one female spawned from dry *T. suecica*, dry *T. suecica+S. costatum* and dry *T. suecica+I. galbana* broodstocks. However egg number was higher in dry *T. suecica+S. costatum*, similar in dry *T. suecica+D. tertiolecta* and dry *T. suecica+I. galbana* and much lower in dry *T. suecica.*

2nd broodstock 1992

Attempts to spawn the clams were made 3 times (spawning I, II, III) after 5 (462 D^o), 6 (553 D^o) and 7 (643 D^o) weeks of conditioning in the second broodstock (Table 3.58). Spawning condition was reached at 643 D^o of conditioning.

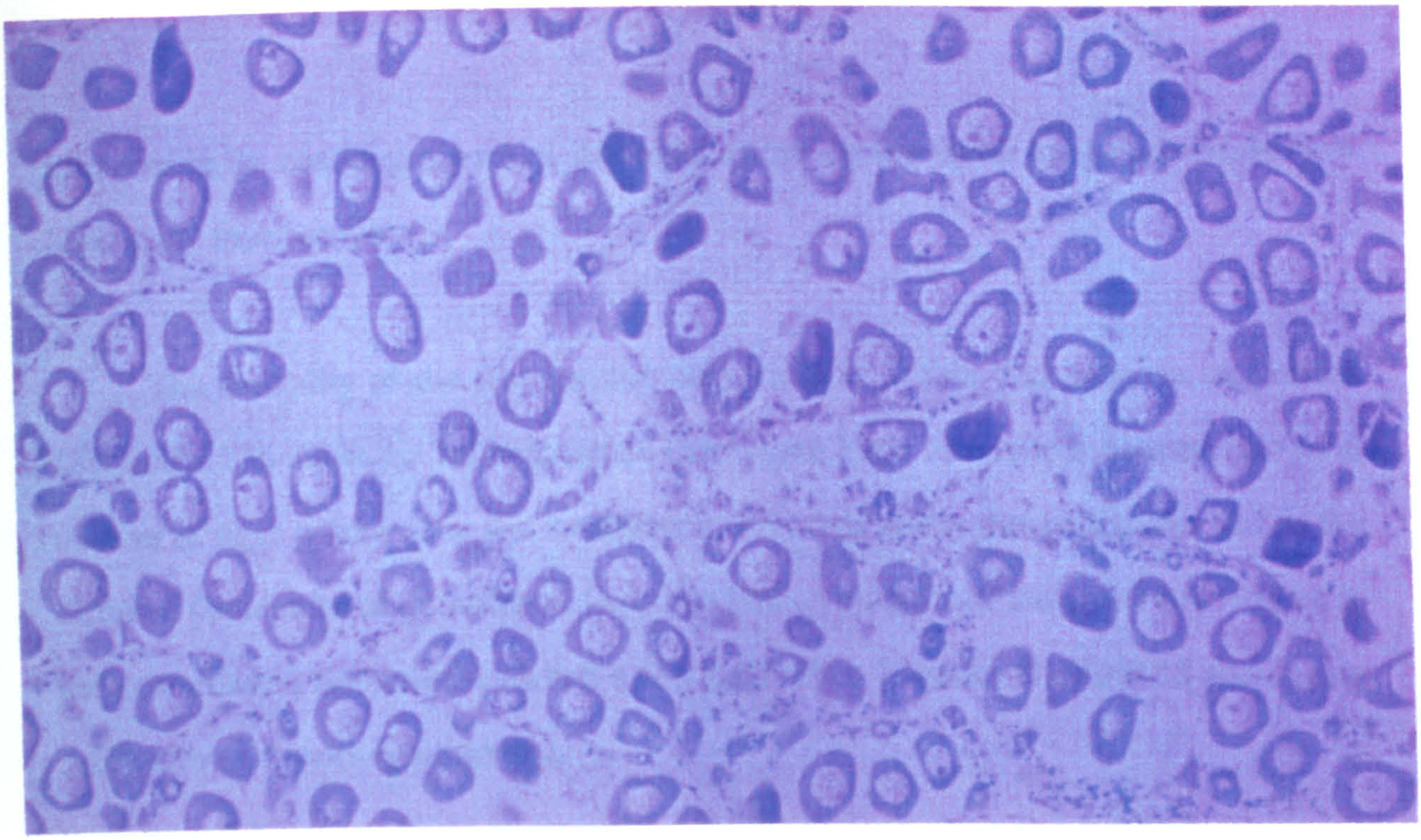
After 462 D^o and 553 D^o weeks 5 and 6 (spawning I, II) the clams did not spawn, which suggested an insufficient period of conditioning. This was an unexpected result since in the 1st broodstock clams spawned after 508 $D^o$and the clams were of a similar size, mean length 44.27±2.43 mm in 1st broodstock and 40.30±2.02 mm in the 2nd broodstock. Analysis of the gonad sections showed that almost all clams had spawned, they were at stage IV and stage V (Plate 3.2 and Appendix 14a). This must have been

Table 3.58. Fecundity of T. philippinarum after being conditioned for 5 (Spawning I), 6 (Spawning II) and 7 (Spawning III) weeks on a mixed diet of 70% dry T. suecica and 30% live diet. For abbreviations and other details see Table 3.42.

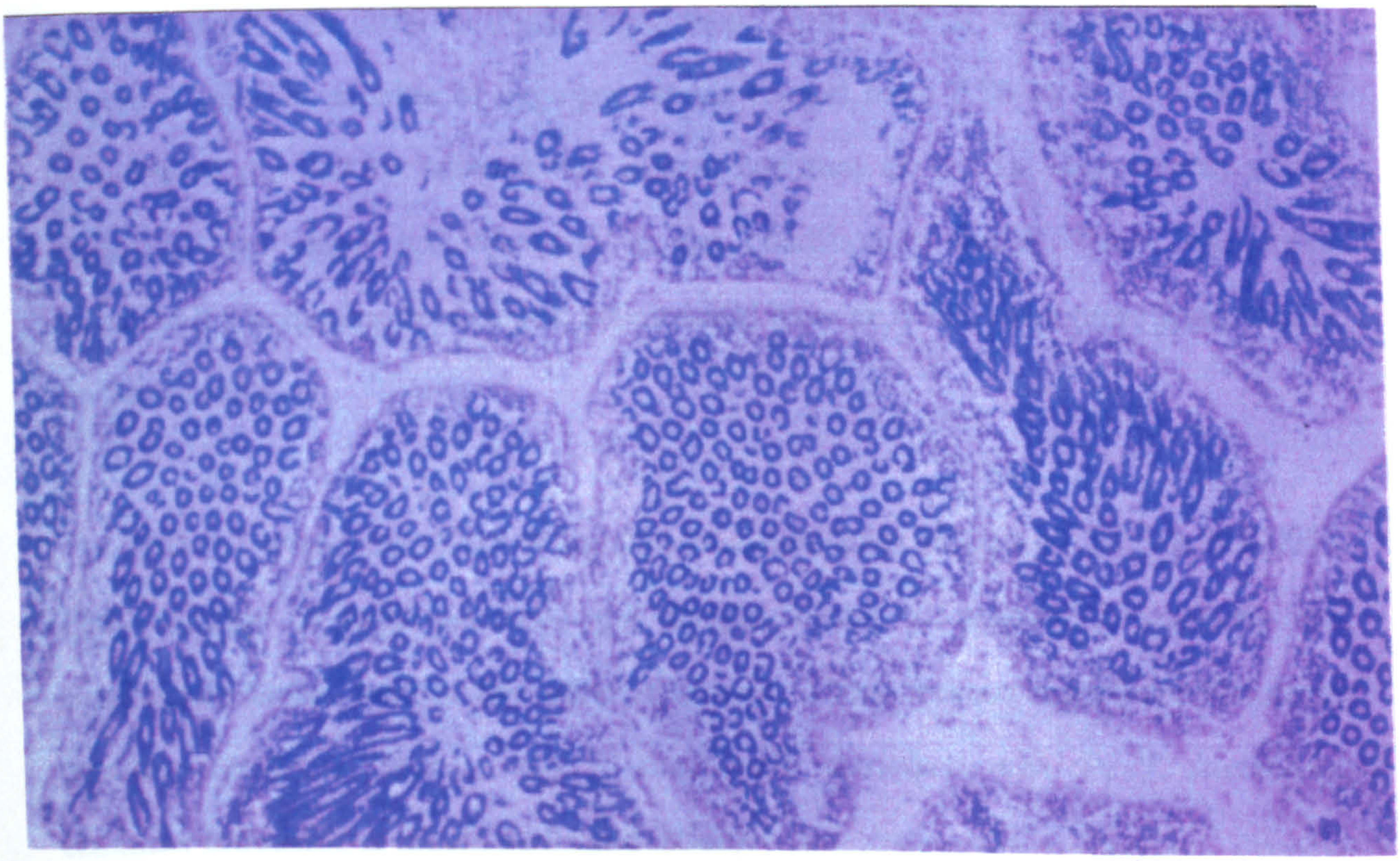
2nd Broodstock conditioning - 1992

Spawning	Treatment	Nº of clams induced	Total nº spawned	Female/male spawned	Total nº of eggs produced (in millions)	Eggs mean per female (in millions)
I	*	15	0	0	0	0
II	*	15	0	0	0	0
III	Fsw	15	1	1/0	0.253	0.253
	Dry Tet	15	11	9/2	15.449	1.717 (± 0.57)
	Dry Tet + Dun	15	12	8/4	11.912	1.489 (± 0.53)
	Dry Tet + Ske	15	13	8/5	21.233	2.654 (± 0.94)
	Dry Tet + Iso	15	11	6/5	25.935	4.323 (± 1.76)

* the same as spawning III



a)



b)

Plate 3.2. Mature stage (2nd broodstock 1992)

a) Female clam with large detached eggs.

b) Male clam with ripe sperm.

triggered in the conditioning tanks. Attempts to induce the clams to spawn should have been made much earlier.

The fecundity of the females was very variable within and between treatments (Appendix 13). As in the 1st broodstock there were large differences between the total number of eggs produced in this broodstock. This was partly due to the diet and to the different fecundities of the females. Only one female clam from the unfed broodstock in the sand-filtered sea water spawned a few eggs after 7 weeks (Table 3.58 and Figure 3.25) of warm water conditioning.

Within the spawning period (approximately 7 hrs), the percentage of clams which spawned in the four different diets ranged from 73 to 87% at week 7 (spawning III) (Figure 3.26). The number of eggs per female ranged from 0.25 to 4.3 million. The fecundity of the 2nd broodstock was almost half that of the 1st broodstock, which suggested that clams had spawned in the conditioning tanks. There was more chlorophyll a in the 2nd broodstock which could have provided even more food than in the 1st broodstock.

After 7 weeks the effect of the diet was significant [$F_{3,27} = 7.57$, $p < 0.001$]. The number of eggs produced per female in dry *T. suecica+I. galbana* was significantly higher than in any other treatment. There was no significant difference between the dry *T. suecica*, dry *T. suecica+D. tertiolecta* and dry *T. suecica+S. costatum* broodstocks (Table 3.58a and 3.58b).

Although Manila clams could be conditioned using only dried algae, mixed diets of live (30%, of *D. tertiolecta, S. costatum or I. galbana*) and spray-dried (70%, T. suecica) algae were generally better. The fecundity of broodstock fed with these diets was greater than for unfed animals.

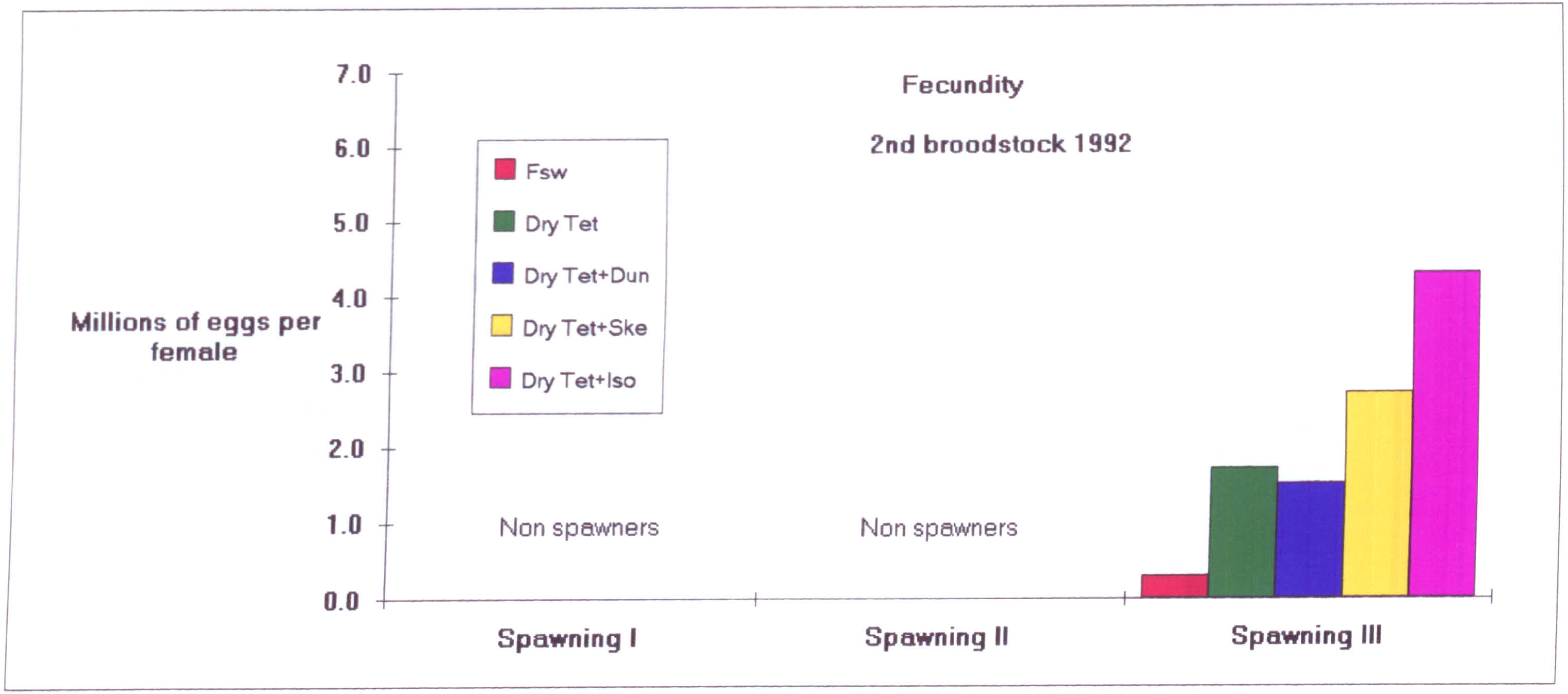


Figure 3.25. Mean fecundity of <u>T. philippinarum</u> in relation to diet after being conditioned for 5 (spawning I), 6 (spawning II) and 7 (spawning III) weeks (2nd broodstock 1992). For clarity, standard errors (given in Table 3.58) are not included in this Figure. For abbreviations see Table 3.42.

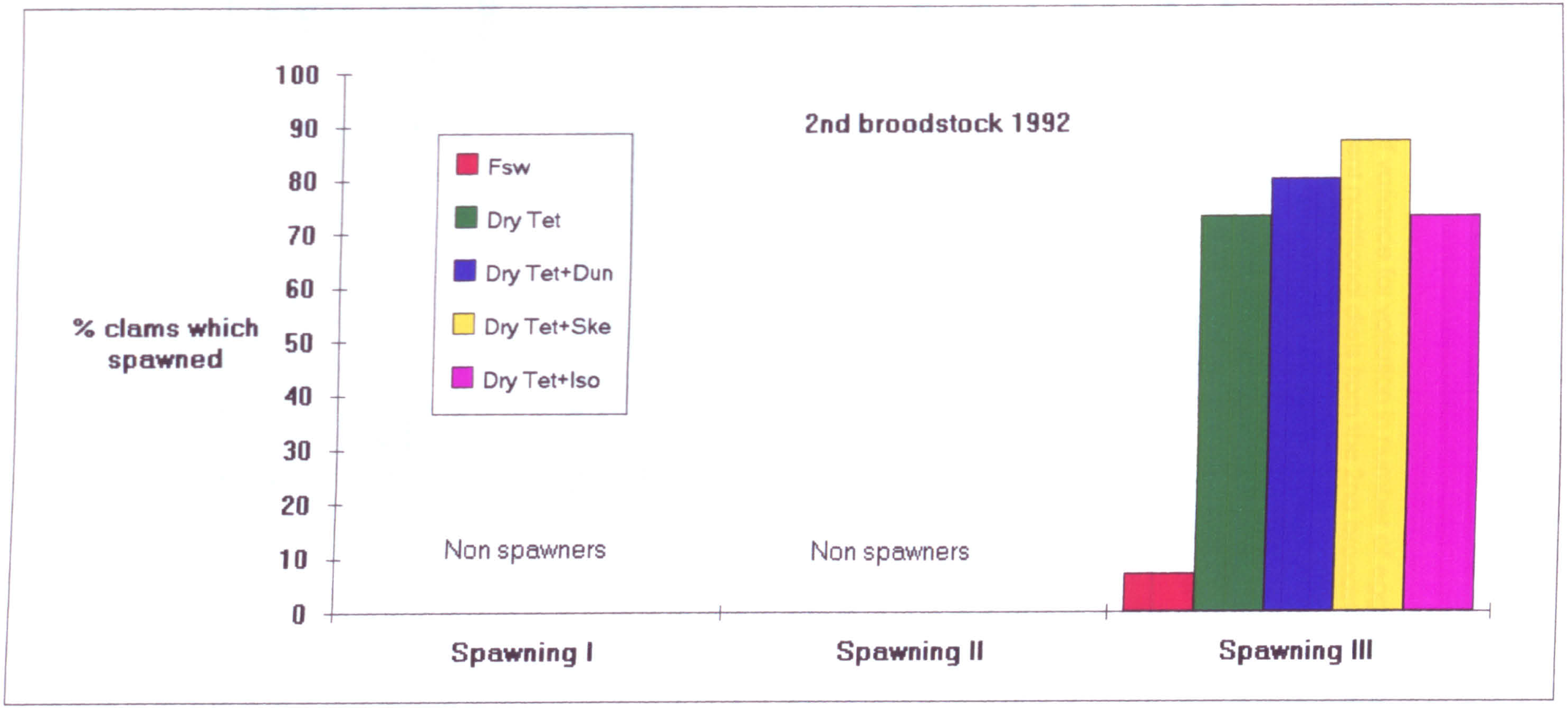


Figure 3.26. Percentage of <u>T. philippinarum</u> which spawned in relation to diet after being conditioned for 5 (spawning I), 6 (spawning II) and 7 (spawning III) weeks (2nd broodstock 1992). For abbreviations see Table 3.42.

Table 3.58a. Analysis of variance for variation in number of eggs per female of T. philippinarum conditioned with different nutritional diets from the 2nd broodstock 1992. For abbreviations and other details see Table 3.7.

2nd broodstock 1992

7 weeks conditioning

Source of variation	d.f.	Sum of squares	Mean square	F Value	P	S
Treatment	3	3.35358E+13	1.11786E+13	7.57	0.0008	***
Residual	27	3.98492E+13	1.47590E+12			
Total	30	7.33850E+13				

Table 3.58b. Results of the t-test (LSD) for the number of eggs per female of T. philippinarum conditioned with different nutritional diets from the 2nd broodstock 1992. For abbreviations and other details see Table 3.7 and 3.42.

2nd broodstock 1992

7 weeks conditioning

	Dry Tet	D-Tet+Dun	Dry Tet+ Ske
Dry Tet+Dun	n.s.		
Dry Tet+Ske	n.s.	n.s.	
Dry Tet+Iso	*	*	*

3.3.4. Egg and larval quality

3.3.4.1. Survival of D-larvae

The percentage survival of D-larvae after 24 h of incubation in natural sea water in the 1st and 2nd broodstock 1992 is shown in Table 3.59 and Figure 3.27. In the 1st broodstock the quality of the eggs from the dry *T. suecica* treatment seemed to be constant in all the spawnings since percentage survival of D-larvae ranged from 70 - 77%. The percentage survival of D-larvae between all treatments was not significantly different [$F_{3,6}$ = 4.66, p>0.05]. Between spawnings the percentage survival was significantly higher in spawning I than the other two [$F_{2,6}$ = 7.10, p<0.05]. In the 2nd broodstock there was a decrease to 31% in the dry *T. suecica* treatment which may have been due to seasonal changes in the natural sea water quality. In this broodstock, it was not possible to carry out any statistical analysis because there were not enough data. In the 1st (spawning II) and 2nd broodstock, there was a relationship between the mean fecundity of clams and the percentage survival of D-larvae in all treatments such that, the percentage survival of D-larvae decreased as the number of eggs produced per female increased (Table 3.56, 3.58 and 3.59).

The quality of the egg, expressed as percentage recovery of D-larvae in artificial sea water in both broodstocks, was very variable between females (Table 3.60). In the 1st broodstock after 6 [$F_{3,18}$ = 1.52, p = 0.24] and 7 weeks [$F_{3,13}$ = 0.24, p = 0.86] there was no significant difference in the percentage survival of D-larvae from the treatments. There was also no significant difference between spawnings [$F_{2,6}$ = 1.90, p = 0.23]. In the 2nd broodstock after 7 weeks there was no difference in the % survival of D-larvae from the broodstock diet treatments. The unfed treatment was not included for ANOVA because it had only one value. The artificial sea water is a more stressful medium, therefore we might have expected the percentage recovery to be lower

Table 3.59. Percentage survival of T. philippinarum D-larvae after 24 h of incubation from the 1st and 2nd broodstock 1992. Clams were conditioned for 6 (Spawning I), 7 (Spawning II) and 8 (Spawning III) weeks in 1st broodstock and 7 (Spawning III) weeks conditioned in 2nd broodstock. For abbreviations see Table 3.42.

Spawning	Treatment	No. embryos incubated (in millions)	No. D-larvae recovered (in millions)	% survival from D-larvae
1st broodstock 1992				
I	Fsw	*	*	*
	Dry Tet	5.520	4.196	76.0
	Dry Tet+Dun	3.156	2.809	89.0
	Dry Tet+Ske	4.183	3.622	86.6
	Dry Tet+Iso	5.698	3.861	67.8
II	Fsw	0	0	0
	Dry Tet	2.000	1.547	77.34
	Dry Tet+Dun	2.000	1.054	52.69
	Dry Tet+Ske	4.000	1.653	41.33
	Dry Tet+Iso	2.836	0.629	22.17
III	Fsw	0	0	0
	Dry Tet	0.604	0.426	70.51
	Dry Tet+Dun	7.757	4.436	57.18
	Dry Tet+Ske	5.168	3.484	67.42
	Dry Tet+Iso	0.533	0.145	27.16
2nd broodstock 1992				
III	Fsw	*	*	*
	Dry Tet	8.651	2.714	31.37
	Dry Tet+Dun	4.935	1.656	33.56
	Dry Tet+Ske	7.961	1.466	18.41
	Dry Tet+Iso	10.390	1.861	17.91

* not enough embryos to incubate

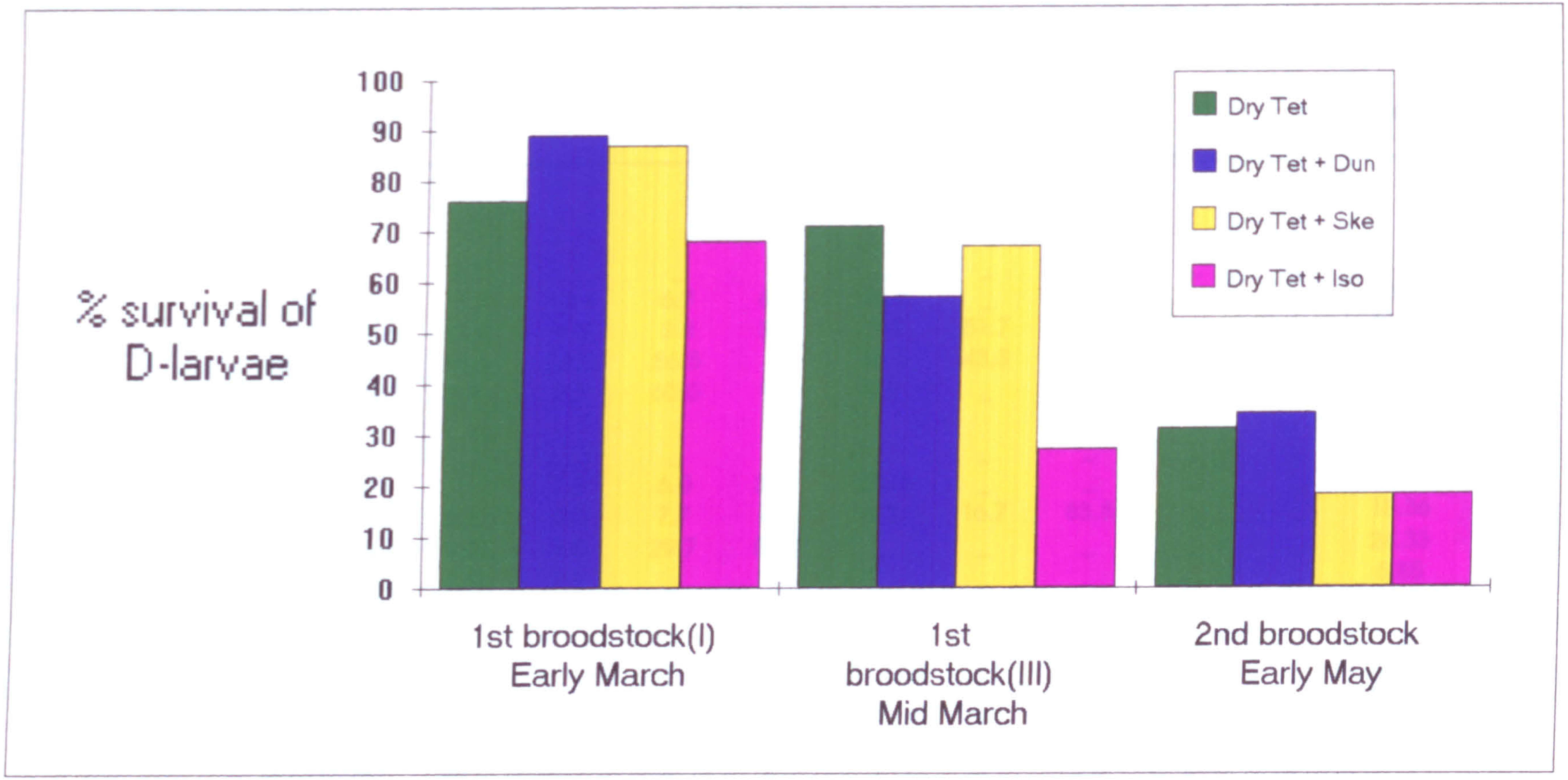


Figure 3.27. Seasonal percentage survival of T. philippinarum D-larvae in the 1st (spawning I and III) and 2nd (spawning III) broodstock 1992. For abbreviations see Table 3.42.

Table 3.60 Percentage survival of T. philippinarum D-larvae after 24 h of incubation from the bioassay in artificial sea water in the 1st and 2nd broodstock. Clams were conditioned for 6 (Spawning I), 7 (Spawning II) and 8 (Spawning III) weeks in 1st broodstock and 7 (Spawning III) weeks conditioned in 2nd broodstock. For abbreviations see Table 3.42.

		Females nº										
Spawning	Treatment	1	2	3	4	5	6	7	8	9	Mean	SE
1st broodstock 1992												
I	Fsw	2.7	-	-	-	-	-	-	-	-	2.70	-
	Dry Tet	11.7	12.7	6.7	61.8	24.0	-	-	-	-	23.38	10.01
	Dry Tet+Dun	43.8	4.0	3.8	39.7	3.8	51.7	41.0	-	-	26.83	8.24
	Dry Tet+Ske	15.2	58.0	56.5	*	50.7	43.3	-	-	-	44.74	7.82
	DryTet+Iso	2.7	2.3	60.0	9.0	9.8	-	-	-	-	16.76	10.92
II	Fsw	-	-	-	-	-	-	-	-	-	-	-
	Dry Tet	*	7.3	6.0	34.7	25.0	-	-	-	-	18.25	6.99
	DryTet+Dun	5.8	4.0	7.7	4.2	6.3	16.7	83.8	-	-	18.36	11.03
	Dry Tet+Ske	9.0	4.8	29.7	53.8	-	-	-	-	-	24.33	11.23
	Dry Tet+Iso	4.5	9.2	-	-	-	-	-	-	-	6.85	2.35
III	Fsw	-	-	-	-	-	-	-	-	-	-	-
	Dry Tet	35.0	-	-	-	-	-	-	-	-	35.00	-
	Dry Tet+Dun	7.5	8.2	17.8	-	-	-	-	-	-	11.17	3.32
	Dry Tet+Ske	9.8	-	-	-	-	-	-	-	-	9.80	-
	Dry Tet+Iso	5.7	-	-	-	-	-	-	-	-	5.70	-
2nd broodstock 1992												
III	Fsw	48.0	-	-	-	-	-	-	-	-	48.00	-
	Dry Tet	12.0	13.8	15.2	4.5	10.8	25.0	18.8	12.3	19.3	14.63	1.96
	Dry Tet+Dun	11.5	25.3	5.5	22.2	16.7	5.2	4.8	50.2	-	17.68	5.42
	Dry Tet+Ske	26.3	21.7	4.2	3.2	3.5	3.0	21.0	5.2	-	11.01	3.56
	Dry Tet+Iso	7.2	6.7	11.7	5.3	11.2	7.2	-	-	-	8.22	1.06

* not enough embryos to incubate

compared with natural sea water. Eggs from the same diet treatment, which developed well in natural sea water also developed well in artificial sea water.

3.3.4.2. Lipid and fatty acid analysis

The ash-free dry weight and the lipid content of *T. philippinarum* eggs and D-larvae which developed from them, after the I, II and III spawnings in the 1st broodstock 1992 and after the III spawning in the 2nd broodstock 1992 are shown in Tables 3.61 and 3.62. These larvae were used to start semi-commercial scale trials. The mean shell length of D-larvae from the different diet treatments is also included in the same Tables.

In the 1st broodstock 1992, the lipid content of eggs spawned by *T. philippinarum* from the different broodstock diets was between 4.2 and 7.8 ng egg^{-1} (15 to 38% of ash-free dry weight) from the three spawnings. In spawning I lipid ranged from 5.5 to 7.6 ng egg^{-1} (15 to 23% of Afdwt). In spawning II, lipid ranged from 4.8 to 7.2 ng egg^{-1} (15 to 30% of Afdwt) and in spawning III it ranged from 5.2 to 7.8 ng egg^{-1} (24 to 38% of Afdwt). The results from the sand filtered sea water treatment were not used because only one female spawned. The lipid content of D-larvae was between 2.1 and 6.4 ng $larvae^{-1}$ (6 to 15% of Afdwt) from the three spawnings.

In spawning I, [$F_{3,16} = 0.60$, $p < 0.62$] II, [$F_{3,12} = 2.60$, $p < 0.10$] and III [$F_{3,2} = 0.39$, $p < 0.78$], lipid as ng per egg was not significantly different between broodstock diet treatments. The sand filtered sea water gave lipid levels of 4.2 ng per egg (21.8% of Afdwt). ANOVA was not carried out in this treatment because there was only one value. Between spawnings, lipid per egg was not significantly different [$F_{2,6} = 0.18$, $p = 0.84$].

Table 3.61. Ash-free dry weight (Afdwt) and lipid content of eggs and D-larvae of T. philippinarum in the 1st broodstock 1992. Clams were conditioned for 6 (Spawning I), 7 (Spawning II) and 8 (Spawning III) weeks with different diets. For abbreviations see Table 3.42.

Broodstock diet	Eggs			D-larvae				% utilization of lipid during egg development
	Afdwt (ng egg)	Lipid (ng egg)	Lipid (% Afdwt)	Mean larval size (µm)	Afdwt (ng D-larva)	Lipid (ng D-larva)	Lipid (% Afdwt)	
Spawning I (6 weeks)								
Fsw	19.43	4.23	21.77	*	*	*	*	*
Dry Tet	35.02	6.57	18.76	97.20	40.62	5.91	14.55	10.05
Dry Tet + Dun	35.78	6.20	17.33	95.25	47.74	5.93	12.42	4.35
Dry Tet + Ske	35.70	5.50	15.41	95.78	44.36	5.24	11.81	4.73
Dry Tet + Iso	32.36	7.55	23.33	95.92	42.63	6.41	15.04	15.10
Spawning II (7 weeks)								
Fsw	_	_	_	_	_	_	_	_
Dry Tet	21.73	6.17	28.39	98.91	38.39	2.23	5.81	63.86
Dry Tet + Dun	29.41	4.80	16.32	96.61	37.97	2.12	5.58	55.83
Dry Tet + Ske	27.90	7.23	25.91	95.89	42.64	5.63	13.20	22.13
Dry Tet + Iso	22.67	6.70	29.55	98.55	39.22	5.02	12.80	25.07
Spawning III (8 weeks)								
Fsw	_	_	_	_	_	_	_	_
Dry Tet	22.04	5.19	23.55	98.95	46.24	4.32	9.34	16.76
Dry Tet + Dun	29.03	7.24	24.94	97.24	35.97	5.48	15.23	24.31
Dry Tet + Ske	24.03	6.34	26.38	97.98	48.47	4.80	9.90	24.29
Dry Tet + Iso	20.59	7.80	37.88	98.11	*	5.29	*	32.18

_ clams did not spawn * Insufficient numbers of D-larvae

Table 3.62. Ash-free dry weight (Afdwt) and lipid content of eggs and D-larvae of T. philippinarum in spawning III (7 weeks) of the 2nd broodstock 1992. Clams were conditioned with different diets. For abbreviations see Table 3.42.

Broodstock diet	Eggs			D-larvae				% utilization of lipid during egg development
	Afdwt (ng egg)	Lipid (ng egg)	Lipid (% Afdwt)	Mean larval size (µm)	Afdwt (ng D-larva)	Lipid (ng D-larva)	Lipid (% Afdwt)	
Spawning III (7 weeks)								
Fsw	*	*	*	*	*	*	*	*
Dry Tet	26.90	7.73	28.74	79.00	40.61	5.84	14.38	24.45
Dry Tet + Dun	31.47	5.07	16.11	79.20	41.41	4.45	10.75	12.23
Dry Tet + Ske	26.22	4.60	17.54	78.80	43.58	3.62	8.31	21.30
Dry Tet + Iso	31.50	6.48	20.57	78.40	45.99	6.01	13.07	7.25

* Only one female spawned

The lipid energy reserves, utilized during development from the egg to the D-larva stage, in the first 24h before feeding, was determined (Table 3.61). The percentage utilization of lipid was significantly higher in spawning II than the others in spawnings I and III [$F_{2,6} = 5.31$, $p < 0.05$].

In the 2nd broodstock 1992, the lipid content of eggs spawned by *T. philippinarum* from the different broodstock diets was between 4.6 and 7.7 ng egg^{-1} (16 to 29% of ash-free dry weight) in spawning III (Table 3.62). However, the highest and lowest values for lipid as ng egg^{-1} did not always correspond with the highest and lowest values for lipid as % Afdwt. Lipid as ng per egg was not significantly different between treatments [$F_{3,23} = 2.76$, $p = 0.07$]. Between the 1st and 2nd broodstocks lipid per egg was not significantly different [$F_{1,3} = 0.37$, $p = 0.58$]. The lipid content of D-larvae was between 3.6 and 6.0 ng larva^{-1} which represented 8 to 14% of Afdwt. As above an assessment of the lipid energy reserves, utilized during development from the egg to the D-larva stage, in the first 24h before feeding, was made. The percentage utilization of lipid ranged from 7.3 to 24.5% between all the fed broodstock treatments (Table 3.62).

Changes in the ash-free dry weight and the lipid content of *T. philippinarum* larvae (ng per larva and % of Afdwt) during development are presented in Table 3.63 and 3.64, from 5 to 19 days in the 1st broodstock 1992 (I and III spawnings; in spawning II it was not possible to set up bins for growth of larvae) and from 7 to 14 days in the 2nd broodstock 1992 (III spawning) from the different broodstock treatments. In spawning III (2nd broodstock) there were only enough D-larvae to grow larvae from the dry *T.suecica+D. tertiolecta* and dry *T. suecica+S. costatum* treatments. The quantity of lipid (ng larva^{-1}) and the shell length in spawning I is shown in Figure 3.28. In spawning III there were not enough data to draw a Figure. In the 1st broodstock 1992 (spawnings I and III) larvae from the different broodstock treatments were grown in natural sea water and measurements made from day 5 to day

Table 3.63. Ash-free dry weight (Afdwt) and lipid content of larvae of T. philippinarum in the 1st broodstock 1992. Clams were conditioned for 6 (Spawning I) and 8 (Spawning III) weeks with different diets. For abbreviations see Table 3.42.

Broodstock diet	Day 5 Afdwt (ng D-larva)	Day 5 Lipid (ng D-larva)	Day 5 Lipid (% Afdwt)	Day 9 Afdwt (ng D-larva)	Day 9 Lipid (ng D-larva)	Day 9 Lipid (% Afdwt)	Day 12 Afdwt (ng D-larva)	Day 12 Lipid (ng D-larva)	Day 12 Lipid (% Afdwt)
Spawning I (6 weeks)									
Dry Tet	175.75	9.46	5.38	–	–	–	544.50	69.06	12.68
Dry Tet + Dun	180.99	8.91	4.92	–	–	–	576.40	64.08	11.12
Dry Tet + Ske	138.75	7.81	5.63	–	–	–	409.25	47.58	11.63
Dry Tet + Iso	154.70	9.97	6.44	–	–	–	510.50	62.80	12.30
Spawning III (8 weeks)									
Dry Tet + Dun	214.63	20.28	9.45	311.55	45.76	14.69	531.90	61.69	11.60
Dry Tet + Ske	187.18	15.26	8.15	500.35	59.28	11.85	866.10	127.27	14.69

(continued)

Table 3.63. (continued)

Broodstock diet	Day 14			Day 17			Day 19		
	Afdwt (ng D-larva)	Lipid (ng D-larva)	Lipid (% Afdwt)	Afdwt (ng D-larva)	Lipid (ng D-larva)	Lipid (% Afdwt)	Afdwt (ng D-larva)	Lipid (ng D-larva)	Lipid (% Afdwt)
Spawning I (6 weeks)									
Dry Tet	853.50	130.18	15.25	820.40	116.08	14.15	_	_	_
Dry Tet + Dun	677.90	95.04	14.02	928.70	128.27	13.81	1159.30	150.52	12.98
Dry Tet + Ske	638.10	95.94	15.04	734.40	60.40	8.22	830.30	135.13	16.27
Dry Tet + Iso	716.10	115.17	16.08	774.40	102.72	13.26	939.00	106.82	11.38
Spawning III (8 weeks)									
Dry Tet + Dun	1592.4	224.46	14.10	1775.6*	236.14*	13.30	_	_	_
Dry Tet + Ske	1030.1	153.11	14.86	1190.4*	128.6*	10.80	1747.6	163.69	9.37

* clams at day 16

Table 3.64. Ash-free dry weight (Afdwt) and lipid content of larvae of T. philippinarum in Spawning III of the 2nd broodstock 1992. For abbreviations see Table 3.42.

Broodstock diet	Day 7			Day 14		
	Afdwt (ng D-larva)	Lipid (ng D-larva)	Lipid (% Afdwt)	Afdwt (ng D-larva)	Lipid (ng D-larva)	Lipid (% Afdwt)
Spawning III (7 weeks)						
Dry Tet	707.30	89.73	12.69	1229.70	144.07	11.72
Dry Tet + Dun	257.95	24.27	9.41	1158.10	125.55	10.84
Dry Tet + Ske	286.60	25.32	8.83	906.30	119.37	13.17
Dry Tet + Iso	665.15	92.36	13.89	965.60	141.08	14.61

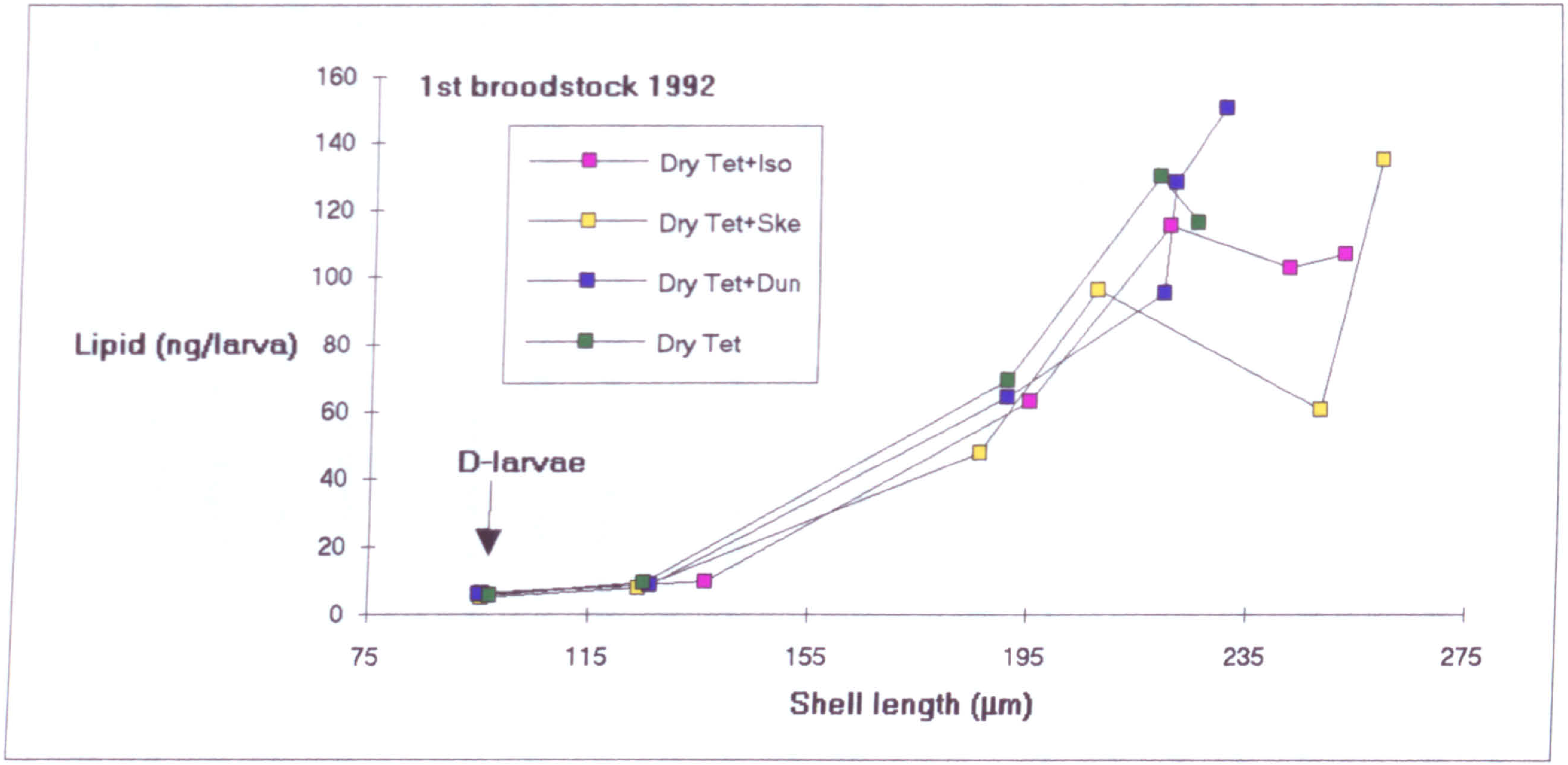


Figure 3.28. Lipid content of T. philippinarum larvae from the 1st broodstock 1992 (spawning I) conditioned on different diets. Successive points from left to right on each line represent values at days 5, 12, 14, 17 and 19. For abbreviations see Table 3.42.

19 and in the 2nd broodstock, measurements were made from day 7 to day 14. In the 1st broodstock 1992 (spawning I and III), there was an increase in the quantity of lipid, as % of Afdwt, from day 5 to day 14, in larvae from all the broodstock treatments followed by a decrease from day 14 to day 17 (with the exception of dry *T. suecica+D. tertiolecta* which was still increasing at 19). The decrease in lipid suggested that larvae had reached the stage of metamorphosis and utilised lipid as an energy source. At 14 days, lipid as % of Afdwt was similar between broodstock diets within spawnings. In the 2nd broodstock 1992 and as in the 1st broodstock, there was an increase in the quantity of lipid as % of Afdwt from day 7 to day 14 in larvae from all the broodstock diet treatments. At 14 days the trend was the same as that for the 1st broodstock 1992. There was no evidence of the utilisation of lipid reserves, suggesting that larvae had not reached the energy consuming stages of metamorphosis.

The fatty acid content of eggs, D-larvae and 14 and 17 day-old larvae (as % of total fatty acids) from spawnings I, II and III in the 1st broodstock 1992 is shown in Table 3.65 and Appendix 15, 15a and 15b. In the 2nd broodstock 1992 the fatty acid content of eggs, D-larvae and 14 day-old larvae from the spawning III is shown in Table 3.66 and Appendix 16. The fatty acid composition of the lipid reserves in eggs was generally dependent on diet quality, ie levels of the essential PUFAs 20:5w3 and 22:6w3 in the eggs were low if the broodstock diet was deficient in these fatty acids.

In the 1st broodstock 1992 (all spawnings), eggs from the dry *T. suecica+D. tertiolecta* treatment had a high level of 18:3w3 and low levels of 20:5w3 and 22:6w3 (reflecting the low levels in the algae). Eggs from dry *T. suecica+S. costatum* fed broodstock had higher levels of 20:5w3. Dry *T. suecica+I. galbana* had higher levels of 22:6w3. In both treatments this reflected the PUFA composition of the algae.

In spawning I, during larval development there was a decrease in the levels of 20:5w3 and 22:6w3 (with the exception of dry *T. suecica+D. tertiolecta*) (Table 3.65).

Table 3.65. Fatty acid composition of eggs and larvae produced by T. philippinarum broodstock fed on different diets. Values are percentages of total fatty acid. For abbreviations see Table 3.42.

1st broodstock 1992	Eggs				D-larvae			
	Dry Tet	Dry Tet+Dun	Dry Tet+Ske	Dry Tet+Iso	Dry Tet	Dry Tet+Dun	Dry Tet+Ske	Dry Tet+Iso
Spawning I								
18:3w3	0.19	5.23	0.22	1.34	0.74	3.87	0.00	1.30
20:5w3	0.91	2.45	6.76	1.41	1.29	1.93	5.06	1.88
22:6w3	1.13	2.02	1.36	4.30	3.39	1.97	0.00	5.74
Polyenoic	8.10	19.19	11.33	13.28	9.02	13.33	6.82	14.00
Spawning II								
18:3w3	0.21	5.57	0.36	1.37	0.00	1.38	0.43	0.37
20:5w3	1.50	2.43	11.74	2.61	1.19	0.00	3.91	3.30
22:6w3	1.18	2.14	3.43	5.98	0.00	0.00	2.90	2.94
Polyenoic	12.85	20.86	23.10	22.55	9.79	10.81	17.28	16.68
Spawning III								
18:3w3	1.10	6.58	2.65	2.49	2.27	4.97	0.86	*
20:5w3	0.00	2.32	5.64	17.82	2.10	1.54	5.06	*
22:6w3	1.55	2.07	3.56	1.56	3.13	1.98	2.08	*
Polyenoic	11.01	23.08	18.97	29.8	14.22	17.84	15.56	*

* contaminated sample
_ No sample

Table 3.65. (continued)

14 day larvae Dry Tet	Dry Tet+Dun	Dry Tet+Ske	Dry Tet+Iso	17 day larvae Dry Tet	Dry Tet+Dun	Dry Tet+Ske	Dry Tet+Iso
1.76	1.63	1.45	1.90	1.25	1.66	-	1.55
5.94	4.71	4.83	4.91	5.27	6.12	-	4.81
8.12	4.09	6.86	7.65	7.31	6.77	-	6.99
31.68	26.18	27.73	30.89	28.69	29.39	-	28.98
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	1.61	1.91	-	-	1.54	0.94	-
-	8.70	6.05	-	-	5.03	3.88	-
-	6.43	7.45	-	-	6.43	5.13	-
-	34.09	32.40	-	-	28.54	23.14	-

Table 3.66. Fatty acid composition of eggs and larvae produced by T. philippinarum broodstock fed on different diets. Values are percentages of total fatty acid. For abbreviations see Table 3.42.

2nd broodstock 1992 - Spawning III	Eggs				D-larvae				14 day larvae			
	Dry Tet	Dry Tet+Dun	Dry Tet+Ske	Dry Tet+Iso	Dry Tet	Dry Tet+Dun	Dry Tet+Ske	Dry Tet+Iso	Dry Tet	Dry Tet+Dun	Dry Tet+Ske	Dry Tet+Iso
Fatty acid												
18:3w3	0.56	7.51	0.00	1.76	0.00	1.37	0.26	0.71	1.98	1.74	1.67	1.97
20:5w3	1.58	2.51	4.10	3.66	0.00	1.18	2.59	1.59	9.57	6.40	8.22	9.66
22:6w3	3.29	1.83	0.00	8.61	0.67	1.24	1.88	4.37	8.38	8.53	6.79	8.39
Polyenoic	11.41	21.93	8.34	27.10	5.74	12.97	13.77	16.05	35.93	30.72	29.69	34.51

In spawning II, there was an increase in 20:2w6 from egg to D-larvae in all the treatments. This may have been related to the quality of the fine particles in the sand filtered sea water. There was a decrease in 20:5w3 and 22:6w3 from egg to D-larvae in dry *T. suecica+D. tertiolecta* and dry *T.suecica+S.costatum* treatments. In the dry *T. suecica* and dry *T. suecica+I. galbana* there was an increase in 20:5w3 and 22:6w3. In spawning III, it was of interest that there were high levels of 20:5w3 in eggs from the dry *T. suecica+I. galbana* treatment because 20:5w3 is very low in these algae. During larvae development generally there was a decrease in 20:5w3 and 22:6w3. In all spawnings there were not enough eggs from the unfed broodstock for analysis.

In the 2nd broodstock 1992 (spawning III), as above, eggs from the dry *T. suecica+D. tertiolecta* treatment had a high level of 18:3w3 and low levels of 20:5w3 and 22:6w3. Eggs from dry *T. suecica+S. costatum* fed broodstock had high levels of 20:5w3. Dry *T. suecica+I. galbana* had higher levels of 22:6w3. In all treatments this reflected the PUFA composition of the algae. There was a utilisation of 20:5w3 and 22:6w3 (with the exception 22:6w3 in the dry T. *suecica+S. costatum*) during development from egg to D-larvae. There was an increase in 18:2w6 from egg to D-larvae in all the treatments and as above, this could have been related to the quality of fine particles in the sand filtered sea water.

Overall the polyenoic fatty acids generally decreased from egg to D-larvae throughout treatments (this is evident in the 1st broodstock 1992, spawning II). Differences in total polyenoic fatty acid in eggs were found between treatments [$F_{3,6} = 5.60$, $p < 0.05$] and spawnings [$F_{2,6} = 5.14$, $p < 0.05$] in the 1st broodstock. The dry *T. suecica* treatment was significantly lower than other treatments. Values were significantly lower in spawning I than spawning II or III. There was an increase in the percentage of these polyenoic fatty acids during development, from D-larvae to 14 days, which corresponded to an increase in lipid as a % of Afdwt (Table 3.65). There was a decrease during development from day 14 to day 17, and this could have been evidence of utilisation during metamorphosis. In the 2nd broodstock it was not possible to carry out any statistical analysis because there were not enough data.

3.3.4.3. Effect of salinity

Salinity stress was used to test whether egg quality was important to survival of larvae from the different broodstock treatments. Salinity was thought to be a better indicator than temperature stress. D-larvae used for this experiment came from the 2nd broodstock 1992 and from spawning III.

The effect of salinity on the growth of Manila clam larvae is shown in Table 3.67. After 7 days at 10 psu growth was significantly lower than other salinities [$F_{2,6}$ = 96.81, $p < 0.001$]. There was no significant differences between broodstock diet treatments [$F_{3,6} = 2.81$, $p = 0.13$]. After 14 days, with 15 and 30 psu there was no significant difference between diet treatments [$F_{3,3} = 3.49$, $p = 0.17$]. Between salinities growth was significantly less at 15 psu [$F_{1,3} = 10.53$, $p < 0.05$].

All clams had died after 14 days when reared at the lowest salinity of 10 psu and high mortalities were evident at day 7. At 15 psu and 30 psu most of the larvae had grown to the stage of metamorphosis. The survival of larvae at day 7 was significantly different between salinities [$F_{2,6} = 20.53$, $p < 0.01$]. The survival was significantly lower at 10 psu, followed by 15 and 30 psu. The survival of larvae from the dry *T. suecica+D. tertiolecta* and dry *T. suecica+S. costatum* treatments were not significantly different. Also the dry *T. suecica* and dry *T. suecica+I. galbana* treatment were not significantly different and both were significantly lower than the other two treatments [$F_{3,6} = 5.18$, $p = 0.04$]. At 14 days, the survival at 15 psu was significantly lower than 30 psu [$F_{1,3} = 13.89$, $p = 0.03$]. Between treatments there was a significant difference in the survival of larvae [$F_{3,3} = 35.93$, $p < 0.01$], the differences were the same as those described above for 7 days.

Table 3.67. Effect of salinity (psu) on the growth of T. philippinarum larvae from different broodstock treatments reared in beakers over 7 days and 14 days. Values are mean shell length (μm). All experiments had two replicates per treatment. Standard error in parentheses. For abbreviations see Table 3.42.

Broodstock treatment	D-larvae	7 day larvae 10 psu	7 day larvae 15 psu	7 day larvae 30 psu	14 day larvae 10 psu	14 day larvae 15 psu	14 day larvae 30 psu
Growth							
Dry Tet	79.0 (±1.26)	107.3 (±1.32)	156.9 (±0.94)	182.9 (±1.14)	0	205.2 (±0.95)	216.5 (±1.21)
Dry Tet+Dun	79.2 (±1.19)	117.8 (±1.22)	169.2 (±0.95)	179.3 (±1.33)	0	205.2 (±1.33)	222.2 (±1.41)
Dry Tet+Ske	78.8 (±1.28)	106.8 (±1.14)	158.6 (±1.53)	156.1 (±1.83)	0	195.5 (±1.02)	198.2 (±1.31)
Dry Tet+Iso	78.4 (±1.37)	102.7 (±3.14)	153.6 (±0.69)	172.8 (±1.05)	0	199.7 (±0.91)	221.1(±0.93)
% survival							
Dry Tet		5	25	33	0	24	29
Dry Tet+Dun		19	59	83	0	54	67
Dry Tet+Ske		4	66	89	0	58	80
Dry Tet+Iso		3	35	50	0	26	38

3.3.4.4. Growth of larvae

Results for the initial shell length of the *T. philippinarum* D-larvae, larval shell length on day 7 through to day 19 and the percentage survival of larvae from the 1st and 2nd broodstock 1992 are shown in Table 3.68. The growth coefficients of the larvae at day 10, 14 and 19 in both 1992 broodstocks are shown in Table 3.69.

The initial shell length of the larvae in the 2nd broodstock was significantly smaller than larvae from the 1st broodstock [$F1,3 = 1433.6$, $p < 0.001$]. In both broodstocks the initial shell length of larvae was not significantly different between diet treatments [$F3,3 = 0.94$, $p = 0.52$] In the 1st broodstock 1992 (spawning I), the initial shell lengths ranged from 95.3 µm to 97.2 µm and from 97.2 µm to 99.0 µm (spawning III). In the 2nd broodstock (spawning III), the initial shell lengths ranged from 78.4 µm to 79.2 µm (Table 3.69).

The shell length of larvae at day 14 in the 1st (spawning I) and 2nd broodstock (spawning III) 1992 was not significantly different [$F_{1,3} = 0.33$, $p = 0.61$]. In both broodstocks, larvae from dry *T. suecica*+ *S. costatum* fed broodstock were significantly smaller than larvae from the other treatments [$F_{3,3} = 10.73$, $p < 0.05$] (Figure 3.29). Although D-larvae in the 2nd broodstock were smaller than D-larvae in the 1st broodstock (Table 3.69), they grew to a similar size by day 14. The growth coefficient of larvae to day 14 in the 1st (spawning I) and 2nd (spawning III) broodstock 1992 was significantly lower in the dry *T. suecica*+*S. costatum* than other treatments [$F_{1,3} = 10.22$, $p < 0.05$]. In the 2nd broodstock 1992 the growth coefficient was significantly higher than in the 1st broodstock [$F_{1,3} = 217.61$, $p < 0.001$].

Table 3.68. Larval performance in natural sea water in relation to broodstock diet quality. Shell length (µm) and percentage survival of T. philippinarum larvae from both 1992 broodstocks are presented. Standard error in parentheses. For abbreviations see Table 3.42.

Spawning	Conditioning diet	% survival to D-larvae	Larvae (at day 10) % survival	Larvae (at day 10) Shell length (µm)	Larvae (at day 14) % survival	Larvae (at day 14) Shell length (µm)	Larvae (at day 19) % survival	Larvae (at day 19) Shell length (µm)
1st broodstock 1992								
I	Fsw	*	*	*	*	*	*	*
	Dry Tet	76.0	38.0	177 (±0.93)	38.0	218 (±1.60)	29	247 (±1.78)
	Dry Tet+Dun	89.0	55.8	176 (±0.15)	55.7	219 (±1.40)	26.7	230 (±3.63)
	Dry Tet+Ske	86.6	71.2	170 (±0.94)	71.2	207 (±1.32)	31.1	259 (±1.93)
	Dry Tet+Iso	67.8	64.4	182 (±0.98)	64.4	220 (±1.46)	36.8	252 (±1.95)
III	Fsw	0	0	0	0	0	0	0
	Dry Tet	70.5	*	*	*	*	*	*
	Dry Tet+Dun	57.2	30.9	218 (±1.31)	28.0	–	27.1	–
	Dry Tet+Ske	67.4	61.1	195 (±1.32)	42.0	257 (±1.93)	36.2	315 (±2.21)
	Dry Tet+Iso	27.2	*	*	*	*	*	*
2nd broodstock 1992								
III	Fsw	*	*	*	*	*	*	*
	Dry Tet	31.4	^32.5	^183 (±1.03)	28.8	217 (±2.54)	–	–
	Dry Tet+Dun	33.6	^82.5	^179 (±0.88)	67.0	222 (±1.83)	–	–
	Dry Tet+Ske	18.4	^88.8	^156 (±1.23)	80.0	198 (±1.65)	–	–
	Dry Tet+Iso	17.9	^50.0	^173 (±0.93)	37.5	221 (±1.82)	–	–

* not enough embryos to incubate

^ Larvae at day 7

Table 3.69. Growth coefficients (G) of T. philippinarum larvae in natural sea water in relation to broodstock diet quality from both 1992 broodstocks. For abbreviations see Table 3.42.

Spawning	Conditioning diet	Initial Shell length (μm)	Growth coefficients (G) Larvae (at day 10)	Larvae (at day 14)	Larvae (at day 19)
1st broodstock 1992					
I	Fsw	*	*	*	*
	Dry Tet	97.20	0.0599	0.0577	0.0491
	Dry Tet+Dun	95.25	0.0614	0.0595	0.0464
	Dry Tet+Ske	95.78	0.0574	0.0550	0.0524
	Dry Tet+Iso	95.92	0.0640	0.0593	0.0508
III	Fsw	0	0	0	0
	Dry Tet	98.95	*	*	*
	Dry Tet+Dun	97.24	0.0897	_	_
	Dry Tet+Ske	97.98	0.0765	0.0689	0.0615
	Dry Tet+Iso	98.11	*	*	*
2nd broodstock 1992					
III	Fsw	*	*	*	*
	Dry Tet	79.00	^0.1200	0.0722	_
	Dry Tet+Dun	79.20	^0.1165	0.0736	_
	Dry Tet+Ske	78.80	^0.0976	0.0658	_
	Dry Tet+Iso	78.40	^0.1131	0.0740	_

* not enough embryos to incubate
^ - Larvae at day 7

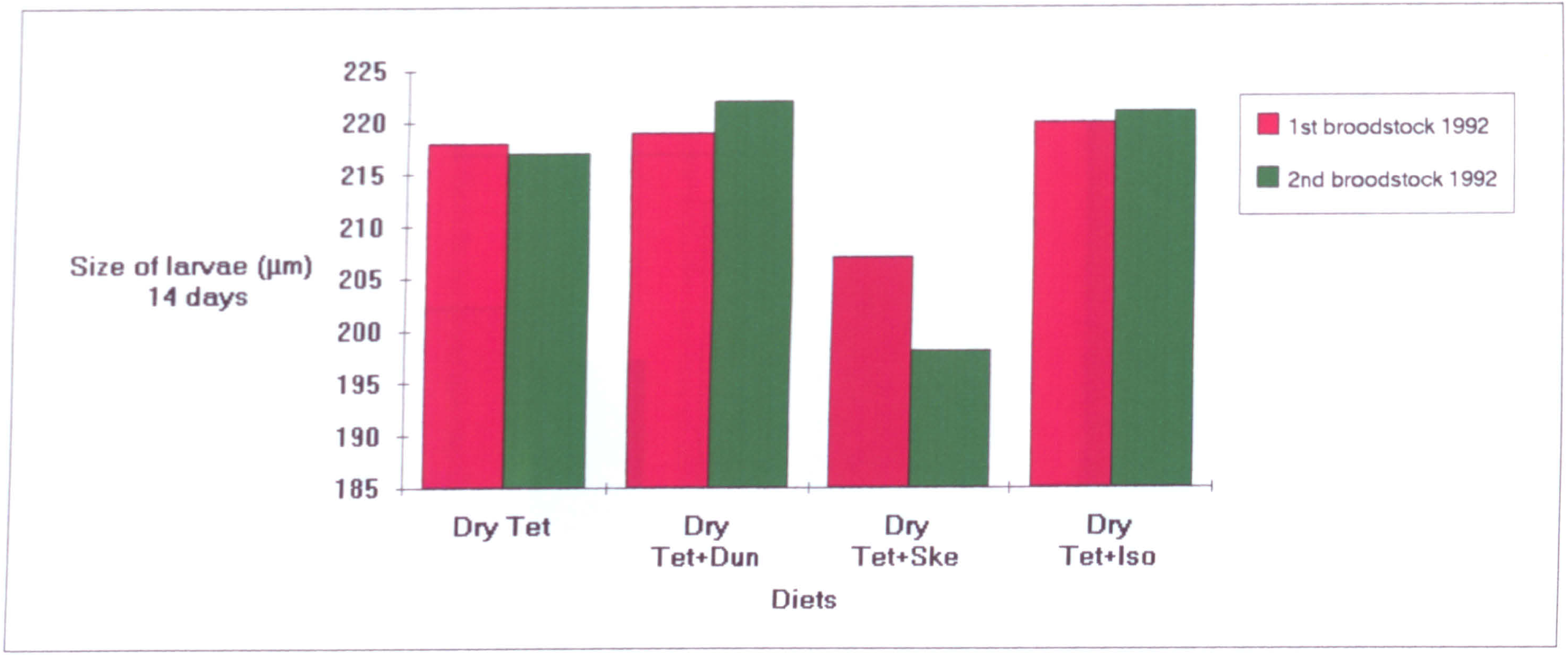


Figure 3.29. Size at 14 days in natural sea water of T. philippinarum larvae derived from adults conditioned in different diets in the 1st and 2nd broodstock 1992. For clarity, standard errors (given in Table 3.68) are not included in this Figure. For abbreviations see Table 3.42.

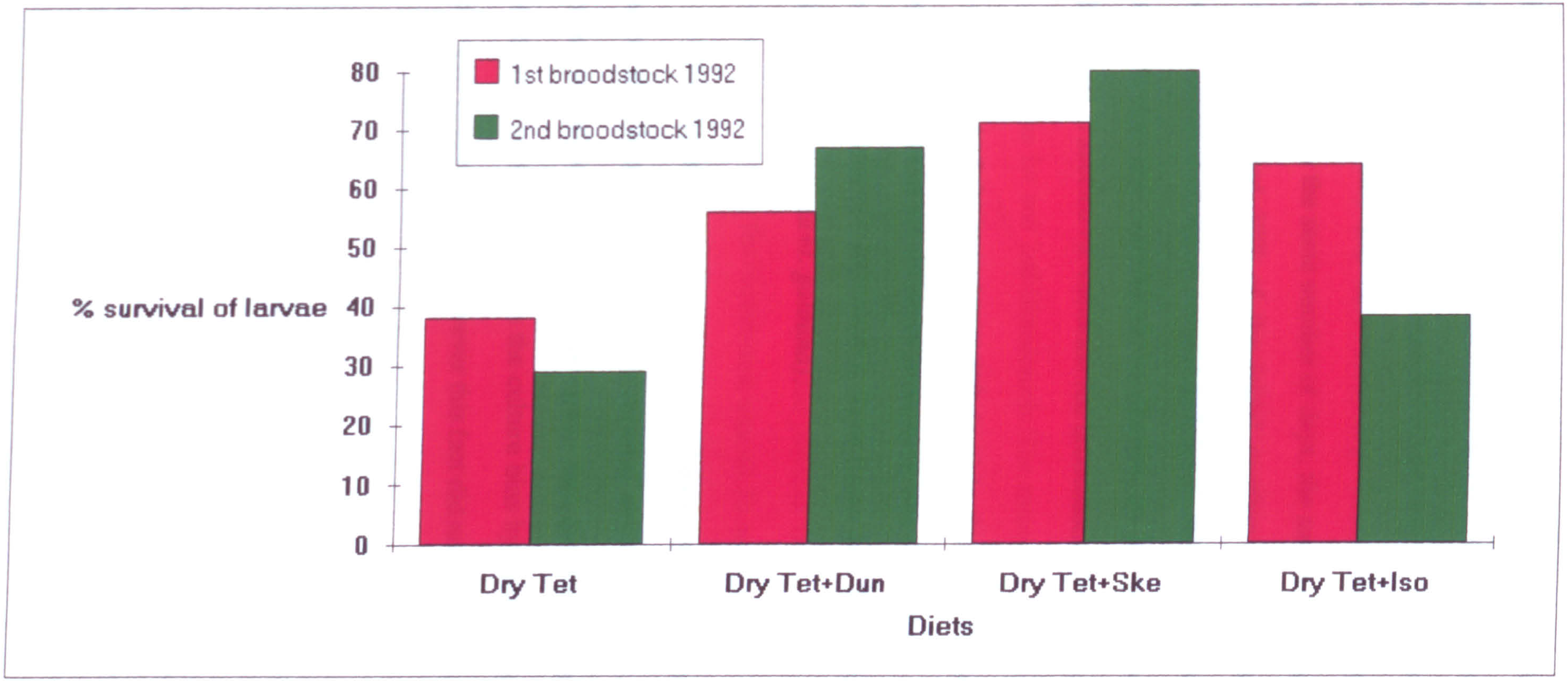


Figure 3.30. Percentage survival at 14 days in natural sea water of T. philippinarum larvae derived from adults conditioned in different diets in the 1st and 2nd broodstock 1992. For abbreviations see Table 3.42.

In the 1st broodstock 1992 (spawning I), the survival of larvae at day 14 was 38% (from dry *T. suecica*), 56% (from dry *T. suecica+D. tertiolecta*), 71% (from dry *T. suecica+S. costatum*) and 64% from dry *T. suecica+I. galbana* of the initial number (1.25 million). In spawning III, the survival of larvae was 28% (from dry *T. suecica+D. tertiolecta*) and 42% (from dry *T.suecica+S. costatum*). In the 2nd broodstock 1992 for the same number of days, the survival of larvae was respectively 29%, 67%, 80% and 38% of the initial number (0.45 million). The percentage survival of larvae from dry *T. suecica* and dry *T. suecica+I. galbana* fed broodstock decreased in the 2nd broodstock in relation to larvae from dry *T. suecica+D. tertiolecta* and *T. suecica+S. costatum* fed broodstock. (Figure 3.30.)

At the end of the experiment (19 days) in the 1st broodstock , the percentage survival of larvae in the different treatments was 29% (from dry *T. suecica*), 27% (from dry *T. suecica+D. tertiolecta*), 31% (from dry *T. suecica+S. costatum*) and 37% (from dry *T. suecica+I. galbana*).

In assessing survival, the numbers of larvae removed from each treatment for measuring shell length and percentage survival were taken into consideration. At the end of all experiments many of the larvae had developed into pediveligers which tended to crawl on the bottom on the culture bins by means of a foot and attached to surfaces with byssus threads, however this fact did not affect the survival estimations.

3.3.5. Sea water quality

3.3.5.1. Suspended particulate material

(i) Size composition and number of particles in suspension

The size distribution of suspended particulate material between 2.5 and 10 μm diameter, in sand-filtered sea water in the 1st and 2nd broodstock conditioning 1992 is shown in Appendix 17. Particles of 2.5 - 5.0 μm diameter accounted for 85.8 and 86.9% , while particles between 5.0 - 10 μm diameter represented 14.2 and 13.1% respectively in the 1st and 2nd broodstock. The mean total values (± standard error) of the three size groups 0.22 - 1, 2.5 - 5.0 and 5.0 - 10 μm diameter in both waters from 1st and 2nd broodstock are given in Table 3.70.

The mean total number of suspended particles (2.5 - 10 μm diameter) in the sand-filtered sea water during the experiment was 49.70 and 65.05 per μl respectively in the 1st and 2nd broodstock conditioning (Table 3.70).

The amount of suspended particulate material, between 2.5 - 5.0 and 5.0 - 10 μm, varied between weeks in both broodstock conditioning. The amount of 2.5 - 5.0 [$F_{1,14}$ = 0.94, p = 0.35] and 5.0 - 10 μm particles [$F_{1,14}$ = 0.31, p = 0.58] was not significantly different between broodstocks.

(ii) Dry weight, ash-free dry weight, lipid and PUFA content

The dry weights, ash-free dry weights, lipid and PUFA content of the suspended particulate material in the sand filtered sea water passing through the conditioning tanks is presented in Appendix 17 and Tables 3.70 and 3.71. The dry weights (> 1 μm diameter) were 9.33 and 10.97 mgl^{-1} and percentage ash-free dry weight content of

Table 3.70. Mean number, weight, lipid content and chlorophyll a of particles in sand-filtered sea water flowing through tanks in the hatchery (1st and 2nd broodstock 1992). Standard error in parentheses.

	Sand-filtered sea water
1st broodstock 1992	
Number of particles (/µl)	
2.5 - 5.0 µm	42.66 (± 9.28)
5.0 - 10 µm	7.04 (± 1.74)
Total dry weight (mg/l)	
0.22 - 1 µm	15.63 (± 2.01)
> 1 µm	9.33 (± 0.82)
Ash-free dry weight (mg/l)*	1.84 (± 0.23)
% ash-free dry weight*	19.71 (± 1.32)
Lipid (% Ash-free dry weight)	4.72 (± 0.63)
Chlorophyll a (µg/l)	1.14 (± 0.19)
2nd broodstock 1992	
Number of particles (/µl)	
2.5 - 5.0 µm	56.50 (± 10.84)
5.0 - 10 µm	8.55 (± 2.06)
Total dry weight (mg/l)	
0.22 - 1 µm	16.94 (± 1.80)
> 1 µm	10.97 (± 1.73)
Ash-free dry weight (mg/l)*	2.32 (± 0.30)
% ash-free dry weight*	22.37 (± 2.05)
Lipid (% Ash-free dry weight)	4.55 (± 0.47)
Chlorophyll a (µg/l)	2.18 (± 0.35)

* 2.5 - 5.0 µm diameter

Table 3.71. Fatty acid composition of the suspended particulates in sand-filtered sea water in both 1992 broodstocks. Values are percentages of total fatty acids.

Fatty acid	1st broodstock 1992	2nd broodstock 1992
14:0	31.68	32.84
16:0	27.34	23.22
18:0	9.38	5.73
16:1w9	0.00	0.00
16:1w7	2.90	5.24
18:1w9	20.45	19.13
18:1w7	0.00	0.99
20:1w9	0.00	0.00
18:2w6	2.04	2.15
18:3w3	0.00	0.00
18:4w3	0.00	1.94
20:2w6	6.20	3.41
20:3w3	0.00	0.00
20:4w6	0.00	1.85
20:5w3	0.00	2.41
22:5w3	0.00	0.51
22:6w3	0.00	0.59
Saturated	68.40	61.79
Monoenoic	23.35	25.36
Polyenoic	8.24	12.86

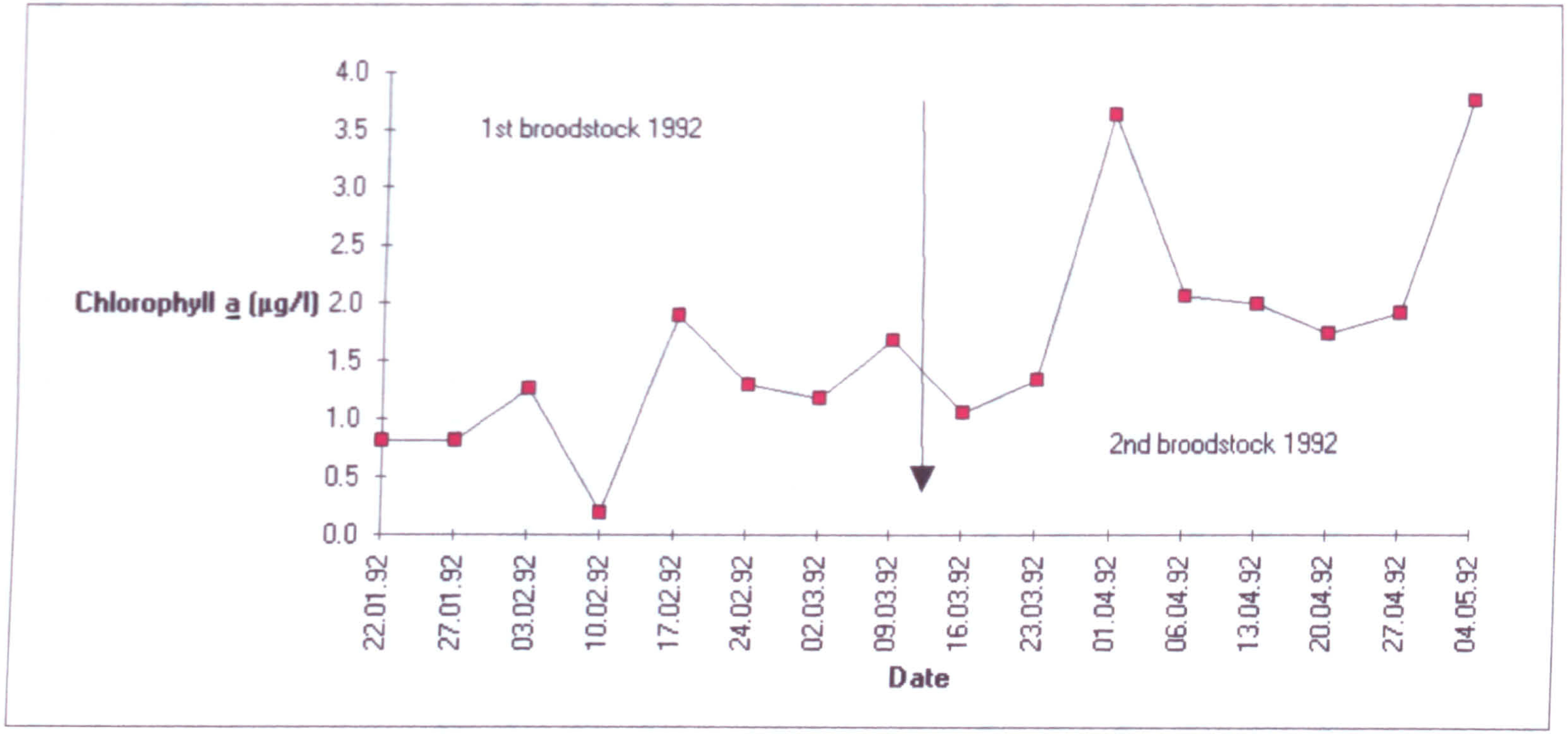


Figure 3.31. Chlorophyll a levels in sand-filtered sea water in 1st and 2nd broodstock 1992.

suspended particulates in the 1st and 2nd broodstock conditioning experiments were 19.71% and 22.37% respectively. Between broodstocks there were no significant differences in the dry weight [$F_{1,14} = 0.73$, $p = 0.41$] and percentage ash-free dry weight [$F_{1,14} = 1.19$, $p = 0.29$] of particles.

The lipid content of suspended particulate material in the sea water expressed as a percentage of the ash-free dry weight is shown in Appendix 17. Lipid content was usually around 5% of the ash-free dry weight of particles in both waters. Lipid content of sea water in the hatchery was variable and low. Lipid content was not significantly different in both broodstocks [$F_{1,14} = 0.05$, $p = 0.83$]. The suspended particulate material in sand-filtered sea water on both 1992 broodstocks was very low in PUFAs (Table 3.71).

3.3.5.2. Chlorophyll a determination

The occurrence of chlorophyll a in the sand-filtered sea water is shown in Table 3.70, Figure 3.31 and Appendix 17. Chorophyll a was variable in both broodstocks but was significantly higher in the 2nd broodstock conditioning because it was later in the year when there was more phytoplankton in the sea water taken from the Conwy estuary [$F_{1,14} = 6.84$, $p < 0.05$].

3.3.6. Summary of results

Once it had been confirmed that the nutritional value of dry *T.suecica* and live *T. suecica* were similar, the dry diet was used in the third part of this study. Dry *T.suecica* was used in combination with three live algae, namely *I. galbana*, *S. costatum* and *D. tertiolecta* which were each supplied as 30% of the diet by weight.

In the 1st and 2nd broodstock 1992 with 6 % food ration attempts to spawn the clams were made 3 times after 6, 7 and 8 weeks and after 5, 6 and 7 weeks of conditioning. Spawning condition was reached within 508 to 655 degree days (1st broodstock) and at 643 degree days (2nd broodstock) of conditioning. However, in this last broodstock clams had already spawned in the tanks at 5 weeks (462 D°).

The fecundity of the females was very variable within and between treatments in both broodstocks. Only one female clam from the unfed broodstock spawned a few eggs in both broodstocks. In all spawnings, all the fed treatments produced an appreciable number of eggs compared with the unfed control. After 7 weeks, in the 1st broodstock, significant higher numbers of eggs were produced in the dry *T.suecica+I. galbana* treatment followed by dry *T. suecica+S. costatum, T.suecica+D. tertiolecta* and dry *T. suecica* treatment. In the 2nd broodstock significantly higher numbers of eggs were produced in dry *T.suecica+I. galbana* than in any other treatments.

There were significant differences for meat dry weight, condition index, % ash-free dry weight, lipid and carbohydrate in clams from the different diet treatments in both broodstocks. In general live weight, length and breadth were the same. There were significant differences between male, female and non-spawning clams.

The lipid content of eggs spawned by *T. philippinarum* was not significantly different between broodstock diet treatments and between spawnings. Lipid content of larvae at day 14 as % of Afdwt was not significantly different from all the broodstock diet treatments.

The fatty acid composition of the lipid reserves in eggs was generally dependent on diet quality, ie levels of the essential PUFAs 20:5w3 and 22:6w3 in the eggs were low if the broodstock diet was deficient in these fatty acids. For example in the 1st broodstock 1992 (spawning I and II), eggs from the dry *T. suecica+D. tertiolecta* treatment had a high level of 18:3w3 and low levels of 20:5w3 and 22:6w3, eggs from dry *T. suecica+S. costatum* had higher levels of 20:5w3 and eggs from dry *T. suecica+I.galbana* had higher levels of 22:6w3, reflecting the PUFA composition of the algae.

The growth of larvae from all broodstock treatments was not significantly different between treatments (exception of dry *T. suecica+S. costatum*) and between spawnings.

Salinity stress was used (10, 15 and 30 psu) to test whether egg quality was important to survival of larvae from the different broodstock treatments. After 7 days, growth at 10 psu was significantly lower than other salinities. After 14 days with 15 and 30 psu, growth was significantly smaller than 30 psu. All clams had died after 14 days when reared at the lowest salinity of 10 psu.

Although Manila clams could be conditioned using only dried algae, mixed diets of live (30%, of *D. tertiolecta, S. costatum or I. galbana*) and spray-dried (70%, T. suecica) algae were generally better. The fecundity of broodstock fed with these diets was greater than for unfed animals.

Chapter 4

Chapter 4

Discussion

Gametogenesis in the Manila clam, as in other bivalves, is dependent on the sea water temperature and the availability of food. In the wild in temperate latitudes, gonad development in the Manila clam is related to the rise in sea water temperature in the spring. Millican and Williams (1985) showed that $10^{o}C$ was the minimum temperature to initiate gametogenesis in this species. It is likely that the fecundity of the clams and their condition after spawning would be dependent on the natural phytoplankton productivity.

Mann (1979) suggested that the time for conditioning based on the number of "day-degrees" (D^{o}) is critical for gametogenesis in bivalves. In this study, histological samples showed that none of the clam broodstocks in any of the treatments were ready for spawning before 5 weeks of hatchery conditioning (with the exception of the 2nd broodstock 1992). With a higher diet ration, 6% of a live or dry T. *suecica+S. costatum,* five weeks (equivalent to 385 D^{o}) was the minimum period of conditioning (2nd broodstock 1991,) before clams could be induced to spawn. Six weeks (equivalent to 508 D^{o}) was the minimum period of conditioning (1st broodstock 1992) before clams were induced to spawn with dry T. *suecica* on its own or mixed with *I. galbana*, *S. costatum* or *D. tertiolecta*. In the 2nd broodstock 1992, according to histology samples clams had partially spawned in the tanks at 5 weeks (equivalent to 462 D^{o}) or before. However seven weeks (equivalent to 643 D^{o}) was the minimum period of conditioning before these clams were induced to spawn again with dry T. *suecica* on its own or mixed with live algae. With a 3% diet of live *D. tertiolecta* or *S. costatum,* seven weeks (equivalent to 644 D^{o}) was the minimum period of conditioning (1st broodstock 1991) before clams could be induced to spawn. However, very few eggs were spawned. Clams with 3% live diet had a requirement of 738 D^{o} to produce larger numbers of good quality eggs.

Overall, the requirement for conditioning Manila clams (32 mm shell length) to spawn with live or dry T. *suecica+S. costatum* was 500 to 700 day D^{o}, equivalent to about 7 to 9 weeks. With dry T. *suecica* on its own or mixed with *I. galbana, S. costatum* or *D. tertiolecta*, it was 500 to 600 day D^{o} (44 mm shell length) equivalent to about 6 to 7 weeks. Beninger and Lucas (1984) found similar results for Manila clams in a commercial rearing pond in northern France, successful spawning occurred between 500 - 600 D^{o}. Studies in the laboratory at Conwy where food was never limiting, have shown that Manila clams can be spawned successfully after conditioning for 460 D^{o} (Kersuzan, 1989) with mixed diets of *T. pseudonana* and *T. suecica*.

Bivalves grown in good environmental conditions have high reserves of lipid and carbohydrate (Shaw et al., 1967; Quayle, 1969; Walne, 1970) and a higher fecundity than animals of similar size in poor conditions (Walne, 1964). Helm et al., (1973) and Rogers (1983) showed that *O. edulis* broodstock receiving a food supplement of *T. suecica* or a mixture of algal species during laboratory conditioning produced more larvae than oysters receiving only the natural phytoplankton in the sea water supply. Breber (1981) working on the carpet shell, *T. decussatus*, clearly showed the need to feed clams during conditioning.

The unfed broodstock produced no eggs or only a few compared with the broodstock conditioned with cultured algae supplements (2nd broodstock 1991 and in the 1st and 2nd broodstock 1992). The eggs produced from the unfed broodstock, indicated that at the time of the investigation some nutritional value was obtained from the natural phytoplankton to satisfy food requirements. The spring phytoplankton bloom had begun by the time that conditioning of the 2nd broodstocks in 1991 and 1992 was started. Chlorophyll a data show that a gradual increase in phytoplankton occurred during the trials. The higher concentration of phytoplankton during the 2nd broodstock 1991 compared with the others probably accounted for the production of eggs from the filtered sea water treatment in this experiment. Higher particle numbers (2nd broodstock 1991) in all the treatments increased the filtration rates of broodstock.

In filtered sea water, there were significantly higher concentrations of suspended particles (2.5-5.0 μm and 5-10 μm) during the conditioning of the 2nd broodstock 1991. Particles, which represented 34.64% of Afdwt were likely to have been phytoplankton cells which would have acted as food sources. Dissolved organic material is another source of nutrition in bivalves (Stewart, 1979; Wright, 1982) and would have been similar in all treatments within trials. Also, the role of bacteria in the feeding of bivalves has been referred to by Reid (1983), and bacteria may have been included in the conditioning of broodstock in the unfed treatment. Bayne et al. (1975), found that in mussels (*Mytilus edulis*) when they were starved more carbon was transferred to the eggs than when they were well fed. Clams which were fed, may have utilised food mainly for maintenance metabolism while unfed animals, with a reduced metabolic demand, transferred the carbon mainly to the developing eggs. The carbohydrate level of the unfed broodstock generally declined with advancing weeks of conditioning, while the lipid level was maintained. The total amount of lipid in the eggs from unfed broodstocks was similar to that in the eggs of fed broodstock.

The fecundity, expressed as total number of eggs produced per female was very low when the quantity of the diet was reduced to a 3% food ration (1st broodstock 1991). The effect of the quantity of live unialgal diets (3% and 6%) of *D. tertiolecta* and *S. costatum* is shown in Figure 4.1. Data for the 6% food ration were obtained from Leal (1990). This clearly shows that fecundity is related to food availability.

In Figure 4.2, 6% live, dry and mixed live and dry diets were compared. Data for *D. tertiolecta* and *T. suecica* were from Leal (1990) and for live *S. costatum* from Laing (pers. comm.). Fecundity was similar when each of the live diets was fed singly. The dry *T. suecica* proved to be a good diet on its own compared with live *T. suecica*.

When dry *T. suecica* was mixed with live algae, female clams generally produced more eggs. The exception was dry *T. suecica+D. tertiolecta*. There were very few eggs produced per female from the unfed treatment.

The mean number of eggs, either as the total per broodstock treatment or as per female clam, generally increased as the quantity and/or quality of lipid in the broodstock diet increased. As reported by Holland (1978), Waldock and Nascimento (1979) and Chu and Dupuy (1980) the lipid rather than the protein and carbohydrate content determined the food value of an algal species. Female clams from the *S. costatum* treatment (1st broodstock 1991) and from the dry *T. suecica+S. costatum* and dry *T. suecica+I. galbana* (1st and 2nd broodstock 1992, after 567 to 643 D^{o}) produced significantly higher numbers of eggs. These algae were higher value species in terms of lipid quality compared with the others tested.

Generally the initial condition index of adult clams was similar in all the broodstock conditioning trials since clams were collected prior to the period of conditioning. The exception was the 1st broodstock 1991, where the initial condition index was lower.

A decrease was found in the condition index of clams from the 1st broodstock 1991 at the end of the conditioning period in all diet treatments which was assumed to be related to the 3% diet fed to the broodstock. The condition index of clams in the 2nd broodstock 1991 had increased at the end of the conditioning period. Clams fed on live and dry *T. suecica+S. costatum* improved in condition, suggesting that lipid was incorporated into body reserves and both diets produced significantly higher numbers of eggs. In the 1st broodstock 1992, clams fed on dry *T. suecica* alone were conditioned faster than clams fed dry *T. suecica* mixed with live diets. Overall during the conditioning of Manila clam broodstocks there was a clear trend of carbohydrates decreasing with time, and the lipids either staying the same or increasing as the length of the conditioning period increased.

In the 1st broodstock 1992 at the end of the conditioning period, clams fed on dry *T. suecica+I. galbana* had improved in condition, suggesting as above that lipid was incorporated into body reserves to produce the higher numbers of eggs. Clams fed on dry *T. suecica* and dry *T. suecica+S.costatum* maintained their condition, yet still produced large numbers of eggs. A loss in condition index was found in clams in filtered sea water.

The lipid content of eggs spawned by *T. philippinarum* during this investigation was 6.5 - 8.8 ng egg-1 (20 and 36% of ash-free dry weight) in the 1st broodstock 1991, and 7.2 - 9.1 ng egg-1 (24 and 36% of ash-free dry weight) in the 2nd broodstock 1991. In the 1st and 2nd broodstock 1992 lipid was 4.2 - 7.8 ng egg-1 (15 and 38% of ash-free dry weight) and 4.6 - 7.7 ng egg-1 (18 and 29% of ash-free dry weight. These results were low compared with previous data of Lovatelli (1985) where total lipid values were 9.3 - 13.5 ng egg^{-1} (at 560 D^{o}).

The amount of lipid in each egg (as ng) was not significantly different in any of the treatments and was not related to broodstock diet. However, the number of eggs released could have been related to the amount of lipid available in the diet of the female clams. It was of note that lipid per egg was higher, by approximately 1 ng egg-1, in spawning III compared with spawning II in both 1991 broodstocks. This may have resulted from the higher concentrations of nanoplankton and picoplankton (as shown by chlorophll a data) or dissolved organic material in the sea water. There may have been an alternative or additional reason for the higher lipid per egg in spawning III compared with spawning II. Glycogen reserves in broodstock clams which were higher in spawning II (2nd broodstock 1991), may have been utilised during further gametogenesis and converted to lipid in eggs in spawning III.

Helm (1977) showed that lipid in *C. gigas* eggs varied with time of year and between years. This variation was correlated with chlorophyll-a in the sea water. Such changes in egg quality resulting from seasonal changes in the nutritional value of naturally occurring phytoplankton, filtered by adult bivalves are important to the viability of embryonic and larval stages. Lipid is the major energy resource of many marine invertebrate larvae, by virtue of its ease of storage and its abundance in the marine environment (Holland, 1978). For example, lipid is the primary energy reserve of *O. edulis* larvae (Millar and Scott, 1967; Holland and Spencer, 1973). The viability of *O. edulis* larvae is related to their lipid content at the time of liberation (Helm et al. 1973) which in turn is dependent on the conditions under which broodstock are held during gametogenesis.

In *Mercenaria mercenaria* and *Crassostrea virginica*, 48% and 55% respectively, of the egg lipid reserves were utilized within 24 h of fertilization at 25°C (Gallagher et al., 1986). In this study, the utilisation of lipid during egg development of Manila clams was between 35 and 50% (1st broodstock 1991), 14 and 26% (2nd broodstock 1991), 4 and 64% (1st and 2nd broodstock 1992) of the egg reserves. During development to the D-larvae, there was generally a reduction in the polyenoic fatty acids.

The total lipid content of the D-larvae in this study was between 3.3 and 5.6 ng larva^{-1} (1st broodstock 1991), 5.1 and 7.7 ng larva^{-1} (2nd broodstock 1991) and 2.1 and 6.4 ng larva^{-1} (1st and 2nd brodstock 1992). This was also low when compared with values of 6.1-13.9 ng larva^{-1} for *T. philippinarum* D-larvae which yielded good survival in earlier trials (Lovatelli, 1985).

Certain polyunsaturated fatty acids, in particular, eicosapentaenoic acid, 20:5w3, and docosahexaenoic acid, 22:6w3, are considered important in the nutrition of bivalve larvae (Webb and Chu, 1983). However, Helm and Laing (1987) and Laing et al.

(1990) suggested that Manila clams have no dietary requirement for these fatty acids and that they can synthesize them by chain elongation (ie, Manila clam larvae have the ability to elongate fatty acids of medium chain length, such as 18:3w3, and produce 22:6w3). The ability to elongate and desaturate short-chain fatty acids has been demonstrated also by Waldock and Holland (1984) with the oyster *C. gigas*.

Although the quantity of lipid was not affected by broodstock diet, the fatty acid composition of bivalve eggs can be manipulated through diet. In spawning II and III (2nd broodstock 1991) the eggs from *T.suecica+S.costatum*, dry *T. suecica+S. costatum* and the filtered sea water treatment contained high quantities of 22:6w3. It was also interesting that the fatty acid composition of eggs released from the filtered sea water treatment in spawning II and III (2nd broodstock 1991) were high in 20:5w3 and 22:6w3. It was impossible to assess whether these polyunsaturated fatty acids came from the sea water during the conditioning period in particles < 1μm such as bacteria or from initial reserves laid down in broodstock prior to laboratory conditioning. Replicate samples of *T. philippinarum* meats from the filtered sea water treatment (2nd broodstock 1991, spawning II) gave different values for 20:5w3 and 22:6w3.

Generally eggs contained fatty acids similar to those provided in the diets during conditioning. For example, in spawning II (1st broodstock 1991) the higher levels of 20:5w3 in eggs from the *S. costatum* broodstock and of 18:3w3 in eggs from the *D. tertiolecta* treatment were probably derived from the diet. However, the eggs from the *D. tertiolecta* treatment also contained some 20:5w3 and 22:6w3. As this alga does not have C_{20} and C_{22} PUFAs, these fatty acids in this treatment may have come from the sea water (from nannoplankton, picoplankton, bacteria or dissolved organic material), from maternal sources (from reserves laid down in the parent before hatchery conditioning) or from elongation and desaturation (Helm and Laing, 1987). The presence of 20:4w6 in eggs from the *D. tertiolecta* broodstock also may have resulted from the pathway of chain elongation.

T. suecica has limited amounts of lipid and of long-chain polyunsaturated fatty acids (e.g. 20:5w3 and 22:6w3) (Webb and Chu, 1983). The nutritional value of *T. suecica* was enhanced by feeding it as part of a mixed diet with, for example, *S. costatum* (Laing and Millican, 1986). In this study live *T. suecica* and spray dried *T. suecica* were compared in mixtures consisting of 70% *T.suecica* and 30% *S. costatum*. Laing and Millican (1991) found that this mixture gave the best growth for Manila clam juveniles. Once it had been confirmed that the nutritional value of live *T. suecica* and spray-dried T. suecica were similar, the dry diet was used in the third part of this study. Dry *T. suecica* was used in combination with three live algae, namely *I.galbana, S. costatum* and *D. tertiolecta* which were each supplied as 30% of the diet by weight.

Differences in lipid composition of eggs did not result in growth variations of larvae when cultured on a semi-commercial scale in natural sea water (1st and 2nd broodstock 1991, 1st and 2nd broodstock 1992 (with the exception of *S. costatum*)). Larvae reared to the pediveliger stage in natural sea water generally grew at similar rates, irrespective of broodstock treatment. Work on bivalve nutrition suggests that growth performance of bivalve larvae was not only related to total lipids in algal diets, but the more important factor determining the nutritional value of algae was their polyunsaturated fatty acid content (Langdon and Waldock, 1981). These authors showed that a dietary deficiency of 20:5w3 and 22:6w3 fatty acids limited growth. As cultured algae can be deficient in one or more nutrients required by bivalves, a mixed diet was fed in the semi-commercial scale trials since these support better growth than single species diets (Helm, 1977; Walne, 1970; Epifanio, 1976; Laing and Millican, 1986). The combination of *I. galbana* and *C. calcitrans* was fed to larvae in the present study since Helm and Laing (1987) found this to be the best diet for Manila clam larvae.

Metamorphosis of bivalve larvae may be dependent on a minimum level of stored lipid rather than on larval size. In the natural environment differences in polyunsaturated fatty acids content and composition of *O.edulis* larvae may account for irregular and unreliable recruitment, particularly when the quality and quantity of diets available to adults and larvae are less than optimal (Helm, et al., 1991). It was interesting that, prior to the utilisation of lipid during metamorphosis, larvae from the *S. costatum* or *D. tertiolecta* treatments (1st broodstock 1991) accumulated the same amount of lipid (148 ng larva^{-1}) before the drop in lipid was observed. The same happened for live or dry *T. suecica* (2nd broodstock 1991) that accumulated the same amount of lipid (80 ng) before metamorphosis started.

The semi-commercial scale trials were carried out under optimal conditions. However, sea water varies considerably in its ability to support larval growth and development (Millar and Scott, 1967; Helm, 1971; Utting and Helm, 1985). This indicates that there are factors of water quality which override the effect of lipid reserves in influencing larval vigour. Larvae with poor initial nutrient reserves might be more susceptible to additional stresses in the environment, including those imposed by changes in water quality. Therefore, larvae were grown under salinity stress and after 7 days, growth at 10 and 15 psu was reduced compared with 30 psu There were no significant differences between broodstock diet treatments. Robinson and Breese (1984) showed that at low salinities of 10 and 15 psu larval growth was slow and larvae did not survive to metamorphosis. After 14 days, larvae were significantly smaller at 15 psu than 30 psu but there was no significant difference between diet treatments at either salinity. All clams had died after 14 days when reared at 10 psu. This showed that broodstock diet did not affect growth of larvae.

Even though diet manipulation caused changes in fecundity, condition of broodstock and in fatty acid composition of the eggs produced, the growth and survival of larvae in a hatchery situation, as shown by semi-commercial scale trials, was not reduced provided a diet containing 20:5w3 and 22:6w3 was fed to larvae.

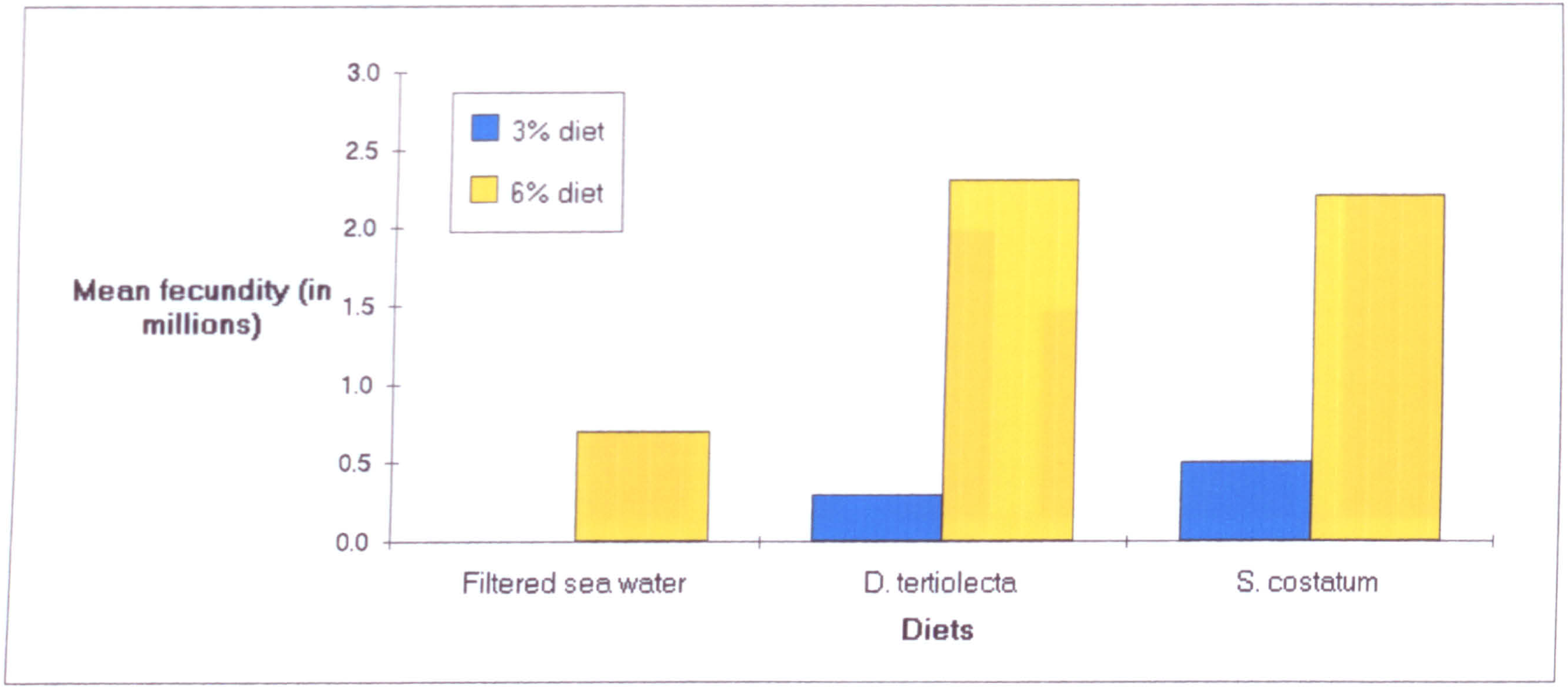


Figure 4.1. Effect of diet quantity on fecundity of T. philippinarum. Diets were 3% and 6% of clam dry meat weight of D. tertiolecta or S. costatum per day.

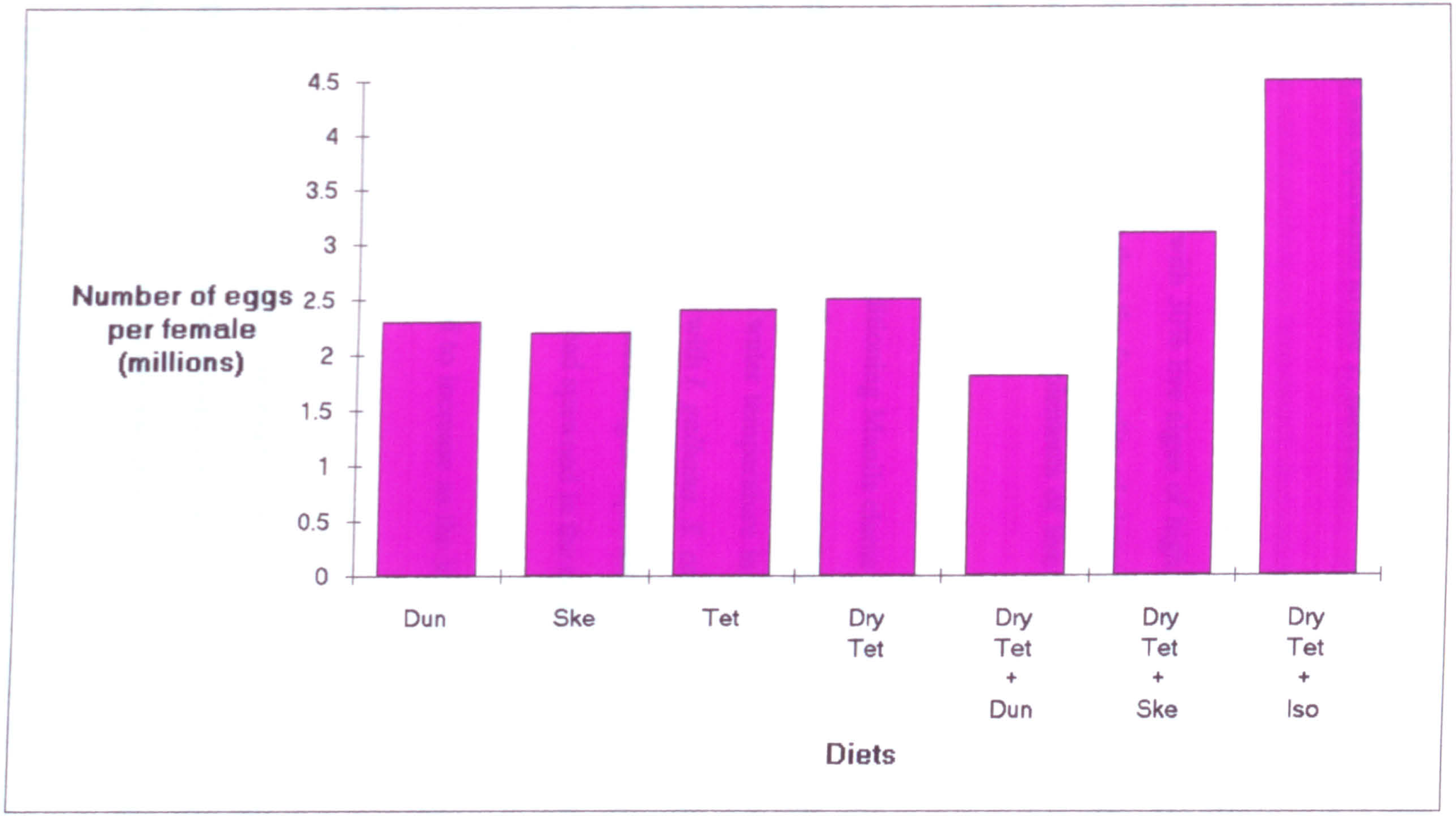


Figure 4.2. Effect of diet quality provided during conditioning on fecundity of T. philippinarum.
For abbreviations see Table 3.21 and 3.42.

Conclusion

1. The dry *T. suecica* was a suitable diet for conditioning broodstock Manila clams in hatcheries, and is more convenient and economical to use. As a single species diet dry *T. suecica* was equivalent to live *T. suecica*.

2. This dry diet can be used mixed with live algae. The fecundity of clams fed a mixed diet of 70% dry algae with 30% live algae of high nutritional value (ie *S. costatum* and *I. galbana*) was higher than the fecundity of clams on a diet of 100% dry algae. Manila clams produced more eggs if supplements of live algae were added (*I. galbana* > *S. costatum*>*D. tertiolecta)*.

3. The requirement for conditioning Manila clams (32 mm mean shell length) to spawn with live or dry T. *suecica*+*S. costatum* was 500 to 700 day D^{o}, equivalent to about 7 to 9 weeks at a constant sea water temperature in the laboratory of 21^{o}C. With dry T. *suecica* on its own or mixed with *I. galbana*, *S. costatum* and *D. tertiolecta* it was 500 to 600 day D^{o} (44 mm mean shell length) equivalent to about 6 to 7 weeks. In one trial (2nd broodstock 1992) clams had spawned in the tanks before 462 D^{o}.

4. Fecundity of clams tended to increase as the level of essential fatty acids in the diet increased.

5. The total amount of lipid in the eggs varied between 4 to 9 nanograms per egg and within trials was not related to broodstock diet treatment. The number of eggs released was generally related to the amount and quality of lipid available to the female clam.

6. The profile of the polyunsaturated fatty acids in the eggs was generally a reflection of the broodstock diet. For example, 18:3w3 was high in eggs from *D. tertiolecta*,

20:5w3 from *S. costatum* and 22:6w3 was high in eggs from *I. galbana* all of which reflected broodstock diet composition.

7. Growth rates of larvae under good environmmental conditions were usually similar and irrespective of broodstock diet treatment. Even under salinity stress, growth of larvae was not dependent on broodstock diet treatment.

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Appendices

Appendix 1 .

Analysis of polyunsaturated fatty acids (PUFA) of eggs and larva (Lepage and Roy,1984)

Extraction of total lipid

1. Use the egg and larval samples preserved for PUFA analysis in the freezer.
2. Place in conical tube and extract with 5 ml of chloroform: methanol (2:1 v/v).
3. Centrifuge at 2,500 rpm for 5 minutes.
4. Decant supernatent into measuring cylinders. Add 5 ml of chloroform: methanol, decant and repeat.
5. Add the required amount of chloroform: methanol up to the 25 ml mark on the measuring cylinder.
6. Pipette 5 ml of 0.017 % of Magnesium chloride into the soution and replace stoppers.
7. Use nitrogen gas supply to remove the air from within the measuring cylinders.
8. Shake each measuring cylinder for approximately 20 seconds and then loosen stoppers to release the pressure.
9. Transfer the solution into centrifuge tubes.
10. Centrifuge at 2,500 rpm for 5 minutes.
11. Use the suction apparatus to remove the top layer of the separated solutions.
12. Add to the centrifuge tubes 1 ml. of Folch solution and hand swirl for a short period of time.
13. Centrifuge at 2,500 rpm for 5 minutes.
14. Again, remove the top layer of the separated layers using the suction apparatus.
15. Filter the remaining solution into small,round condenser flasks.
16. Connect the condenser flasks to the condenser and evaporate the liquid under vacuum with water bath at 50 ^{o}C.

Transesterification of lipids to FAMES

17. Add 0.5 ml of chloroform to the flasks and hand swirl.
18. Pipette 0.1 ml of the solution into the reacti - vials.
19. Evaporate the solution using the nitrogen gas supply until a small amount of liquid remains.
20. Add 3 ml of acetyl chloride in methanol to reacti - vials.
    (1 ml acetyl chloride in 50 ml methanol).
21. Mix with vortex.
22. Place in the oven for an hour.
23. Mix 6 g of sodium carbonate with 100 ml of distilled water.
24. Pipette 3 ml of sodium carbonate into conical tubes.
25. Add 1 ml of hexane; replace stoppers in tubes and shake.
26. Centrifuge at 2,500 rpm for 5 minutes.
27. Use a Pasteur pipette to remove the top layer and discard the bottom layer.
28. Place the solutions in sample tubes for GC analysis.

Appendix 1. (continued)

Separation of neutral and polar lipid fractions.

To separate the triglycerides and phospholipids from a sample the above procedure is carried out, plus the following ones commensing at stage 20.

Prepare silica gel columns.

a) Push a small piece of cotton wool into a Pasteur pipette.
b) Heat silicic acid in an oven at 120°C for one hour. Remove and allow to cool.
c) Weigh 0.2 g of silicic acid into each conical tube and add 5 ml of chloroform, mix with the vortex.
d) Connect the nitrogen line to the Pasteur pipettes to force the chloroform extract through the column. Pipette 0.4 ml lipid extract into silica gel column.
e) Elute with 5 ml of chloroform, retain chloroform extract.
f) Add 5ml of methanol to the column and force the methanol extract through the column. Retain the methanol extract.
g) Place solutions into the reacti - vials.
h) Evaporate down the solution with the reacti - therm, set at a temperature of 50°C
i) Label sample tubes in the following way:

TAG for chloroform extracts (triglycerides)
PL for methanol extracts (phospholipids)

j) Add 3 ml acetyl chloride in methanol to transesterify lipids (see step 20). Continue the remaining stages from 21 to the end.

Appendix 2. Condition indices and biochemical composition of 1st T. philippinarum broodstock 1991. Values are given for initial and conditioned for 6, 7 and 8 weeks with different diets.

Date: 21.01.91 **Ref.:** 1st broodstock conditioning 1991 - Initial

Species *Tapes philippinarum*

Clams nº	Live weight g	Length mm	Breadth mm	Meat dry weight, g	Condition index	% Afdwt	Lipid (% Afdwt)	Carbohydrate (% Afdwt)	Protein (% Afdwt)
1	9.3	33	24	0.33	56.71	73.50	6.23	6.27	87.50
2	8.4	24	23	0.30	85.20	80.00	5.84	6.65	87.51
3	5.0	28	19	0.21	95.58	75.00	4.52	5.11	90.37
4	8.9	33	24	0.35	79.88	74.40	5.20	3.75	91.05
5	7.5	29	23	0.25	84.85	72.40	5.97	3.95	90.08
6	9.2	33	26	0.34	90.16	78.40	5.22	6.17	88.61
7	8.0	32	22	0.27	81.24	75.10	4.81	3.90	91.29
8	10.2	34	25	0.42	102.48	73.70	6.32	6.06	87.62
9	7.5	33	23	0.34	92.25	73.40	4.88	5.38	89.74
10	8.5	32	24	0.32	82.92	68.70	5.59	3.96	90.45
11	9.0	32	23	0.34	82.22	71.10	5.18	5.71	89.11
12	10.0	35	25	0.40	91.78	75.50	5.66	4.19	90.15
13	7.3	29	22	0.28	89.62	70.40	6.19	4.83	88.98
14	9.3	34	24	0.36	90.67	74.30	4.51	5.42	90.07
15	8.6	33	24	0.33	79.20	71.10	5.56	5.89	88.55
16	9.4	37	26	0.40	93.91	83.80	4.52	6.86	88.62
17	9.3	34	24	0.37	92.64	74.40	4.37	6.99	88.64
18	10.1	36	25	0.41	81.83	73.50	4.73	5.74	89.53
19	6.1	30	21	0.26	90.28	74.40	4.64	4.84	90.52
20	6.8	31	21	0.26	85.82	71.90	5.87	5.91	88.22
21	8.6	31	24	0.32	92.13	74.60	6.11	6.29	87.60
22	7.3	31	23	0.28	81.30	72.50	6.25	6.11	87.64
23	9.2	33	25	0.34	80.88	73.20	5.75	4.90	89.35
24	5.2	28	20	0.19	79.46	68.50	6.00	4.48	89.52
25	7.3	30	22	0.28	86.23	71.70	4.57	3.56	91.87
26	10.1	34	25	0.35	77.62	72.00	6.31	7.36	86.33
27	6.7	30	22	0.26	81.20	73.20	5.29	8.47	86.24
28	9.6	34	24	0.36	85.63	73.50	4.99	5.99	89.02
29	9.5	35	24	0.37	80.45	75.40	5.42	4.60	89.98
30	4.6	27	19	0.17	81.96	73.20	6.35	3.29	90.36
Mean	8.21	31.75	23.20	0.32	85.20	73.76	5.43	5.42	89.15
SE	0.28	0.52	0.34	0.01	1.47	0.55	0.12	0.23	0.26

Appendix 2a. Broodstock of T. philippinarum conditioned for 6 weeks with a diet of D. tertiolecta.

Date:	6.03.91			Ref.: 1st broodstock conditioning 1991 - 1st spawning (6 weeks)					
Species	*T. philippinarum*			Diet: *D. tertiolecta*					
Clams nº	**Live weight g**	**Length mm**	**Breadth mm**	**Meat dry weight, g**	**Condition index**	**% Afdwt**	**Lipid (% Afdwt)**	**Carbohydrate (% Afdwt)**	**Protein (% Afdwt)**
D. tertiolecta									
Non-spawners									
1	8.1	32	22	0.23	71.03	82.33	16.52	6.10	77.38
2	8.5	33	25	0.41	105.82	85.12	16.21	5.78	78.01
3	9.0	34	24	0.37	105.31	79.44	17.12	6.80	76.08
4	8.6	32	24	0.27	51.37	85.19	18.55	5.02	76.43
5	9.2	34	24	0.27	63.45	84.22	15.44	5.50	79.06
6	8.7	34	25	0.28	59.76	83.53	13.53	6.52	79.95
7	9.6	34	24	0.22	43.00	84.75	16.40	4.45	79.15
8	9.0	32	26	0.32	69.93	81.87	16.12	6.50	77.38
9	9.3	33	25	0.28	63.91	84.10	12.13	8.10	79.77
10	8.4	33	24	0.27	65.00	83.93	14.54	5.59	79.87
11	7.8	32	26	0.23	60.55	78.50	16.43	6.11	77.46
12	8.5	33	24	0.29	69.00	85.07	15.28	5.60	79.12
13	8.2	32	24	0.23	56.73	84.23	14.72	4.95	80.33
14	7.2	29	24	0.21	67.29	83.72	17.92	6.32	75.76
15	8.1	33	23	0.19	49.85	82.69	15.48	5.60	78.92
Mean	8.55	32.67	24.27	0.27	66.80	83.25	15.76	5.93	78.31
SE	0.16	0.33	0.27	0.02	4.55	0.52	0.42	0.23	0.39
D. tertiolecta (replicate)									
Non-spawners									
1	8.3	32	25	0.35	69.49	86.29	15.07	8.60	76.33
2	9.8	35	24	0.37	90.93	82.78	13.65	5.03	81.32
3	9.0	32	24	0.29	67.07	86.07	17.66	7.18	75.16
4	11.0	36	29	0.35	62.23	84.15	16.16	5.21	78.63
5	10.2	36	27	0.42	80.19	82.80	13.89	6.85	79.26
6	8.8	33	24	0.30	72.26	80.86	18.06	6.25	75.69
7	9.5	34	23	0.36	83.49	83.57	17.23	5.70	77.07
8	7.7	32	23	0.29	78.84	79.64	15.95	5.59	78.46
9	11.7	36	28	0.45	76.41	82.72	13.90	5.83	80.27
10	7.2	31	24	0.24	65.22	80.38	13.81	6.02	80.17
11	8.2	32	25	0.29	71.46	82.76	11.12	5.63	83.25
12	7.9	31	22	0.27	75.63	83.75	13.36	5.08	81.56
13	11.0	36	29	0.39	69.32	85.29	13.13	4.58	82.29
14	11.3	37	25	0.36	76.68	84.99	9.65	5.05	85.30
15	7.8	31	22	0.26	74.97	83.35	11.04	7.92	81.04
Mean	9.31	33.60	24.93	0.33	74.28	83.29	14.25	6.03	79.72
SE	0.38	0.57	0.60	0.02	1.91	0.50	0.64	0.30	0.75

Appendix 2a (continued). Broodstock of T. philippinarum conditioned for 6 weeks with a diet of S. costatum.

Date:	6.03.01			Ref.: 1st broodstock conditioning 1991 - 1st spawning (6 weeks)					
Species	*T. philippinarum*			Diet: *S. costatum*					
Clams nº	Live weight g	Length mm	Breadth mm	Meat dry weight, g	Condition index	%Afdwt	Lipid (%Afdwt)	Carbohydrate (%Afdwt)	Protein (%Afdwt)
S. costatum									
Non-spawners									
1	11.1	34	26	0.35	68.04	85.73	10.03	5.80	84.17
2	10.2	33	25	0.31	65.83	82.24	10.82	5.06	84.12
3	8.8	32	23	0.33	87.74	86.17	7.89	9.77	82.34
4	7.9	32	24	0.28	76.32	85.08	9.40	4.56	86.04
5	9.3	33	26	0.38	88.60	81.61	10.29	5.15	84.56
6	7.5	32	23	0.31	83.70	84.96	7.42	6.93	85.65
7	10.3	35	25	0.42	84.02	85.52	11.81	4.91	83.28
8	8.0	32	23	0.27	68.15	83.69	10.40	3.76	85.84
9	8.6	32	25	0.29	71.40	80.62	9.68	3.91	86.41
10	8.3	34	25	0.30	67.41	80.93	8.28	3.04	88.68
11	8.8	32	23	0.30	70.74	82.99	8.19	5.10	86.71
12	9.1	32	25	0.32	73.41	81.09	9.00	6.79	84.21
13	8.7	32	24	0.33	79.76	83.83	8.59	4.64	86.77
14	8.5	32	23	0.30	77.77	74.73	7.90	3.29	88.81
15	8.0	31	25	0.30	79.97	83.36	8.76	6.13	85.11
Mean	8.89	32.53	24.33	0.32	76.19	82.84	9.23	5.26	85.51
SE	0.26	0.27	0.29	0.01	1.96	0.75	0.32	0.44	0.47
S. costatum (replicate)									
Non-spawners									
1	9.1	34	27	0.33	73.69	79.55	8.55	5.49	85.96
2	9.3	33	24	0.34	77.70	80.29	8.22	4.02	87.76
3	10.6	34	27	0.33	69.43	80.49	7.70	3.64	88.66
4	9.1	33	24	0.33	73.89	81.92	7.57	5.14	87.29
5	10.2	34	27	0.31	62.96	79.36	8.95	3.84	87.21
6	7.4	32	23	0.30	78.11	84.49	9.47	3.78	86.75
7	10.1	33	25	0.34	74.59	85.81	9.32	3.92	86.76
8	9.8	36	25	0.34	70.83	80.90	7.91	5.43	86.66
9	9.0	35	25	0.40	91.32	81.00	7.41	5.95	86.64
10	9.7	35	25	0.32	63.35	80.28	7.10	3.43	89.47
11	9.6	33	26	0.36	78.78	81.48	7.36	4.25	88.39
12	10.2	31	25	0.31	70.81	81.61	8.09	3.26	88.65
13	9.1	34	25	0.40	86.93	83.63	8.49	6.92	84.59
14	8.0	33	24	0.21	54.63	78.94	7.73	3.15	89.12
15	7.1	30	22	0.25	82.37	84.75	8.02	5.06	86.92
Mean	9.24	33.33	24.93	0.32	73.96	81.63	8.13	4.49	87.39
SE	0.26	0.40	0.37	0.01	2.43	0.54	0.18	0.29	0.34

Appendix 2a (continued). Broodstock of T. philippinarum conditioned for 6 weeks with filtered sea water.

Date:	6.03.91			Ref.: 1st broodstock conditioning 1991 - 1st spawning (6 weeks)					
Species	*T. philippinarum*			Diet: Filtered sea water (Fsw)					
Clams nº	Live weight g	Length mm	Breadth mm	Meat dry weight, g	Condition index	% Afdwt	Lipid (%Afdwt)	Carbohydrate (% Afdwt)	Protein (% Afdwt)
Fsw									
Non-spawners									
1	10.4	36	25	0.26	66.03	78.22	8.05	3.92	88.03
2	11.7	37	26	0.35	67.96	78.83	7.01	3.31	89.68
3	10.4	34	26	0.22	45.46	81.67	7.71	3.62	88.67
4	7.4	30	23	0.20	51.03	76.55	7.84	3.19	88.97
5	9.8	32	25	0.25	50.53	75.52	7.55	3.46	88.99
6	10.8	37	27	0.29	54.98	80.45	7.21	3.34	89.45
7	9.4	35	26	0.29	61.81	82.56	7.99	3.56	88.45
8	8.7	34	23	0.21	46.86	81.88	7.45	3.10	89.45
9	10.5	37	25	0.23	40.88	79.76	7.27	3.12	89.61
10	7.6	32	24	0.21	61.91	76.81	7.68	3.20	89.12
11	9.8	36	27	0.24	46.94	78.60	7.12	3.44	89.44
12	8.5	33	23	0.21	49.55	78.33	7.40	3.24	89.36
13	6.5	31	24	0.16	47.21	78.81	7.11	2.87	90.02
14	8.7	33	25	0.26	59.58	81.22	7.02	2.39	90.59
15	8.1	32	25	0.24	61.26	80.89	8.28	2.91	88.81
Mean	9.23	33.93	24.93	0.24	54.13	79.34	7.51	3.24	89.24
SE	0.37	0.60	0.34	0.01	2.16	0.54	0.10	0.09	0.16
Fsw (replicate)									
Non-spawners									
1	9.4	35	25	0.24	52.67	80.95	7.78	4.14	88.08
2	9.3	32	25	0.22	48.60	76.07	9.20	2.77	88.03
3	9.5	33	25	0.24	51.04	79.83	8.02	3.38	88.60
4	9.9	34	25	0.26	57.95	78.46	8.92	3.61	87.47
5	8.1	32	24	0.23	59.97	74.01	10.27	2.51	87.22
6	6.8	30	23	0.17	51.18	77.81	8.35	3.12	88.53
7	8.1	34	23	0.21	52.98	78.70	7.75	3.08	89.17
8	8.6	33	24	0.23	55.80	81.19	8.01	3.92	88.07
9	9.9	36	25	0.26	52.08	79.94	9.26	2.98	87.76
10	9.5	33	25	0.29	62.15	80.61	9.80	3.20	87.00
11	8.2	33	24	0.17	42.98	78.20	7.93	3.08	88.99
12	9.8	34	26	0.23	49.02	79.38	5.29	3.78	90.93
13	9.4	34	25	0.26	55.87	75.75	6.47	3.34	90.19
14	9.4	33	26	0.24	53.18	81.28	6.27	4.08	89.65
15	8.8	34	25	0.21	47.07	77.79	5.66	3.46	90.88
Mean	8.99	33.33	24.67	0.23	52.84	78.66	7.93	3.36	88.70
SE	0.22	0.36	0.23	0.01	1.29	0.55	0.38	0.12	0.32

Appendix 2b. Broodstock of T. philippinarum conditioned for 7 weeks with a diet of D. tertiolecta.

Date:	13.03.91				Ref.: 1st broodstock conditioning 1991- 2nd spawning (7 weeks)				
Species	*Tapes philippinarum*				Diet: D. tertiolecta				
Clams nº	**Live weight g**	**Length mm**	**Breadth mm**	**Meat dry weight, g**	**Condition index**	**% Afdwt**	**Lipid (% Afdwt)**	**Carbohydrate (% Afdwt)**	**Protein (% Afdwt)**
D. tertiolecta									
Females									
1	7.5	32	25	0.28	56.31	82.24	16.29	3.90	79.81
2	7.5	31	24	0.23	52.59	82.75	14.74	4.39	80.87
3	6.8	31	23	0.28	82.21	82.08	15.47	3.24	81.29
4	6.9	33	24	0.33	93.97	86.57	14.09	3.97	81.94
Males									
1	5.3	29	22	0.31	97.31	71.31	11.08	2.83	86.09
Non-spawners									
1	7.1	31	22	0.34	125.44	81.22	13.54	4.91	81.55
2	6.7	29	22	0.26	77.56	82.28	13.98	3.23	82.79
3	7.1	31	23	0.25	64.61	86.26	13.68	6.16	80.16
4	7.5	33	26	0.23	53.98	84.72	14.16	3.85	81.99
5	7.3	32	23	0.33	92.44	84.04	14.75	2.82	82.43
6	6.0	34	24	0.37	93.54	89.24	13.45	4.76	81.79
7	7.2	31	22	0.23	70.66	81.22	14.41	3.53	82.06
8	7.7	33	24	0.34	81.43	85.49	11.36	3.53	85.11
9	6.6	30	21	0.27	96.32	84.32	11.74	4.21	84.05
Mean	6.95	31.43	23.21	0.29	81.31	83.12	12.85	3.95	82.28
SE	0.17	0.40	0.37	0.01	5.48	1.10	0.40	0.24	0.47
D. tertiolecta (replicate)									
Females									
1	6.4	30	22	0.16	36.84	81.25	10.22	2.73	87.05
2	7.2	31	23	0.32	75.36	80.82	10.64	3.63	85.73
3	7.3	31	23	0.31	74.38	81.72	10.28	4.67	85.05
4	9.8	33	26	0.44	85.31	81.55	10.06	2.92	87.02
Males									
1	8.2	33	26	0.31	55.58	82.61	9.68	3.47	86.85
Non-spawners									
1	8.2	32	22	0.26	65.87	77.95	10.65	3.12	86.23
2	7.0	31	22	0.34	87.10	82.72	10.64	6.81	82.55
3	6.8	31	23	0.29	86.06	80.15	10.23	2.78	86.99
4	7.9	30	24	0.23	57.03	79.91	11.01	4.35	84.64
5	7.3	30	25	0.23	63.00	82.46	11.40	5.72	82.88
6	8.3	31	24	0.34	85.53	79.61	11.56	7.99	80.45
7	8.5	31	25	0.36	75.25	78.22	11.76	4.44	83.80
8	8.2	31	22	0.25	70.22	81.39	11.80	4.26	83.94
9	6.7	31	23	0.24	62.18	79.50	11.32	4.28	84.40
10	4.3	26	19	0.21	94.77	79.10	12.26	4.17	83.57
Mean	7.49	30.80	23.27	0.29	71.63	80.60	10.90	4.36	84.74
SE	0.32	0.42	0.47	0.02	3.95	0.40	0.19	0.39	0.50

Appendix 2b (continued). Broodstock of T. philippinarum conditioned for 7 weeks with a diet of S. costatum.

Date:	13.03.91				Ref.:	1st broodstock conditioning 1991- 2nd spawning (7 weeks)			
Species	*Tapes philippinarum*				Diet:	*S. costatum*			
Clams nº	Live weight g	Length mm	Breadth mm	Meat dry weight, g	Condition index	% Afdwt	Lipid (% Afdwt)	Carbohydrate (% Afdwt)	Protein (% Afdwt)
S. costatum									
Females									
1	7.9	32	24	0.21	53.08	83.71	11.23	4.41	84.36
2	7.9	31	25	0.33	62.40	79.31	11.98	3.01	85.01
3	8.5	31	25	0.34	68.88	82.96	12.66	3.35	83.99
4	7.5	32	22	0.26	66.46	82.09	9.14	3.95	86.91
5	8.0	32	24	0.33	74.48	83.57	11.01	2.76	86.23
6	8.6	32	24	0.36	76.00	80.62	12.40	4.02	83.58
Males									
1	8.7	32	25	0.32	68.45	77.37	11.89	3.54	84.57
2	4.7	27	20	0.14	39.41	77.10	11.93	3.92	84.15
3	7.5	32	23	0.36	82.50	78.97	10.00	3.00	87.00
Non-spawners									
1	7.6	31	25	0.30	89.53	82.28	9.97	2.94	87.09
2	8.4	33	25	0.33	95.91	79.77	9.65	2.95	87.40
3	7.1	32	24	0.26	62.34	79.51	9.31	3.36	87.33
4	5.5	30	21	0.26	94.67	79.24	10.35	2.88	86.77
5	6.5	30	23	0.26	79.18	80.65	10.04	2.10	87.86
6	8.4	34	25	0.39	55.65	82.19	10.46	3.87	85.67
Mean	7.53	31.40	23.67	0.30	71.26	80.62	10.80	3.34	85.86
SE	0.30	0.41	0.41	0.02	4.08	0.54	0.30	0.16	0.38
S. costatum (replicate)									
Females									
1	8.1	34	27	0.32	67.89	82.03	9.87	2.13	88.00
2	9.9	34	26	0.32	59.60	78.71	8.26	3.07	88.67
3	8.6	34	24	0.22	44.68	81.56	9.07	2.62	88.31
4	7.2	31	23	0.29	73.50	85.32	9.02	3.95	87.03
5	8.0	30	24	0.31	70.32	80.92	8.77	2.92	88.31
6	7.5	32	27	0.31	75.85	80.41	9.58	3.27	87.15
Males									
1	8.7	34	27	0.26	50.75	81.15	10.84	2.75	86.41
2	7.5	33	24	0.34	74.64	83.11	11.10	4.44	84.46
Non-spawners									
1	9.1	34	24	0.25	48.84	81.13	9.86	2.69	87.45
2	7.5	32	23	0.30	71.48	79.02	8.35	3.29	88.36
3	7.7	33	27	0.40	72.45	83.57	8.50	3.21	88.29
4	6.7	31	22	0.19	63.73	81.11	8.26	4.23	87.51
5	8.2	33	24	0.30	64.45	78.68	9.28	3.42	87.30
6	7.2	32	24	0.30	74.53	84.45	9.12	2.74	88.14
7	8.0	32	24	0.27	59.71	83.36	8.38	2.27	89.35
Mean	8.01	32.60	24.67	0.29	64.83	81.64	9.22	3.13	87.65
SE	0.21	0.34	0.43	0.01	2.62	0.52	0.23	0.17	0.30

Appendix 2b (continued). Broodstock of T. philippinarum conditioned for 7 weeks with filtered sea water.

Date: 13.03.91 — **Ref.:** 1st broodstock conditioning 1991- 2nd spawning (7 weeks)

Species *Tapes philippinarum* — **Diet:** Filtered sea water (Fsw)

Clams nº	Live weight g	Length mm	Breadth mm	Meat dry weight, g	Condition index	% Afdwt	Lipid (% Afdwt)	Carbohydrate (% Afdwt)	Protein (% Afdwt)
Fsw									
Non-spawners									
1	8.9	33	24	0.27	75.53	73.84	11.65	2.56	85.79
2	6.8	31	23	0.20	63.23	72.42	11.05	•	•
3	8.4	33	24	0.28	47.00	74.95	10.67	2.35	86.98
4	7.4	32	24	0.23	69.55	76.06	10.52	2.45	87.03
5	8.3	33	23	0.29	71.00	76.09	9.07	3.68	87.25
6	7.7	31	23	0.21	51.75	76.16	10.24	2.32	87.44
7	10.8	37	26	0.26	46.57	72.66	10.60	2.38	87.02
8	9.2	33	25	0.21	46.77	69.93	9.87	2.27	87.86
9	7.9	32	24	0.18	46.16	78.33	10.09	2.25	87.66
10	6.3	31	22	0.18	65.25	79.31	8.70	2.60	88.70
11	7.6	31	24	0.18	54.03	71.65	10.05	2.88	87.07
12	6.7	31	23	0.25	80.03	69.65	10.19	2.24	87.57
13	7.5	31	25	0.18	55.48	71.46	9.66	2.16	88.18
14	7.0	32	24	0.18	53.12	70.40	9.09	•	•
15	6.5	31	22	0.23	70.73	72.73	9.76	1.99	88.25
Mean	7.81	32.13	23.73	0.22	59.75	73.71	10.08	2.47	87.45
SE	0.31	0.41	0.28	0.01	3.00	0.78	0.20	0.11	0.19
Fsw (replicate)									
Non-spawners									
1	7.5	32	23	0.21	73.14	72.39	9.26	•	
2	9.3	35	23	0.33	65.84	74.25	7.27	2.38	90.35
3	8.7	33	25	0.25	64.74	76.03	8.42	2.18	89.40
4	6.8	31	23	0.22	64.32	70.95	8.32	2.04	89.64
5	7.4	30	23	0.22	66.73	76.74	7.69	2.18	90.13
6	7.2	30	23	0.14	43.24	72.28	7.89	2.28	89.83
7	5.5	27	20	0.11	41.42	73.27	8.46	•	
8	7.8	32	23	0.18	44.93	70.07	7.71	2.28	90.01
9	7.6	32	24	0.25	71.37	72.51	8.27	1.94	89.79
10	8.6	33	24	0.24	56.42	75.14	8.25	2.10	89.65
11	9.3	33	26	0.29	67.19	77.42	7.62	2.89	89.49
12	8.1	33	25	0.29	73.69	70.40	7.95	2.34	89.71
13	6.2	30	22	0.24	85.43	74.45	7.92	2.07	90.01
14	8.3	33	24	0.16	42.11	76.41	7.85	2.28	89.87
15	8.4	33	25	0.31	74.52	75.71	7.40	3.42	89.18
Mean	7.79	31.80	23.53	0.23	62.34	73.87	8.02	2.34	89.77
SE	0.28	0.50	0.38	0.02	3.53	0.61	0.13	0.10	0.08

Appendix 2c. Broodstock of T. philippinarum conditioned for 8 weeks with a diet of D. tertiolecta.

Date:	20.03.91- 21.03.91				Ref.: 1st broodstock conditioning 1991 - 3rd spawning (8 weeks)				
Species	*Tapes philippinarum*				Diet: D. tertiolecta				
Clams nº	**Live weight g**	**Length mm**	**Breadth mm**	**Meat dry weight, g**	**Condition index**	**% Afdwt**	**Lipid (% Afdwt)**	**Carbohydrate (% Afdwt)**	**Protein (% Afdwt)**
D. tertiolecta									
Females									
1	7.3	30	25	0.28	79.66	77.33	10.34	9.34	80.32
2	6.3	30	22	0.23	117.20	80.29	10.71	9.18	80.11
3	6.0	30	22	0.21	68.45	80.86	11.38	9.03	79.59
4	6.1	30	23	0.24	67.14	80.28	11.46	10.48	78.06
Males									
1	6.4	29	22	0.26	84.90	81.66	10.78	8.33	80.89
2	6.1	30	22	0.19	95.00	81.30	9.72	6.97	83.31
Non-spawners									
1	9.4	35	24	0.34	79.49	77.31	11.12	8.49	80.39
2	9.3	32	25	0.27	67.53	80.98	10.37	6.37	83.26
3	6.1	29	22	0.22	73.53	83.09	10.47	9.04	80.49
4	7.6	32	23	0.31	89.71	81.74	11.62	10.11	78.27
5	8.3	31	24	0.25	62.90	81.40	10.93	10.45	78.62
6	6.9	30	22	0.25	84.59	83.74	11.23	5.99	82.78
Mean	7.14	30.67	23.00	0.25	80.84	80.83	10.84	8.65	80.51
SE	0.36	0.48	0.35	0.01	4.38	0.56	0.16	0.44	0.52
D. tertiolecta (replicate)									
Females									
1	6.3	31	22	0.22	65.67	78.69	9.91	5.06	85.03
2	5.3	31	23	0.25	66.57	82.99	9.76	5.12	85.12
3	7.9	34	24	0.24	56.40	78.10	9.35	6.09	84.56
4	9.5	35	26	0.31	61.48	81.30	10.95	7.88	81.17
5	6.6	31	22	0.23	69.36	78.90	10.65	5.64	83.71
6	6.9	32	24	0.25	69.03	83.48	10.06	4.08	85.86
7	8.0	32	24	0.25	66.89	81.58	11.65	8.08	80.27
8	6.3	30	22	0.21	62.73	79.17	10.10	5.79	84.11
9	6.3	31	23	0.25	72.34	82.26	11.31	6.31	82.38
Male									
1	9.9	35	25	0.33	63.48	80.99	11.36	6.22	82.42
Non-spawners									
1	6.5	33	23	0.22	47.63	83.36	10.68	6.19	83.13
2	6.5	31	22	0.18	68.88	81.25	10.71	9.18	80.11
3	8.6	31	23	0.16	39.80	78.63	9.41	5.56	85.03
4	8.0	32	24	0.27	73.03	77.33	9.83	6.41	83.76
Mean	7.32	32.07	23.36	0.24	63.09	80.57	10.41	6.26	83.33
SE	0.36	0.43	0.32	0.01	2.52	0.55	0.20	0.36	0.49

Appendix 2c (continued). Broodstock of T. philippinarum conditioned for 8 weeks with a diet of S. costatum.

Date:	20.03.91- 21.03.91			**Ref.:**	1st broodstock conditioning 1991- 3rd spaning (8 weeks)				
Species	*Tapes philippinarum*			**Diet:**	*S. costatum*				
Clams nº	**Live weight g**	**Length mm**	**Breadth mm**	**Meat dry weight, g**	**Condition index**	**% Afdwt**	**Lipid (% Afdwt)**	**Carbohydrate (% Afdwt)**	**Protein (% Afdwt)**
S. costatum									
Females									
1	8.1	33	23	0.31	74.54	81.78	13.57	4.54	81.89
2	8.5	31	23	0.31	77.60	78.12	11.78	7.08	81.14
3	7.6	32	24	0.31	87.46	79.11	12.39	5.60	82.01
Males									
1	8.7	33	24	0.33	90.83	80.91	11.37	3.52	85.11
2	8.4	32	23	0.33	89.51	77.61	10.57	3.85	85.58
3	8.3	30	23	0.28	80.89	79.01	11.01	4.59	84.40
Non-spawners									
1	7.4	31	23	0.22	59.03	78.85	11.03	5.03	83.94
2	7.6	31	23	0.28	71.92	82.92	10.73	7.57	81.70
3	8.8	32	24	0.33	82.72	81.55	10.67	7.28	82.05
4	7.8	32	23	0.31	91.44	80.24	9.97	7.85	82.18
5	7.4	32	23	0.25	72.23	82.77	11.36	7.32	81.32
6	8.8	33	23	0.29	73.33	80.58	11.79	5.42	82.79
7	9.9	34	24	0.36	77.24	85.14	10.45	8.26	81.29
Mean	8.25	32.00	23.31	0.30	79.13	80.66	11.28	5.99	82.72
SE	0.20	0.30	0.13	0.01	2.59	0.60	0.26	0.45	0.42
S. costatum (replicate)									
Males									
1	7.6	33	24	0.29	70.56	81.05	13.70	4.54	81.76
2	8.4	33	24	0.42	98.65	71.52	13.00	3.90	83.10
3	7.1	29	22	0.29	94.61	84.11	12.25	2.41	85.34
4	5.6	30	21	0.24	78.16	78.21	13.68	4.30	82.02
5	7.4	30	21	0.28	91.29	82.30	13.61	5.03	81.36
Non-spawners									
1	8.3	33	23	0.34	91.46	84.40	15.88	6.67	77.45
2	10.1	35	25	0.36	74.92	82.04	14.63	5.84	79.53
3	9.1	33	23	0.30	77.61	81.75	13.82	5.11	81.07
4	10.0	34	24	0.34	72.62	79.74	13.42	5.61	80.97
5	4.1	27	19	0.16	75.62	81.24	11.20	2.93	85.87
6	8.3	32	23	0.33	87.76	81.59	16.18	6.03	77.79
7	6.2	29	21	0.17	63.96	79.15	12.26	3.93	83.81
8	10.8	35	25	0.35	72.27	80.68	14.87	5.89	79.24
Mean	7.92	31.77	22.69	0.30	80.73	80.60	13.73	4.78	81.49
SE	0.53	0.70	0.50	0.02	2.98	0.90	0.39	0.35	0.72

Appendix 2c (continued). Broodstock of T. philippinarum conditioned for 8 weeks with filtered sea water.

Date:	20.03.91- 21.03.91			Ref.: 1st broodstock conditioning 1991- 3rd spawning (8 weeks)					
Species	*Tapes philippinarum*			**Diet:** Filtered sea water (Fsw)					
Clams nº	**Live weight g**	**Length mm**	**Breadth mm**	**Meat dry weight, g**	**Condition index**	**% Afdwt**	**Lipid (% Afdwt)**	**Carbohydrate (% Afdwt)**	**Protein (% Afdwt)**
Fsw									
Non-spawners									
1	8.8	34	25	0.13	31.90	74.37	9.28	2.37	88.35
2	9.1	32	24	0.17	41.54	75.24	8.77	2.42	88.81
3	6.1	33	24	0.09	20.63	79.84	9.89	1.75	88.36
4	5.8	29	22	0.11	39.41	74.18	9.30	2.12	88.58
5	4.4	27	20	0.06	29.24	73.30	9.14	2.46	88.40
6	6.5	29	22	0.13	41.58	73.21	8.74	2.51	88.75
7	8.2	33	23	0.16	44.05	75.06	8.13	2.44	89.43
8	8.9	34	24	0.22	54.42	75.08	9.32	3.36	87.32
9	7.0	29	23	0.11	36.90	75.15	8.25	3.05	88.70
10	7.9	33	24	0.17	48.22	75.14	9.05	2.44	88.51
11	8.0	32	24	0.14	37.00	73.20	9.29	2.42	88.29
12	8.3	33	24	0.20	53.03	77.80	9.64	3.53	86.83
Mean	7.41	31.50	23.25	0.14	39.83	75.13	9.07	2.57	88.36
SE	0.42	0.68	0.39	0.01	2.81	0.56	0.15	0.15	0.20
Fsw (replicate)									
Non-spawners									
1	8.2	32	23	0.20	62.19	75.25	10.63	3.19	86.18
2	8.8	33	24	0.20	43.47	78.74	10.29	2.91	86.80
3	5.5	26	20	0.12	49.58	75.32	9.29	2.66	88.05
4	8.9	36	24	0.21	49.50	78.15	10.75	2.99	86.26
5	9.0	35	24	0.21	47.32	74.59	10.06	2.55	87.39
6	6.1	27	20	0.10	36.81	74.16	9.30	3.21	87.49
7	9.4	34	25	0.23	53.26	72.97	8.91	2.91	88.18
8	8.4	34	24	0.17	41.85	70.06	9.42	2.37	88.21
9	8.2	33	24	0.16	40.74	72.06	10.41	2.28	87.31
10	5.8	28	22	0.16	56.66	70.00	9.14	3.16	87.70
11	8.4	34	23	0.18	62.76	76.95	9.49	2.46	88.05
12	5.4	27	21	0.13	53.33	69.73	8.75	3.23	88.02
13	10.1	35	26	0.22	45.21	76.60	9.14	2.94	87.92
14	8.1	33	24	0.18	43.83	74.94	8.81	3.12	88.07
15	8.8	34	24	0.21	54.90	66.97	8.81	2.40	88.79
Mean	7.93	32.07	23.20	0.18	49.43	73.77	9.55	2.83	87.63
SE	0.39	0.86	0.45	0.01	1.99	0.88	0.18	0.09	0.19

Appendix 3. Gonadal development and sex ratio of T. philippinarum conditioning with different diets in the 1st broodstock 1991. For abbreviations see Table 3.3.

	Initial	Conditioning period					
		2 weeks			5 weeks		
		Dun	Ske	Fsw	Dun	Ske	Fsw
1st broodstock 1991							
Male	9 (90%)	9 (90%)	4 (40%)	2 (20%)	3 (30%)	4 (40%)	1 (10%)
Female	1 (10%)	1 (10%)	6 (60%)	8 (80%)	7 (70%)	6 (60%)	9 (90%)
Stage I	10 (100%)	6 (60%)	4 (40%)	10 (100%)	-	-	10 (100%)
Stage II	-	4 (40%)	6 (60%)	-	5 (50%)	7 (70%)	-
Stage III	-	-	-	-	2 (20%)	1 (10%)	-
Stage IV	-	-	-	-	3 (30%)	2 (20%)	-
Stage V	-	-	-	-	-	-	-
Stage VI	-	-	-	-	-	-	-

Appendix 3a. Gonadal development and sex ratio of T. philippinarum conditioning with different diets in the 2nd broodstock 1991. For abbreviations see Table 3.21.

	Initial	Conditioning period					
		2 weeks			4 weeks		
		Tet+Ske	Dry Tet+Ske	Fsw	Tet+Ske	Dry Tet+Ske	Fsw
2nd broodstock 1991							
Male	5 (50%)	5 (50%)	5 (50%)	4 (40%)	4 (40%)	4 (40%)	3 (30%)
Female	5 (50%)	5 (50%)	5 (50%)	6 (60%)	6 (60%)	6 (60%)	7 (70%)
Stage I	10 (100%)	4 (40%)	4 (40%)	10 (100%)	-	-	1 (10%)
Stage II	-	5 (50%)	6 (60%)	-	1 (10%)	1 (10%)	7 (70%)
Stage III	-	1 (10%)	-	-	8 (80%)	7 (70%)	2 (20%)
Stage IV	-	-	-	-	1 (10%)	2 (20%)	-
Stage V	-	-	-	-	-	-	-
Stage VI	-	-	-	-	-	-	-

Appendix 4. Number of eggs produced per female (in millions) T. philippinarum after being conditioned for 6 (Spawning I), 7 (Spawning II) and 8 (Spawning III) weeks on a 3% live diet of D. tertiolecta or S. costatum. For abbreviation see Table 3.3.

1st Broodstock conditioning - 1991

Spawning	Females Nº	Treatment Dun	Dun-R	Ske	Ske-R	Fsw	Fsw-R
I	-	-	-	-	-	-	-
II	1	0.070	0.015	0.034	0.079	-	-
	2	0.019	0.387	0.703	0.224	-	-
	3	0.063	0.014	0.450	0.051	-	-
	4	0.079	0.229	0.843	0.655	-	-
	5	-	-	0.478	0.026	-	-
	6	-	-	0.116	0.022	-	-
III	1	0.877	1.141	1.398	-	-	-
	2	0.242	0.239	0.128	-	-	-
	3	0.240	1.165	0.772	-	-	-
	4	0.074	0.431	-	-	-	-
	5	-	1.493	-	-	-	-
	6	-	1.308	-	-	-	-
	7	-	1.806	-	-	-	-
	8	-	0.459	-	-	-	-
	9	-	0.655	-	-	-	-

Appendix 5. Fatty acid composition of eggs and D-larvae produced by T. philippinarum broodstock fed on 3% of different diets. Values are percentages of total fatty acid.

1st broodstock 1991 - Spawning II	**Eggs**		**D-larvae**	
Algae	*D. tertiolecta*	*S. costatum*	*D. tertiolecta*	*S. costatum*
Fatty acid				
14:0	28.47	25.32	*	31.30
16:0	28.72	27.84	*	28.07
18:0	8.38	7.51	*	8.24
16:1w9	0.00	0.00	*	0.00
16:1w7	2.75	6.92	*	4.74
18:1w9	14.47	10.98	*	13.48
18:1w7	1.86	3.61	*	2.43
20:1w9	0.00	0.00	*	0.00
18:2w6	3.57	2.22	*	2.48
18:3w3	5.62	0.00	*	0.00
18:4w3	0.00	1.05	*	0.00
20:2w6	2.26	0.30	*	0.00
20:3w3	0.40	0.92	*	0.86
20:4w6	1.34	0.00	*	0.00
20:5w3	1.56	9.25	*	5.36
22:5w3	0.00	0.00	*	0.00
22:6w3	0.60	4.08	*	3.04
Saturated	65.57	60.67	*	67.61
Monoenoic	19.08	21.51	*	20.65
Polyenoic	15.35	17.82	*	11.74

* not enough eggs to incubate

Table 5a. Fatty acid composition of eggs and larvae produced by T. philippinarum broodstock fed on 3% of D. tertiolecta. Values are percentages of total fatty acid.

1st broodstock 1991 - Spawning III

D. tertiolecta

	Eggs	D-larvae	Age of larvae 5	7	9	12	16	21	26
Fatty acid									
14:0	30.94	32.03	16.49	26.33	21.62	15.77	22.07	31.40	31.77
16:0	28.27	28.68	19.08	22.42	22.10	20.85	23.06	24.76	25.19
18:0	8.22	7.93	6.44	6.32	5.37	4.47	5.40	6.67	7.73
16:1w9	1.30	1.54	0.00	0.00	0.00	7.99	0.00	0.00	1.82
16:1w7	2.08	0.93	6.43	4.45	7.75	12.71	6.80	3.70	1.65
18:1w9	14.17	13.80	11.39	14.71	13.10	5.46	13.85	14.82	14.59
18:1w7	1.27	1.38	5.19	3.19	4.27	2.28	4.64	3.40	3.21
20:1w9	0.86	1.51	2.76	1.69	1.63	0.00	1.75	1.32	1.51
18:2w6	4.06	3.24	3.95	3.24	3.31	3.51	3.15	3.00	3.13
18:3w3	5.12	4.38	2.71	2.71	1.67	2.81	1.49	1.23	1.13
18:4w3	0.00	0.00	5.81	3.90	5.17	6.63	4.34	1.94	0.95
20:2w6	1.43	2.05	2.91	1.70	1.65	1.58	1.44	1.05	1.55
20:3w3	0.00	0.00	0.77	0.00	0.00	0.00	0.00	0.00	0.00
20:4w6	1.42	1.33	0.46	0.00	0.00	0.00	0.00	0.00	0.00
20:5w3	0.86	1.19	4.10	2.63	4.57	5.23	4.11	2.40	2.36
22:5w3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
22:6w3	0.00	0.00	11.51	6.71	7.79	10.71	7.90	4.31	3.41
Saturated	67.43	68.64	42.01	55.07	49.09	41.09	50.53	62.83	64.69
Monoenoic	19.68	19.16	25.77	24.04	26.75	28.44	27.04	23.24	22.78
Polyenoic	12.89	12.19	32.22	20.89	24.16	30.47	22.43	13.93	12.53

Appendix 5b. Fatty acid composition of eggs and larvae produced by T. philippinarum broodstock fed on 3% of S. costatum. Values are percentages of total fatty acid.

1st broodstock 1991 - Spawning III
S. costatum

	Eggs	D-larvae	Age of larvae			
			5	7	10	14
Fatty acid						
14:0	34.57	37.72	41.60	23.35	29.05	20.39
16:0	28.21	28.19	27.89	22.83	23.31	25.03
18:0	7.25	8.32	7.94	7.72	5.87	9.26
16:1w9	0.00	0.00	0.00	0.00	0.00	0.00
16:1w7	5.06	3.39	4.22	6.99	5.99	10.57
18:1w9	13.20	15.89	18.35	13.64	14.63	12.85
18:1w7	1.70	0.00	0.00	3.77	2.64	3.55
20:1w9	0.00	0.00	0.00	1.40	0.78	1.18
18:2w6	2.43	3.48	0.00	3.03	2.89	2.46
18:3w3	0.00	0.00	0.00	1.72	1.03	1.20
18:4w3	0.00	0.00	0.00	4.36	5.16	2.68
20:2w6	0.00	0.00	0.00	0.94	1.09	1.18
20:3w3	0.00	0.00	0.00	0.00	0.00	0.00
20:4w6	0.00	0.00	0.00	0.00	0.00	0.00
20:5w3	6.11	3.01	0.00	3.92	3.02	5.40
22:5w3	0.00	0.00	0.00	0.00	0.00	0.00
22:6w3	1.47	0.00	0.00	6.33	4.54	4.25
Saturated	70.03	74.23	77.43	53.90	58.23	54.68
Monoenoic	19.96	19.28	22.57	25.80	24.04	28.15
Polyenoic	10.01	6.49	0.00	20.30	17.73	17.17

Appendix 6. Sand-filtered sea water quality in the hatchery, 1st and 2nd broodstock 1991
(Afdwt = Ash-free dry weight ; Dwt = Dry weight ; Chl = Chlorophyll)

Date	Particulates							CHL
	2.5 - 5.0 µm (µl/l)	5.0 - 10 µm (µl/l)	Dwt (> 1 µm) (mg/l)	Afdwt (mg/l)	% Afdwt	Lipid (% Afdwt)	Dwt (0.22 - 1 µm) (mg/l)	(µg/l)
1st broodstock 1991								
(23.01.91)	13.31	3.98	3.17	1.06	33.49	9.03	55.00	0.57
(28.01.91)	8.84	1.5	18.38	3.43	18.69	1.86	60.00	0.35
(4.02.91)	29.6	5.13	24.32	4.36	17.94	1.8	23.00	0.19
(11.02.91)	110.53	14.7	13.70	2.01	14.65	5.09	25.00	1.24
(18.02.91)	17.08	2.98	10.56	2.33	22.05	2.87	3.00	1.25
(25.02.91)	67.41	8.64	12.95	2.24	17.32	4.35	17.50	2.15
(4.03.91)	26.12	5.49	12.70	2.59	20.41	3.44	11.50	0.93
(11.03.91)	6.48	1.20	17.22	3.26	18.95	2.58	66.00	0.64
(18.03.91)	4.59	1.36	5.72	1.49	26.00	3.65	17.00	1.10
Mean	31.55	5.00	13.19	2.53	21.06	3.85	30.89	0.94
SE	11.77	1.46	2.14	0.34	1.88	0.74	7.71	0.20
2nd broodstock 1991								
(22.04.91)	19.72	4.07	7.84	1.41	18.00	7.36	6.50	1.58
(29.04.91)	14.31	2.47	24.40	5.05	20.70	2.02	10.00	2.54
(6.05.91)	43.90	10.92	18.194	5.28	29.03	3.54	20.00	3.19
(14.05.91)	57.50	33.33	10.42	4.48	43.01	12.3	68.00	3.13
(20.05.91)	26.79	5.94	6.07	2.68	44.21	6.76	12.00	1.97
(29.05.91)	70.69	17.76	16.45	8.07	49.06	6.26	24.00	3.81
(03.06.91)	89.64	13.67	17.72	8.07	38.49	4.44	70.00	3.14
Mean	46.08	12.59	14.44	5.01	34.64	6.10	30.07	2.77
SE	10.58	4.02	2.47	0.94	4.59	1.26	10.28	0.29

Appendix 7. Condition indices and biochemical composition of T. philippinarum initial 2nd broodstock 1991 and conditioned for 5, 7 and 9 weeks with different diets.

Date: 17.04.91				**Ref.:** 2nd broodstock conditioning 1991 - Initial					
Spec.: *Tapes philippinarum*									
Clams nº	**Live weight g**	**Length mm**	**Breadth mm**	**Meat dry weight, g**	**Condition index**	**% Afdwt**	**Lipid (% Afdwt)**	**Carbohydrate (% Afdwt)**	**Protein (% Afdwt)**
1	8.2	31	23	0.40	120.27	80.82	13.86	11.45	74.69
2	8.4	31	26	0.37	98.65	78.89	12.93	10.25	76.82
3	8.3	31	24	0.30	81.46	80.05	10.62	9.76	79.62
4	9.0	33	25	0.42	100.29	72.98	12.61	10.95	76.44
5	8.9	33	25	0.40	101.13	76.79	10.29	9.75	79.96
6	9.2	34	26	0.42	94.61	79.63	11.55	12.08	76.37
7	7.6	33	25	0.38	97.13	78.20	10.74	12.16	77.10
8	9.3	32	25	0.46	107.81	79.10	12.01	12.20	75.79
9	10.1	33	25	0.50	108.15	76.58	12.14	12.89	74.97
10	8.7	32	24	0.36	97.43	78.63	9.67	9.45	80.88
11	8.1	30	24	0.43	122.77	78.72	10.54	10.84	78.62
12	9.4	33	26	0.49	117.36	74.34	11.43	11.64	76.93
13	8.2	34	25	0.45	101.84	80.51	11.18	10.47	78.35
14	9.0	34	24	0.23	53.64	77.24	8.16	2.43	89.41
15	8.9	34	24	0.26	58.34	73.60	10.19	3.26	86.55
16	9.6	33	24	0.48	100.25	80.26	13.21	13.68	73.11
17	9.2	34	24	0.46	106.00	79.46	12.46	13.87	73.67
18	9.2	32	25	0.44	98.11	76.79	13.02	11.75	75.23
19	9.3	33	25	0.46	105.00	70.34	13.22	12.37	74.41
20	8.4	32	23	0.39	99.23	79.84	12.15	10.37	77.48
21	7.3	32	23	0.33	104.31	77.41	11.37	13.56	75.07
22	8.6	31	25	0.38	100.82	77.73	11.19	10.14	78.67
23	9.5	35	24	0.47	103.47	77.67	12.75	13.13	74.12
24	9.5	31	25	0.45	102.52	77.22	11.91	13.79	74.30
25	8.5	32	25	0.46	111.88	78.69	12.20	13.04	74.76
26	8.3	30	25	0.39	109.53	78.69	12.07	10.38	77.55
27	8.3	31	24	0.37	102.11	79.06	12.90	13.04	74.06
28	9.2	33	24	0.46	108.88	79.77	12.66	13.70	73.64
29	8.3	30	23	0.40	105.71	76.82	12.11	12.51	75.38
30	8.3	33	24	0.40	98.22	79.61	11.81	12.51	75.68
Mean	8.76	32.33	24.47	0.41	100.56	77.85	11.77	11.25	76.99
SE	0.11	0.25	0.16	0.01	2.64	0.44	0.22	0.48	0.66

Appendix 7a. Broodstock of T. philippinarum conditioned for 5 weeks with a diet of live T. suecica+S. costatum.

Date: 21.05.91-22.05.91				Ref: 2nd broodstock 1991 - 1st spawning (5 weeks)					
Spec.: *Tapes philippinarum*				Diet: *T. suecica + S. costatum (Tet+Ske)*					
Clams nº	Live weight g	Length mm	Breadth mm	Meat dry weight, g	Condition index	% Afdwt	Lipid (% Afdwt)	Carbohydrate (% Afdwt)	Protein (% Afdwt)
Tet + Ske									
Females									
1	13.0	36	29	0.66	114.17	84.91	13.66	22.63	63.71
Non-spawners									
1	10.0	34	25	0.62	129.15	83.90	13.35	18.54	68.11
2	13.7	37	28	0.70	111.13	86.16	13.58	21.78	64.64
3	10.6	35	25	0.61	120.14	88.57	10.95	18.38	70.67
4	9.4	33	25	0.53	131.60	83.61	11.48	23.66	64.86
5	11.2	35	25	0.64	130.76	87.15	8.72	17.21	74.07
6	9.9	35	25	0.48	109.57	85.33	7.73	20.09	72.18
7	10.3	35	27	0.53	114.37	87.69	10.72	15.63	73.65
8	11.1	35	26	0.64	120.96	87.08	10.11	20.19	69.70
9	9.9	34	25	0.54	115.34	83.51	10.18	20.67	69.15
10	10.1	34	27	0.61	121.06	87.64	10.73	19.35	69.92
11	12.1	38	28	0.68	115.05	82.91	8.08	18.49	73.43
12	12.7	35	27	0.64	120.49	83.05	8.43	16.22	75.35
13	9.5	33	25	0.53	116.73	86.51	10.17	16.00	73.83
14	12.2	35	27	0.68	125.41	84.78	10.97	21.97	67.06
Mean	11.06	34.93	26.27	0.61	119.73	85.52	10.59	19.39	70.02
SE	0.36	0.34	0.36	0.02	1.79	0.48	0.49	0.64	0.97
Tet + Ske (replicate)									
Non-spawners									
1	10.9	37	26	0.71	126.82	84.32	10.08	22.57	67.35
2	10.4	37	25	0.66	144.02	83.37	9.96	19.30	70.74
3	11.6	36	26	0.74	131.34	86.22	10.67	20.77	68.56
4	9.0	33	25	0.50	116.60	86.09	10.22	22.58	67.20
5	10.9	34	27	0.65	135.29	83.76	10.15	20.41	69.44
6	9.3	34	25	0.60	138.42	85.73	11.08	19.75	69.17
7	11.9	35	25	0.72	131.35	85.53	12.86	17.98	69.16
8	13.0	36	29	0.70	127.25	83.56	12.57	17.11	70.32
9	11.3	35	26	0.67	141.62	84.85	10.61	22.41	66.98
10	10.5	33	24	0.51	117.35	86.07	11.04	21.58	67.38
11	10.5	33	26	0.37	123.02	79.75	10.78	19.96	69.26
12	12.6	35	27	0.76	138.71	86.55	10.63	25.00	64.37
13	11.1	35	25	0.57	115.57	85.38	10.54	19.82	69.64
14	10.8	33	27	0.59	125.28	85.89	11.41	17.07	71.52
15	9.4	34	26	0.30	66.64	77.90	11.17	18.32	70.51
Mean	10.88	34.67	25.93	0.60	125.29	84.33	10.92	20.31	68.77
SE	0.29	0.36	0.32	0.04	4.79	0.64	0.22	0.58	0.48

Appendix 7a (continued). Broodstock of T. philippinarum conditioned for 5 weeks with a diet of dry T. suecica+S. costatum.

Date:	21.05.91-22.05.91			Ref: 2nd broodstock 1991 - 1st spawning (5 weeks)					
Spec.:	*Tapes philippinarum*			Diet: Dry T. suecica + S. costatum (Dry Tet+Ske)					
Clams nº	**Live weight g**	**Length mm**	**Breadth mm**	**Meat dry weight, g**	**Condition Index**	**% Afdwt**	**Lipid (% Afdwt)**	**Carbohydrate (% Afdwt)**	**Protein (% Afdwt)**
Dry Tet + Ske									
Females									
1	11.2	32	27	0.63	94.76	85.31	9.30	22.03	68.67
Males									
1	8.4	31	25	0.45	107.83	85.52	10.52	18.33	71.15
Non-spawners									
1	9.9	34	25	0.51	118.28	83.89	10.49	19.47	70.04
2	10.9	35	27	0.64	120.15	85.41	10.30	21.29	68.41
3	10.6	36	28	0.59	98.20	86.03	10.69	21.61	67.70
4	10.5	35	25	0.58	123.83	82.10	10.72	20.89	68.39
5	11.2	36	28	0.61	107.25	83.58	8.73	20.09	71.18
6	10.1	33	26	0.47	104.67	83.53	13.05	21.78	65.17
7	8.7	32	24	0.42	106.79	82.01	10.49	21.97	67.54
8	12.2	39	27	0.66	111.02	86.00	9.88	25.35	64.77
9	9.7	34	25	0.64	126.24	88.08	12.26	28.42	59.32
10	10.7	34	26	0.55	117.17	87.28	11.00	24.23	64.77
11	13.1	37	26	0.67	124.24	82.80	9.54	26.01	64.45
12	12.1	36	27	0.61	117.69	85.05	9.17	23.02	67.81
13	10.4	35	25	0.53	122.91	84.21	9.98	22.84	67.18
Mean	10.66	34.60	26.07	0.57	113.40	84.72	10.41	22.49	67.10
SE	0.32	0.54	0.32	0.02	2.54	0.46	0.29	0.68	0.79
Dry Tet + Ske (replicate)									
Non-spawners									
1	9.8	33	26	0.58	120.63	83.43	10.31	22.09	67.60
2	11.2	34	25	0.60	145.15	84.09	8.92	23.78	67.30
3	11.3	36	27	0.60	108.58	82.15	9.62	24.35	66.03
4	8.8	32	23	0.44	122.17	84.51	9.94	20.47	69.59
5	12.9	37	27	0.69	125.04	85.51	11.23	26.42	62.35
6	11.1	37	27	0.58	135.51	84.35	10.31	19.53	70.16
7	11.3	34	28	0.56	104.79	79.37	8.95	25.16	65.89
8	10.8	33	26	0.54	119.64	82.71	12.45	28.45	59.10
9	11.3	35	25	0.58	109.85	85.61	8.64	23.88	67.48
10	9.7	34	25	0.51	115.65	83.59	8.97	25.35	65.68
11	8.7	35	25	0.61	126.13	88.91	11.58	24.72	63.70
12	10.5	34	26	0.58	119.14	83.96	10.84	20.53	68.63
13	10.4	36	26	0.52	108.69	81.54	9.20	23.08	67.72
14	11.0	36	26	0.54	100.74	85.30	8.82	21.48	69.70
15	12.5	37	27	0.61	109.70	83.35	10.56	24.99	64.45
Mean	10.76	34.87	25.93	0.57	118.09	83.89	10.02	23.62	66.36
SE	0.30	0.41	0.32	0.01	3.07	0.55	0.30	0.63	0.78

Appendix 7a (continued). Broodstock of T. philippinarum conditioned for 5 weeks with filtered sea water.

Date: 21.05.91-22.05.91			Ref: 2nd broodstock 1991 - 1st spawning (5 weeks)						
Spec.: *Tapes philippinarum*			Diet: Filtered sea water (Fsw)						
Clams nº	**Live weight g**	**Length mm**	**Breadth mm**	**Meat dry weight, g**	**Condition index**	**% Afdwt**	**Lipid (% Afdwt)**	**Carbohydrate (% Afdwt)**	**Protein (% Afdwt)**
Fsw									
Males									
1	11.5	36	26	0.63	117.94	84.13	8.08	15.53	76.39
Non-spawners									
1	9.5	33	25	0.52	118.93	84.23	9.97	13.99	76.04
2	12.0	37	28	0.55	108.33	84.88	8.84	10.06	81.10
3	8.2	32	24	0.36	90.40	85.22	8.80	15.37	75.83
4	11.1	35	27	0.58	118.24	85.64	7.94	18.20	73.86
5	9.7	35	27	0.51	106.42	86.98	9.31	18.22	72.47
6	10.2	35	26	0.62	131.00	87.10	9.53	15.46	75.01
7	8.5	34	24	0.42	100.36	84.32	8.42	17.15	74.43
8	10.1	35	25	0.46	81.46	84.05	8.33	10.69	80.98
9	11.3	39	27	0.66	140.15	87.49	10.17	10.59	79.24
10	9.7	34	26	0.50	131.74	85.21	11.03	17.87	71.10
11	7.7	31	24	0.40	85.30	92.07	9.34	14.38	76.28
12	10.7	35	25	0.52	102.65	85.44	9.36	13.04	77.60
13	11.0	35	26	0.54	93.09	83.57	7.90	15.44	76.66
14	11.4	36	27	0.56	96.50	81.98	9.51	15.53	74.96
Mean	10.18	34.80	25.80	0.52	108.17	85.49	9.10	14.77	76.13
SE	0.33	0.50	0.33	0.02	4.59	0.60	0.23	0.70	0.72
Fsw (replicate)									
Non-spawners									
1	12.4	37	26	0.66	113.00	85.69	10.37	14.26	75.37
2	10.8	34	26	0.56	106.94	85.16	10.92	15.97	73.11
3	10.9	34	27	0.58	116.36	76.60	8.88	16.39	74.73
4	10.5	34	26	0.56	140.75	84.41	11.97	18.11	69.92
5	8.8	32	25	0.49	110.11	84.96	11.77	17.13	71.10
6	8.5	33	24	0.43	106.18	85.28	10.25	15.41	74.34
7	10.1	31	25	0.48	121.00	85.88	11.29	17.49	71.22
8	11.0	34	26	0.56	115.98	84.08	10.92	18.71	70.37
9	11.4	34	25	0.49	114.81	84.73	11.92	17.56	70.52
10	8.7	34	24	0.42	100.69	81.84	11.61	16.65	71.74
11	9.5	33	25	0.48	101.98	85.00	10.94	10.06	79.00
12	10.4	34	25	0.44	126.43	82.35	11.17	17.99	70.84
13	9.1	31	24	0.46	97.81	85.49	11.35	15.04	73.61
14	10.1	33	25	0.44	103.33	83.05	11.08	15.76	73.16
15	9.6	32	25	0.56	130.16	81.92	10.25	10.64	79.11
Mean	10.13	33.33	25.20	0.51	113.70	83.76	10.98	15.81	73.21
SE	0.29	0.39	0.22	0.02	3.10	0.62	0.21	0.66	0.75

Appendix 7b. Broodstock of T. philippinarum conditioned for 7 weeks with a diet of live T. suecica+S. costatum.

Date: 04.06.91-05.06.91				**Ref:** 2nd broodstock 1991- 2nd spawning (7 weeks)					
Spec.: *Tapes philippinarum*				**Diet:** *T. suecica + S. costatum (Tet+Ske)*					
Clams nº	**Live weight g**	**Length mm**	**Breadth mm**	**Meat dry weight, g**	**Condition index**	**% Afdwt**	**Lipid (% Afdwt)**	**Carbohydrate (% Afdwt)**	**Protein (% Afdwt)**
Tet + Ske									
Females									
1	10.3	34	25	0.62	132.47	85.90	12.23	21.32	66.45
2	11.4	35	24	0.71	128.71	86.38	11.87	21.54	66.59
Males									
1	11.2	35	25	0.72	146.67	85.32	10.99	22.73	66.28
2	12.4	37	27	0.79	131.15	84.96	10.46	23.42	66.12
3	12.3	36	27	0.85	144.64	85.99	10.84	22.34	66.82
4	8.9	33	24	0.58	137.14	87.77	11.60	20.26	68.14
5	10.7	33	27	0.61	134.62	84.15	11.57	18.10	70.33
6	13.1	37	26	0.90	136.88	88.68	11.97	20.54	67.49
7	10.9	35	25	0.68	128.98	84.42	11.34	21.94	66.72
Non-spawners									
1	13.8	39	28	0.98	147.83	87.52	10.63	26.88	62.49
2	11.9	38	26	0.70	123.32	84.32	9.79	15.70	74.51
3	10.2	33	26	0.74	165.53	85.19	13.54	17.96	68.50
4	13.4	38	26	0.83	121.41	85.75	9.46	19.43	71.11
5	10.0	33	26	0.69	156.57	86.16	13.31	16.59	70.10
6	10.1	34	22	0.63	128.67	83.15	10.10	15.80	74.10
Mean	11.37	35.33	25.60	0.74	137.64	85.71	11.31	20.30	68.38
SE	0.37	0.53	0.39	0.03	3.20	0.38	0.30	0.80	0.82
TET + SKE (replicate)									
Females									
1	12.0	37	26	0.76	120.54	85.12	11.62	14.39	73.99
2	10.1	37	27	0.82	125.86	90.36	12.73	17.89	69.38
3	11.6	35	27	0.79	133.20	87.68	11.56	17.89	70.55
4	11.9	36	27	0.74	138.74	82.89	10.32	15.23	74.45
Males									
1	13.8	38	28	0.97	146.82	86.83	12.16	16.76	71.08
2	14.3	37	28	0.89	132.67	85.99	11.23	18.44	70.33
3	13.2	37	29	0.91	147.27	87.64	11.33	18.17	70.50
4	13.5	39	31	1.01	140.50	89.16	11.82	19.05	69.13
5	12.9	36	27	0.81	147.75	85.78	11.19	22.36	66.45
6	12.8	36	27	0.86	118.48	86.70	9.93	19.19	70.88
7	13.5	37	28	0.73	119.59	83.38	10.98	18.48	70.54
8	13.0	37	29	0.89	161.35	85.96	12.05	19.24	68.71
9	9.8	34	26	0.55	119.89	84.69	12.04	15.93	72.03
Non-spawners									
1	11.5	37	27	0.76	176.05	88.33	11.26	24.19	64.55
2	11.2	36	26	0.64	137.13	86.09	9.79	23.44	66.77
Mean	12.34	36.60	27.53	0.81	137.72	86.44	11.33	18.71	69.96
SE	0.34	0.31	0.35	0.03	4.26	0.52	0.21	0.73	0.68

Appendix 7b (continued). Broodstock of T. philippinarum conditioned for 7 weeks with a diet of dry T. suecica+S.costatum.

Date: 18.06.91 **Ref:** 2nd broodstock 1991- 2nd spawning (7 weeks)
Spec.: *Tapes philippinarum* **Diet:** *Dry T. suecica + S. costatum*

Clams nº	Live weight g	Length mm	Breadth mm	Meat dry weight, g	Condition index	% Afdwt	Lipid (% Afdwt)	Carbohydrate (% Afdwt)	Protein (% Afdwt)
Dry TET + SKE									
Females									
1	11.5	36	27	0.70	124.71	83.76	11.99	21.54	66.47
2	9.2	35	26	0.52	115.36	84.68	13.11	22.00	64.89
3	13.2	39	29	0.77	105.41	82.77	11.02	21.91	67.07
4	10.5	34	26	0.71	169.45	87.57	12.57	25.13	62.30
5	11.1	36	25	0.65	141.26	83.41	13.49	25.58	60.93
6	8.9	33	24	0.59	155.11	86.53	12.49	27.18	60.33
7	10.2	34	25	0.66	126.27	85.61	10.68	18.88	70.44
8	11.4	34	25	0.60	115.29	84.51	13.04	23.42	63.54
9	10.2	34	26	0.64	126.08	87.28	12.57	19.85	67.58
10	10.9	38	26	0.74	139.87	85.55	10.87	21.74	67.39
Males									
1	9.9	34	24	0.69	140.18	86.64	11.47	24.49	64.04
2	11.8	35	27	0.69	125.84	83.51	11.50	25.80	62.70
3	12.9	36	27	0.78	134.12	85.75	12.04	21.82	66.14
Non-spawners									
1	9.2	34	24	0.57	138.05	83.27	10.43	25.34	64.23
2	11.5	37	25	0.80	153.71	87.01	10.54	23.93	65.53
Mean	10.83	35.27	25.73	0.67	134.05	85.19	11.85	23.24	64.91
SE	0.33	0.44	0.36	0.02	4.38	0.41	0.26	0.61	0.71
Dry TET + SKE (replicate)									
Females									
1	9.1	35	25	0.62	122.24	86.61	11.51	20.62	67.87
2	9.1	34	25	0.63	131.44	86.67	13.04	19.12	67.84
3	9.0	34	26	0.57	105.46	84.93	13.01	20.45	66.54
4	10.5	36	27	0.69	133.50	86.26	12.73	23.34	63.93
5	9.4	34	27	0.59	118.46	83.55	11.44	23.43	65.13
6	9.7	34	27	0.56	108.88	83.97	10.57	21.49	67.94
7	15.1	40	29	0.99	148.12	85.67	11.74	19.98	68.28
8	10.2	33	26	0.57	119.19	83.99	11.11	18.64	70.25
Males									
1	10.9	35	27	0.78	143.85	86.82	10.03	21.57	68.40
2	12.4	38	27	0.91	142.92	84.94	10.70	21.63	67.67
3	11.1	35	26	0.64	135.96	83.95	11.23	22.00	66.77
4	11.9	36	28	0.88	137.75	87.15	11.38	22.90	65.72
Non-spawners									
1	10.1	35	26	0.58	102.51	86.24	7.06	25.14	67.80
2	11.2	35	27	0.65	124.35	83.58	10.74	24.10	65.16
3	12.1	38	27	0.78	130.42	86.71	10.67	25.26	64.07
Mean	10.79	35.47	26.67	0.70	127.00	85.40	11.13	21.98	66.89
SE	0.42	0.49	0.27	0.04	3.66	0.34	0.37	0.53	0.46

Appendix 7b (continued). Broodstock of T. philippinarum conditioned for 7 weeks with filtered sea water.

Date: 04.05.91-05.05.91 **Ref:** 2nd broodstock 1991- 2nd spawning (7 weeks)
Spec.: *Tapes philippinarum* **Diet:** Filtered sea water

Clams nº	Live weight g	Length mm	Breadth mm	Meat dry weight, g	Condition index	% Afdwt	Lipid (% Afdwt)	Carbohydrate (% Afdwt)	Protein (% Afdwt)
FSW									
Females									
1	8.6	33	25	0.52	124.64	83.16	10.99	18.00	71.01
2	9.1	34	24	0.60	124.79	84.37	12.11	18.49	69.40
Males									
1	12.8	36	29	0.70	101.39	83.82	10.02	19.88	70.10
2	10.5	36	26	0.69	133.37	84.53	11.57	23.56	64.87
3	9.0	34	24	0.64	132.50	84.87	9.49	18.23	72.28
4	12.7	39	27	0.74	121.72	85.52	11.49	21.47	67.04
5	9.5	32	25	0.53	123.49	81.10	10.68	18.75	70.57
6	10.6	35	24	0.68	138.12	85.15	12.04	19.80	68.16
7	10.1	34	25	0.62	135.09	85.55	11.57	23.18	65.25
8	7.9	32	24	0.44	102.81	84.41	10.87	17.52	71.61
Non-spawners									
1	13.7	39	24	0.95	141.18	84.32	10.47	18.26	71.27
2	10.5	34	25	0.62	131.11	83.11	11.50	19.16	69.34
3	11.9	37	27	0.73	126.17	84.30	9.04	20.12	70.84
4	11.3	36	25	0.66	133.71	83.63	9.43	20.60	69.97
5	12.8	34	26	0.76	125.03	84.47	10.54	22.25	67.21
Mean	10.73	35.00	25.33	0.66	126.34	84.15	10.79	19.95	69.26
SE	0.45	0.56	0.37	0.03	2.94	0.29	0.25	0.49	0.59
FSW (replicate)									
Females									
1	12.1	37	28	0.88	130.81	88.14	10.51	21.81	67.68
2	9.7	33	25	0.58	118.67	82.98	11.04	22.31	66.65
3	8.1	33	25	0.53	111.89	82.99	12.01	19.01	68.98
4	13.4	38	27	0.83	146.26	86.09	11.73	23.26	65.01
5	10.5	35	27	0.65	129.66	83.49	10.44	20.13	69.43
6	10.7	35	26	0.66	139.64	83.10	9.57	17.73	72.70
7	7.8	32	25	0.42	103.39	83.94	10.74	19.41	69.85
Males									
1	10.8	34	25	0.61	118.88	82.41	11.11	18.13	70.76
2	10.6	34	25	0.59	117.34	84.15	10.03	19.60	70.37
3	9.0	33	24	0.55	128.59	88.51	10.70	17.35	71.95
Non-spawners									
1	10.6	37	25	0.70	136.86	80.30	11.23	15.81	72.96
2	10.5	35	25	0.69	128.59	84.36	10.38	21.26	68.36
3	10.8	33	27	0.75	146.57	83.99	7.06	17.98	74.96
4	11.1	34	26	0.71	133.42	83.32	10.74	14.56	74.70
5	13.3	40	29	0.89	138.33	86.24	10.67	20.71	68.62
Mean	10.60	34.87	25.93	0.67	128.59	84.27	10.53	19.27	70.20
SE	0.41	0.58	0.36	0.03	3.22	0.56	0.29	0.62	0.74

Appendix 7c. Broodstock of T. philippinarum conditioned for 9 weeks with a diet of live T.suecica+S.costatum.

Date:	18.06.91			Ref: 2nd broodstock 1991- 3rd spawning (9 weeks)					
Spec.:	*Tapes philippinarum*			Diet: *T. suecica + S. costatum (Tet+Ske)*					
Clams nº	**Live weight g**	**Length mm**	**Breadth mm**	**Meat dry weight, g**	**Condition index**	**% Afdwt**	**Lipid (% Afdwt)**	**Carbohydrate (% Afdwt)**	**Protein (% Afdwt)**
Tet + Ske									
Females									
1	9.9	36	26	0.70	120.05	88.76	15.32	13.63	71.05
2	11.9	37	28	1.14	169.48	91.65	16.91	11.96	71.13
3	13.5	39	28	0.92	159.02	85.27	13.84	18.14	68.02
4	11.5	37	26	0.69	140.20	88.69	13.30	17.93	68.77
5	13.4	38	28	0.87	148.02	84.76	15.22	13.10	71.68
6	9.6	36	26	0.76	119.98	83.06	15.17	17.58	67.25
Males									
1	11.7	37	28	0.86	134.45	89.31	10.97	15.93	73.10
2	10.6	34	27	0.73	137.57	85.07	12.58	12.23	75.19
3	9.7	35	26	0.78	174.33	88.33	12.68	15.38	71.94
4	13.5	39	28	1.47	204.07	86.76	10.83	17.06	72.11
5	10.1	34	26	0.86	161.89	87.35	11.68	10.99	77.33
6	12.4	37	27	0.98	157.81	86.53	10.52	20.62	68.86
7	14.1	38	29	1.04	140.36	87.35	10.30	13.80	75.90
8	9.6	32	25	0.66	128.47	87.40	10.07	15.99	73.94
Non-spawners									
1	10.4	35	26	0.76	155.35	86.46	17.23	12.01	70.76
Mean	11.46	36.27	26.93	0.88	150.07	87.12	13.11	15.09	71.80
SE	0.42	0.51	0.30	0.05	5.80	0.55	0.62	0.73	0.76
Tet + Ske (replicate)									
Females									
1	12.0	36	29	0.69	101.54	83.80	11.10	14.99	73.91
2	11.1	36	26	0.74	125.41	89.20	10.99	18.77	70.24
3	8.4	35	25	0.53	112.60	85.82	12.93	13.11	73.96
4	13.5	40	30	0.87	110.72	88.37	11.66	23.54	64.80
Males									
1	11.8	37	30	0.85	151.82	88.47	11.98	16.39	71.63
2	14.1	37	29	1.00	156.75	87.79	11.05	20.66	68.29
3	8.8	34	26	0.69	192.64	88.15	11.80	15.09	73.11
4	8.5	38	27	0.59	125.19	88.22	11.11	15.41	73.48
5	13.3	39	29	1.11	168.71	88.57	12.19	15.25	72.56
6	12.8	39	30	0.86	162.57	89.34	10.86	13.77	75.37
7	11.4	34	27	0.97	156.85	87.48	10.52	15.68	73.80
8	10.5	37	26	0.89	153.50	87.79	10.48	15.71	73.81
Non-spawners									
1	10.6	34	25	0.80	162.49	87.82	15.03	13.24	71.73
2	10.7	35	26	0.76	136.11	87.26	10.66	13.15	76.19
3	11.0	36	26	0.77	151.51	86.86	9.44	20.94	69.62
Mean	11.23	36.47	27.40	0.81	144.56	87.66	11.45	16.38	72.17
SE	0.46	0.50	0.49	0.04	6.49	0.36	0.33	0.82	0.76

Appendix 7c (continued). broodstock of T. philippinarum conditioned for 9 weeks with a diet of dry T.suecica+S.costatum.

Date:	18.06.91			Ref: 2nd broodstock 1991- 3rd spawning (9 weeks)					
Spec.:	*Tapes philippinarum*			Diet: Dry T. suecica + S. costatum (Dry Tet+Ske)					
Clams nº	**Live weight g**	**Length mm**	**Breadth mm**	**Meat dry weight, g**	**Condition index**	**% Afdwt**	**Lipid (% Afdwt)**	**Carbohydrate (% Afdwt)**	**Protein (% Afdwt)**
Dry Tet + Ske									
Females									
1	11.9	36	26	0.57	104.85	84.63	12.43	18.98	68.59
2	9.6	34	25	0.59	165.17	85.16	10.38	20.27	69.35
3	10.6	36	27	0.55	144.42	84.26	12.21	21.53	66.26
4	12.0	35	25	0.60	91.88	84.45	10.75	21.59	67.66
5	9.2	30	25	0.53	132.33	83.03	11.80	17.76	70.44
6	10.3	36	27	0.55	93.51	83.96	10.37	18.70	70.93
7	8.6	32	26	0.54	104.50	84.25	13.38	19.41	67.21
8	11.0	34	27	0.52	89.29	83.95	11.21	17.33	71.46
Males									
1	9.8	34	24	0.65	134.88	83.94	11.68	16.63	71.69
2	11.5	37	27	0.86	132.80	83.47	11.10	19.83	69.07
3	10.6	34	24	0.63	139.04	84.13	10.67	17.87	71.46
4	10.6	35	25	1.02	243.02	85.24	12.52	18.87	68.61
Non-spawners									
11	12.2	37	27	0.70	140.02	85.32	11.29	24.28	64.43
12	11.6	35	26	0.94	146.34	84.37	17.54	19.38	63.08
13	12.7	36	28	0.78	148.00	84.52	11.40	21.24	67.36
Mean	10.81	34.73	25.93	0.67	134.00	84.31	11.92	19.58	68.51
SE	0.31	0.48	0.32	0.04	9.88	0.16	0.46	0.51	0.67
Dry Tet + Ske (replicate)									
Females									
1	12.6	37	27	0.78	127.07	85.65	10.86	17.06	72.08
2	9.3	35	26	0.57	155.30	85.96	13.09	19.51	67.40
3	12.0	36	27	0.56	96.21	85.11	11.31	21.57	67.12
4	9.0	34	26	0.46	99.20	84.55	10.09	18.44	71.47
5	11.5	36	26	0.65	113.51	83.03	10.24	21.86	67.90
Males									
1	12.6	35	26	0.72	117.39	83.96	9.97	19.55	70.48
2	9.8	36	27	0.75	201.86	84.00	10.32	16.97	72.71
3	11.1	37	27	0.63	123.73	83.90	10.45	23.31	66.24
4	12.4	36	26	0.65	121.85	83.81	10.74	17.48	71.78
5	9.4	32	24	0.56	129.86	85.15	9.98	20.56	69.46
Non-spawners									
1	9.7	33	22	0.44	105.43	82.04	8.78	14.28	76.94
2	12.6	36	26	0.70	142.12	85.23	9.27	22.12	68.61
3	12.8	37	27	0.68	128.87	84.37	10.11	21.42	68.47
4	11.2	33	26	0.95	201.43	84.52	11.00	22.53	66.47
5	11.1	37	27	0.97	167.03	84.38	10.07	17.92	72.01
Mean	11.14	35.33	26.00	0.67	135.39	84.38	10.42	19.64	69.94
SE	0.36	0.42	0.35	0.04	8.52	0.26	0.25	0.66	0.76

Appendix 7c. Broodstock of T. philippinarum conditioned for 9 weeks with filtered sea water.

Date:	18.06.91			**Ref:** 2nd broodstock 1991- 3rd spawning (9 weeks)					
Spec.:	*Tapes philippinarum*			**Diet:** Filtered sea water					
Clams nº	**Live weight g**	**Length mm**	**Breadth mm**	**Meat dry weight, g**	**Condition index**	**% Afdwt**	**Lipid (% Afdwt)**	**Carbohydrate (% Afdwt)**	**Protein (% Afdwt)**
FSW									
Females									
1	9.3	33	25	0.55	128.42	83.39	12.60	14.86	72.54
2	11.7	37	26	0.65	102.83	85.07	9.76	21.88	68.36
Males									
1	11.0	36	26	0.55	105.33	79.33	9.98	15.71	74.31
2	10.8	35	26	0.62	121.78	83.73	9.76	14.79	75.45
Non-spawners									
1	10.0	34	26	0.65	127.75	86.37	12.94	18.53	68.53
2	11.1	39	27	0.67	96.88	80.62	10.34	16.02	73.64
3	10.6	34	26	0.61	130.68	83.06	13.45	14.94	71.61
4	9.4	33	25	0.65	137.36	85.67	14.42	20.58	65.00
5	10.9	35	26	0.62	114.26	82.24	9.81	16.84	73.35
6	10.2	35	25	0.54	115.64	81.87	9.01	18.50	72.49
7	10.5	35	25	0.46	88.33	83.57	9.70	16.84	73.46
8	10.6	36	27	0.79	142.82	84.46	13.65	16.50	69.85
9	9.0	33	25	0.47	103.04	85.69	8.06	15.44	76.50
10	10.2	34	26	0.64	122.96	84.19	10.05	16.08	73.87
11	9.3	36	27	0.57	107.57	85.18	8.10	21.91	69.99
Mean	10.31	35.00	25.87	0.6	116.38	83.63	10.78	17.29	71.93
SE	0.20	0.43	0.19	0.02	4.04	0.51	0.53	0.63	0.79
FSW (replicate)									
Females									
1	9.7	34	26	0.47	97.69	83.58	7.54	19.30	73.16
2	11.0	35	27	0.51	91.05	81.05	10.49	13.02	76.49
3	10.0	34	25	0.63	130.42	85.50	10.99	18.26	70.75
Males									
1	11.3	35	26	0.54	99.78	83.56	11.25	16.83	71.92
2	12.0	36	27	0.73	133.55	83.85	9.82	19.69	70.49
3	9.6	35	25	0.52	112.09	84.25	10.01	12.85	77.14
4	9.7	35	25	0.46	91.48	77.39	10.43	15.32	74.25
5	10.1	34	27	0.50	106.19	84.12	9.99	17.01	73.00
6	11.8	35	26	0.57	109.67	82.11	9.99	11.55	78.46
7	8.9	34	24	0.47	112.05	84.59	9.58	15.39	75.03
8	8.5	32	24	0.37	115.78	79.89	10.64	19.58	69.78
Non-spawners									
1	10.8	37	25	0.61	106.54	82.52	9.09	17.96	72.95
2	11.4	38	26	0.63	122.76	82.72	10.64	15.11	74.25
3	9.2	32	25	0.49	111.39	81.20	8.62	14.41	76.97
4	9.5	34	27	0.50	106.51	84.52	11.12	18.64	70.24
Mean	10.23	34.67	25.67	0.53	109.80	82.72	10.01	16.33	73.66
SE	0.28	0.41	0.27	0.02	3.22	0.55	0.26	0.68	0.71

Appendix 8. Number of eggs produced per female of T. philippinarum (in millions) after being conditioned for 5 (Spawning I), 7 (Spawning II) and 9 (Spawning III) weeks on mixed diets of 70% T. suecica and 30% S. costatum or 70% dry T. suecica and 30% S. costatum.
For abbreviation see Table 3.21.

2nd broodstock 1991

Spawning	Females Nº	Treatment Tet+Ske	Tet+Ske-R	Dry Tet+Ske	Dry Tet+Ske-R	Fsw	Fsw-R
I	1	0.890	0	0.056	0	0	0
II	1	2.971	3.905	3.747	1.478	0.234	0.685
	2	1.887	5.210	3.777	2.243	2.667	0.727
	3	-	0.974	4.025	1.737	-	1.041
	4	-	0.185	0.714	3.393	-	0.118
	5	-	-	0.080	2.005	-	1.296
	6	-	-	0.878	2.696	-	1.216
	7	-	-	0.380	0.074	-	0.598
	8	-	-	0.243	1.326	-	-
	9	-	-	4.043	-	-	-
	10	-	-	3.204	-	-	-
III	1	4.092	9.116	4.070	0.592	1.773	2.681
	2	0.206	1.264	4.998	4.604	0.973	4.212
	3	4.295	5.387	6.861	8.254	-	0.300
	4	0.034	7.204	4.124	5.597	-	-
	5	2.810	-	5.659	5.283	-	-
	6	3.836	-	6.116	-	-	-
	7	-	-	3.405	-	-	-
	8	-	-	8.393	-	-	-

Appendix 9. Fatty acid composition of T. philippinarum eggs and larvae from broodstock fed on different diets. 2nd broodstock 1991, spawning II. Values are percentages of total fatty acid. For abbreviations see Table 3.21.

2nd broodstock 1991 - Spawning II	**Eggs**			**D-larvae**		
Treatment	Tet+Ske	Dry Tet+Ske	Fsw	Tet+Ske	Dry Tet+Ske	Fsw
Fatty acid						
14:0	5.90	5.87	5.96	10.71	7.20	7.32
16:0	24.83	25.35	25.21	24.71	27.47	27.66
18:0	5.36	5.15	5.22	6.40	6.97	5.93
16:1w7/9	6.10	5.86	4.52	1.11	5.69	4.09
18:1w9	11.37	11.55	13.17	14.29	12.14	13.22
18:1w7	3.06	2.96	2.43	2.66	3.06	2.33
20:1w9	4.94	5.08	4.58	7.27	5.37	5.12
18:2w6	1.28	1.20	1.91	1.15	0.95	1.25
18:3w3	3.02	2.71	3.26	5.45	2.34	3.31
18:4w3	8.78	9.25	11.00	5.97	7.68	10.86
20:2w6	1.65	1.11	0.99	3.00	1.79	1.42
20:3w3	0.82	0.66	0.72	0.93	0.00	0.58
20:4w6	1.25	1.08	0.82	0.92	1.00	0.65
20:5w3	9.45	8.76	5.43	6.23	6.75	4.04
22:5w3	0.64	0.67	0.51	0.92	0.30	0.16
22:6w3	12.55	12.73	14.27	8.26	11.31	12.07
Saturated	36.09	36.37	36.39	41.82	41.64	40.91
Monoenoic	25.47	25.45	24.70	25.33	26.26	24.76
Polyenoic	39.44	38.17	38.91	32.83	32.12	34.34

(continued)

Appendix 9. (continued)

5 day larvae			10 day larvae			14 day larvae		
Tet+Ske	Dry Tet+Ske	Fsw	Tet+Ske	Dry Tet+Ske	Fsw	Tet+Ske	Dry Tet+Ske	Fsw
7.76	7.81	9.01	7.81	8.71	12.13	12.55	8.14	7.83
15.04	13.12	15.54	16.97	16.58	18.61	18.49	17.87	19.32
6.65	6.61	6.91	6.81	6.18	7.46	4.62	4.74	4.50
20.06	22.09	22.41	22.93	24.25	17.20	21.97	25.58	24.71
2.95	2.04	3.29	2.84	2.59	7.54	4.72	1.14	1.16
8.71	8.09	7.21	8.85	9.01	6.28	7.94	9.89	10.52
3.44	2.88	2.46	2.31	1.96	1.70	1.63	1.58	1.67
0.39	0.25	0.59	0.62	0.48	1.16	0.73	0.38	0.42
1.00	1.10	0.76	1.05	1.03	1.08	1.31	1.24	1.31
2.98	2.26	2.58	1.84	1.67	1.57	0.79	1.39	1.77
1.39	1.33	1.24	0.89	0.92	2.51	1.65	0.71	0.58
0.00	0.55	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.85	1.57	0.78	0.80	0.57	2.64	1.09	0.52	0.56
21.76	22.27	20.51	21.67	22.36	15.17	19.58	23.56	22.84
0.70	0.94	0.64	0.76	0.64	0.98	0.59	0.64	0.71
6.34	7.09	6.08	3.85	3.05	3.97	2.35	2.62	2.09
29.45	27.54	31.46	31.59	31.47	38.20	35.66	30.75	31.65
35.16	35.10	35.37	36.93	37.81	32.72	36.26	38.19	38.06
35.41	37.36	33.18	31.48	30.72	29.08	28.09	31.06	30.28

Appendix 9a. Fatty acid composition of T. philippinarum eggs and D-larvae from broodstock fed on different diets. 2nd broodstock 1991, spawning III. Values are percentages of total fatty acid. For abbreviations see Table 3.21.

2nd broodstock 1991 - Spawning III	**Eggs**			**D-larvae**		
Treatment	Tet+Ske	Dry Tet+Ske	Fsw	Tet+Ske	Dry Tet+Ske	Fsw
Fatty acid						
14:0	14.67	15.26	19.66	27.38	24.39	21.14
16:0	28.46	29.66	28.42	26.25	26.85	23.35
18:0	9.34	7.18	6.90	5.57	6.39	6.99
16:1w9	0.00	0.00	0.00	0.00	0.00	0.00
16:1w7	8.88	4.78	4.75	4.06	4.05	5.16
18:1w9	15.19	16.74	15.83	14.31	14.92	13.74
18:1w7	2.41	2.87	1.92	1.96	2.15	2.30
20:1w9						
18:2w6	2.22	1.88	1.87	2.33	1.79	2.79
18:3w3	1.72	1.85	2.21	1.77	1.24	2.53
18:4w3	2.87	4.33	4.23	3.32	2.45	3.65
20:2w6	0.00	0.00	0.00	0.00	0.00	0.00
20:3w3	0.00	0.00	0.00	0.00	0.00	0.00
20:4w6	4.06	2.72	0.00	0.00	1.01	1.50
20:5w3	7.26	6.21	4.92	6.12	7.03	7.30
22:5w3	0.00	0.00	0.00	0.00	0.00	0.00
22:6w3	2.94	6.52	9.30	6.91	7.73	9.55
Saturated	52.47	52.10	54.98	59.21	57.63	51.48
Monoenoic	26.47	24.39	22.49	20.33	21.12	21.20
Polyenoic	21.06	23.51	22.53	20.46	21.25	27.32

Appendix 10. Condition indices and biochemical composition of 1st T. philippinarum broodstock 1992. Values are given for initial and broodstock conditioned for 6, 7 and 8 weeks with different diets.

Date: 22.01.92 | Ref: 1st broodstock conditioning 1992 - initial

Species *Tapes philippinarum*

Clams nº	Live weight g	Length mm	Breadth mm	Meat dry weight, g	Condition index	% Afdwt	Lipid (% Afdwt)	Carbohydrate (% Afdwt)	Protein (% Afdwt)
1	24.6	47	33	0.95	106.08	77.83	9.54	13.81	76.65
2	17.4	44	32	0.85	93.79	77.46	10.65	12.15	77.20
3	17.1	43	32	0.73	77.63	74.14	11.01	13.60	75.39
4	16.1	42	32	0.84	103.04	83.59	10.18	21.27	68.55
5	18.0	42	30	0.83	102.16	83.58	9.79	13.13	77.08
6	16.5	42	30	0.75	93.15	78.82	11.39	12.93	75.68
7	16.5	41	31	0.70	82.75	79.93	9.70	15.11	75.19
8	18.2	42	30	0.82	103.39	81.50	8.76	19.99	71.25
9	20.1	46	34	0.80	82.52	79.45	9.57	14.08	76.35
10	25.5	48	34	1.18	93.31	78.10	9.99	17.41	72.60
11	28.6	48	35	1.28	110.73	77.39	10.81	16.73	72.46
12	20.7	45	33	0.93	84.51	76.40	10.37	10.47	79.16
13	14.8	39	29	0.58	98.00	75.68	9.27	13.46	77.27
14	22.5	46	34	1.02	96.15	79.71	10.47	17.25	72.28
15	23.8	45	34	1.26	117.40	81.15	9.51	25.86	64.63
16	25.5	48	34	1.12	95.02	82.50	9.29	19.92	70.79
17	24.1	45	33	1.24	124.39	83.02	8.99	19.36	71.65
18	24.7	45	34	1.08	111.78	79.36	8.80	18.28	72.92
19	20.8	45	34	0.99	95.00	82.36	10.61	18.06	71.33
20	26.8	47	34	1.28	118.29	81.23	10.08	20.32	69.60
21	18.5	44	31	0.85	99.49	81.54	11.47	13.67	74.86
22	22.7	46	33	1.22	123.00	79.87	10.14	26.64	63.22
23	19.3	45	31	0.83	99.49	79.59	9.13	14.58	76.29
24	15.6	40	29	0.75	115.40	78.57	8.83	16.37	74.80
25	17.5	40	30	0.83	98.40	82.13	8.57	19.04	72.39
26	22.5	43	33	0.96	103.33	80.86	8.59	15.85	75.56
27	21.2	44	33	0.93	96.74	77.95	10.16	17.26	72.58
28	22.6	45	35	1.05	98.00	79.53	10.15	18.92	70.93
29	23.7	46	34	0.91	73.13	78.60	8.43	16.15	75.42
30	19.3	45	32	1.09	95.41	81.33	8.61	21.64	69.75
Mean	20.85	44.27	32.43	0.95	99.72	79.77	9.76	17.11	73.13
SE	0.67	0.44	0.32	0.03	2.31	0.43	0.16	0.69	0.67

Appendix 10a. Broodstock of T. philippinarum conditioned for 6 weeks with a diet of dry T. suecica.

Date:	05.03.92				**Ref:** 1st broodstock 1992 - spawning I (6 weeks)				
Species	*Tapes philippinarum*				**Diet:** Dry T. suecica				
Clams nº	**Live weight g**	**Length mm**	**Breadth mm**	**Meat dry weight, g**	**Condition index**	**% Afdwt**	**Lipid (% Afdwt)**	**Carbohydrate (% Afdwt)**	**Protein (% Afdwt)**
Females									
1	23.6	47	35	1.14	101.96	85.36	11.05	21.00	67.95
2	20.2	44	32	1.03	88.66	80.29	10.37	23.55	66.08
3	20.1	46	34	1.00	88.12	82.87	10.63	19.19	70.18
4	18.2	42	34	0.89	86.89	82.28	11.17	19.97	68.86
5	24.0	46	37	1.01	82.00	83.45	11.13	17.56	71.31
Males									
1	24.2	46	34	1.14	91.41	82.23	10.26	23.24	66.50
2	17.5	43	31	1.04	135.12	83.70	10.19	20.54	69.27
3	19.5	44	33	0.95	92.26	79.78	9.25	17.75	73.00
4	24.2	46	33	1.18	99.31	84.23	9.89	14.11	76.00
5	22.2	47	34	1.11	97.25	82.25	11.95	14.31	73.74
6	20.3	45	34	1.07	96.37	83.35	8.87	22.26	68.87
7	24.7	45	35	1.13	99.62	83.78	9.55	21.39	69.06
8	22.2	45	34	1.04	102.26	80.42	11.15	21.71	67.14
9	21.6	45	31	0.87	87.42	80.76	9.15	21.47	69.38
Non-spawners									
1	28.3	48	36	1.53	98.91	80.50	9.21	32.87	57.92
Mean	22.05	45.27	33.80	1.07	96.50	82.35	10.25	20.73	69.02
SE	0.74	0.41	0.43	0.04	3.19	0.43	0.24	1.14	1.06

Appendix 10a (continued). Broodstock of T. philippinarum conditioned for 6 weeks with a diet of dry T. suecica + D. tertiolecta

Date: 05.03.92 **Ref:** 1st broodstock 1992 - spawning I (6 weeks)

Species *Tapes philippinarum* **Diet:** Dry T. suecica + D. tertiolecta

Clams nº	Live weight g	Length mm	Breadth mm	Meat dry weight, g	Condition index	% Afdwt	Lipid (% Afdwt)	Carbohydrate (% Afdwt)	Protein (% Afdwt)
Females									
1	21.2	46	35	0.85	73.38	83.87	10.75	21.37	67.88
2	28.5	51	36	1.03	76.09	81.78	9.99	18.77	71.24
3	15.8	39	29	0.60	94.89	85.67	7.73	18.98	73.29
4	27.8	50	34	1.01	75.10	83.53	9.16	16.69	74.15
5	10.5	36	25	0.41	81.10	87.42	8.80	18.83	72.37
6	26.7	45	36	0.75	64.98	86.87	9.94	16.48	73.58
7	17.2	42	34	0.78	77.03	86.54	10.18	17.57	72.25
Males									
1	20.2	46	32	0.70	69.01	82.52	8.75	12.79	78.46
2	23.5	46	34	1.01	83.00	86.88	9.60	14.53	75.87
3	17.4	41	31	0.64	85.84	85.77	8.81	14.08	77.11
4	32.0	51	38	1.45	82.63	87.67	9.99	12.07	77.94
5	21.5	44	32	0.89	85.93	84.26	9.94	14.33	75.73
6	17.8	44	32	0.79	99.43	87.89	10.19	15.12	74.69
7	17.2	44	31	0.73	105.38	87.76	9.68	15.06	75.26
8	23.3	44	36	1.01	96.65	86.57	10.45	17.75	71.80
Mean	21.37	44.60	33.00	0.84	83.36	85.67	9.60	16.29	74.11
SE	1.47	1.08	0.85	0.06	2.99	0.52	0.21	0.67	0.72

Appendix 10a (continued). Broodstock of T. philippinarum conditioned for 6 weeks with a diet of dry T. suecica + S. costatum

Date: 05.03.92 **Ref:** 1st broodstock 1992 - spawning I (6 weeks)

Species *Tapes philippinarum* **Diet:** Dry T. suecica + S. costatum

Clams nº	Live weight g	Length mm	Breadth mm	Meat dry weight, g	Condition index	% Afdwt	Lipid (% Afdwt)	Carbohydrate (% Afdwt)	Protein (% Afdwt)
Females									
1	20.3	45	35	1.08	104.53	84.32	13.14	14.91	71.95
2	18.0	45	31	0.80	102.59	81.33	12.44	15.39	72.17
3	19.8	46	33	1.03	106.30	79.95	12.31	13.34	74.35
4	22.7	42	32	1.09	86.73	78.05	13.10	12.37	74.53
5	15.8	44	31	0.79	100.67	82.12	12.00	16.09	71.91
6	21.3	46	34	1.04	94.50	79.03	12.17	15.41	72.42
Males									
1	22.2	46	32	0.98	97.51	81.17	12.98	15.01	72.01
2	30.0	42	35	1.28	115.12	81.89	12.03	16.15	71.82
3	24.0	45	34	1.10	121.77	78.40	12.70	14.77	72.53
4	19.6	47	33	1.15	101.60	87.26	10.72	16.00	73.28
5	22.3	44	34	0.99	95.04	82.91	11.53	16.03	72.44
6	21.0	44	32	1.07	103.41	83.43	12.29	16.26	71.45
7	21.2	46	32	0.86	75.76	84.41	10.12	14.57	75.31
8	20.4	44	34	1.11	124.93	85.21	9.99	13.40	76.61
Non-spawners									
1	23.6	45	32	1.02	96.75	84.04	9.17	14.32	76.51
Mean	21.48	44.73	32.93	1.02	101.81	82.23	11.78	14.93	73.29
SE	0.82	0.37	0.34	0.03	3.23	0.68	0.32	0.30	0.45

Appendix 10a (continued). Broodstock of T. philippinarum conditioned for 6 weeks with a diet of dry T. suecica + I. galbana

Date: 05.03.92 **Ref:** 1st broodstock 1992 - spawning I (6 weeks)

Species *Tapes philippinarum* **Diet:** Dry T. suecica + I. galbana

Clams nº	Live weight g	Length mm	Breadth mm	Meat dry weight, g	Condition index	% Afdwt	Lipid (% Afdwt)	Carbohydrate (% Afdwt)	Protein (% Afdwt)
Females									
1	18.2	44	33	0.72	76.06	80.73	10.27	19.83	69.90
2	21.0	44	31	0.97	94.11	83.63	10.88	27.25	61.87
3	24.8	46	36	1.28	120.37	83.44	11.84	23.55	64.61
4	26.4	48	37	1.14	83.79	81.46	10.89	24.59	64.52
5	17.7	43	31	1.01	102.04	84.50	10.68	27.78	61.54
Males									
1	20.5	45	34	1.37	129.55	84.13	10.04	23.91	66.05
2	21.8	47	34	1.47	117.74	86.61	10.60	28.26	61.14
3	26.3	46	36	1.36	114.39	83.99	11.35	23.80	64.85
4	19.4	47	36	0.89	76.00	84.38	11.67	16.12	72.21
5	20.9	44	35	1.15	118.56	82.24	10.26	24.61	65.13
6	21.8	45	35	1.35	123.99	82.77	9.65	23.97	66.38
7	25.6	46	34	1.33	104.86	81.00	9.08	22.51	68.41
8	27.4	46	35	1.39	107.56	84.83	11.82	21.66	66.52
Non-spawners									
1	18.3	43	31	0.80	109.34	78.15	10.85	21.10	68.05
2	21.5	45	34	1.15	104.43	82.96	9.55	19.44	71.01
Mean	22.11	45.27	34.13	1.16	105.52	82.99	10.63	23.23	66.15
SE	0.83	0.38	0.50	0.06	4.32	0.53	0.22	0.85	0.86

Appendix 10a (continued). Broodstock of T. philippinarum conditioned for 6 weeks with filtered sea water.

Date:	05.03.92				**Ref:** 1st broodstock 1992 - spawning I (6 weeks)				
Species	*Tapes philippinarum*				**Diet:** FSW				
Clams nº	**Live weight g**	**Length mm**	**Breadth mm**	**Meat dry weight, g**	**Condition index**	**% Afdwt**	**Lipid (% Afdwt)**	**Carbohydrate (% Afdwt)**	**Protein (% Afdwt)**
Females									
1	16.8	42	31	0.84	96.67	74.83	8.73	13.16	78.11
Males									
1	25.0	47	38	0.81	62.75	83.50	9.86	12.05	78.09
Non-spawners									
1	22.2	46	36	0.69	69.22	78.22	9.62	13.89	76.49
2	24.6	48	36	0.84	64.99	76.03	10.24	6.52	83.24
3	30.9	50	37	1.34	91.62	78.09	9.68	20.35	69.97
4	16.8	42	31	0.69	85.46	77.52	8.99	12.24	78.77
5	24.2	45	36	0.95	81.51	73.29	9.81	9.86	80.33
6	20.7	44	33	0.68	70.26	75.19	7.77	8.51	83.72
7	26.3	44	36	0.85	73.54	74.63	10.88	9.75	79.37
8	19.1	42	31	0.60	70.80	74.66	8.82	7.32	83.86
9	24.2	48	35	0.95	98.61	80.50	9.31	14.46	76.23
10	25.9	48	36	0.97	76.13	77.29	7.74	10.53	81.73
11	17.3	44	31	0.70	55.70	72.28	8.86	6.03	85.11
12	29.1	49	37	1.13	86.87	81.71	9.73	13.83	76.44
13	22.1	44	35	0.76	54.96	75.48	9.27	6.00	84.73
Mean	23.01	45.53	34.60	0.85	75.94	76.88	9.29	10.97	79.75
SE	1.11	0.69	0.65	0.05	3.58	0.81	0.22	1.01	1.07

Appendix 10b. Broodstock of T. philippinarum conditioned for 7 weeks with a diet of dry T. suecica

Date: 10.03.92 **Ref:** 1st broodstock 1992 - spawning II (7 weeks)

Species *Tapes philippinarum* **Diet:** Dry T. suecica

Clams nº	Live weight g	Length mm	Breadth mm	Meat dry weight, g	Condition index	% Afdwt	Lipid (% Afdwt)	Carbohydrate (% Afdwt)	Protein (% Afdwt)
Females									
1	18.9	42	32	0.93	93.52	83.42	12.72	12.76	74.52
2	13.2	41	29	0.71	87.94	84.07	7.56	20.00	72.44
3	22.9	44	34	1.18	119.19	81.81	6.77	27.33	65.90
4	16.0	41	30	0.79	105.63	79.14	7.79	18.93	73.28
5	23.0	47	34	1.18	102.95	80.06	8.07	23.44	68.49
Males									
1	22.4	47	34	1.19	97.10	79.49	7.90	20.41	71.69
2	17.9	45	34	0.89	82.70	87.06	8.34	18.72	72.94
3	21.2	47	34	1.21	118.74	81.44	7.21	19.40	73.39
4	20.8	44	31	1.03	110.54	79.84	9.04	22.97	67.99
Non spawners									
1	19.9	46	33	1.04	92.91	80.88	10.17	21.07	68.76
2	20.4	41	32	1.03	101.17	81.11	9.45	19.81	70.74
3	22.2	44	35	1.27	116.07	82.71	8.64	23.21	68.15
4	21.1	44	32	0.98	109.36	80.05	9.72	17.21	73.07
5	14.5	41	32	0.80	94.68	83.49	10.41	18.19	71.40
6	19.1	44	34	0.89	87.73	79.15	10.82	14.84	74.34
Mean	19.57	43.87	32.67	1.01	101.35	81.58	8.97	19.89	71.14
SE	0.78	0.58	0.44	0.04	3.02	0.57	0.41	0.92	0.69

Appendix 10b (continued). Broodstock of T. philippinarum conditioned for 7 weeks with a diet of dry T. suecica + D. tertiolecta

Date: 10.03.92 **Ref:** 1st broodstock 1992 - spawning II (7 weeks)

Species *Tapes philippinarum* **Diet:** Dry T. suecica + D. tertiolecta

Clams nº	Live weight g	Length mm	Breadth mm	Meat dry weight, g	Condition index	% Afdwt	Lipid (% Afdwt)	Carbohydrate (% Afdwt)	Protein (% Afdwt)
Females									
1	23.0	46	34	0.94	87.16	78.98	14.14	20.11	65.75
2	16.7	43	31	0.63	64.04	80.96	14.26	7.95	77.79
3	25.2	46	35	0.91	72.03	81.77	13.10	14.87	72.03
4	23.6	49	34	1.09	88.23	83.24	11.91	18.60	69.49
5	21.4	47	34	0.94	87.74	80.66	10.46	19.77	69.77
6	20.7	46	34	1.06	89.20	82.02	11.60	16.63	71.77
7	19.9	44	35	0.79	98.99	79.39	12.55	13.28	74.17
Males									
1	21.6	45	34	1.14	112.90	83.61	11.81	20.83	67.36
2	19.6	45	32	1.03	102.90	83.08	10.76	18.71	70.53
3	20.8	44	32	1.10	97.65	80.29	11.83	17.97	70.20
4	19.0	44	30	1.03	116.92	82.60	11.22	17.92	70.86
Non spawners									
1	20.5	44	33	0.97	97.69	82.43	11.72	17.42	70.86
2	23.5	46	32	1.07	92.13	80.57	12.16	14.05	73.79
3	17.8	40	29	0.71	91.51	78.89	12.45	7.30	80.25
4	24.5	47	36	1.24	97.25	83.77	11.52	17.20	71.28
Mean	21.19	45.07	33.00	0.98	93.09	81.48	12.10	16.17	71.73
SE	0.63	0.54	0.51	0.04	3.48	0.43	0.28	1.05	0.95

Appendix 10b (continued). Broodstock of T. philippinarum conditioned for 7 weeks with a diet of dry T. suecica + S. costatum

Date: 10.03.92 — **Ref:** 1st broodstock 1992 - spawning II (7 weeks)

Species *Tapes philippinarum* — **Diet:** Dry T. suecica + S. costatum

Clams nº	Live weight g	Length mm	Breadth mm	Meat dry weight, g	Condition index	% Afdwt	Lipid (% Afdwt)	Carbohydrate (% Afdwt)	Protein (% Afdwt)
Females									
1	21.0	45	34	1.10	78.59	82.33	9.20	22.3	68.50
2	25.0	46	37	1.06	87.58	83.36	9.79	19.81	70.40
3	20.8	46	35	0.84	70.07	81.46	11.49	12.39	76.12
4	17.9	43	31	0.67	74.12	80.76	10.78	14.13	75.09
Males									
1	26.4	45	34	1.07	90.80	83.19	10.25	18.67	71.08
2	18.5	42	32	1.04	110.16	80.15	9.72	21.35	68.93
3	21.1	47	34	1.33	103.60	83.16	10.79	18.06	71.15
Non-spawners									
1	25.0	44	32	0.97	97.05	79.78	9.69	17.57	72.74
2	24.5	49	34	1.16	96.33	82.24	9.02	18.92	72.06
3	23.1	46	34	1.08	91.71	81.89	10.59	16.15	73.26
4	21.8	44	34	1.25	123.49	79.34	12.32	16.72	70.96
5	26.6	45	34	1.16	102.44	82.42	10.91	15.03	74.06
6	22.5	45	34	1.11	105.50	83.79	10.42	14.77	74.81
7	19.6	45	32	1.05	111.18	86.13	11.82	15.95	72.23
8	25.3	46	36	1.22	106.03	83.15	10.64	16.44	72.92
Mean	22.61	45.20	33.80	1.07	96.58	82.21	10.50	17.22	72.29
SE	0.72	0.43	0.40	0.04	3.81	0.45	0.24	0.70	0.57

Appendix 10b (continued). Broodstock of T. philippinarum conditioned for 7 weeks with a diet of dry T. suecica + I. galbana

Date: 12.03.92 **Ref:** 1st broodstock 1992 - spawning II (7 weeks)

Species *Tapes philippinarum* **Diet:** Dry T. suecica + I. galbana

Clams nº	Live weight g	Length mm	Breadth mm	Meat dry weight, g	Condition index	% Afdwt	Lipid (% Afdwt)	Carbohydrate (% Afdwt)	Protein (% Afdwt)
Females									
1	20.4	44	32	1.05	104.79	83.94	11.67	20.25	68.08
2	14.6	40	29	0.61	75.16	81.97	10.89	16.1	73.01
Males									
1	31.4	51	39	1.76	118.65	86.48	11.03	27.62	61.35
2	24.7	47	36	1.2	97.08	81.47	9.27	17.63	73.1
3	24.1	48	35	1.35	95.54	84.7	9.49	24.07	66.44
4	25.5	47	36	1.24	95.17	83.19	11.5	16.89	71.61
5	20.7	43	32	1.44	151.68	87.37	10.04	22.46	67.5
6	33	53	39	1.97	129.46	87.12	8.18	27.29	64.53
7	21.1	46	32	1.36	140.06	85.57	10	23.77	66.23
Non spawners									
1	24.6	45	33	1.65	135.52	85.51	9.73	22.64	67.63
2	24.8	50	35	1.51	124.68	86.64	10.27	22.06	67.67
3	21.1	46	33	1.36	137.59	86.52	10.44	17.38	72.18
4	22.2	47	33	1.15	108.72	85.03	9.48	16.35	74.17
5	24.4	47	36	1.63	117.12	86.92	8.82	22.03	69.15
6	25.3	45	35	1.26	111.12	83.74	9.74	17.74	72.52
Mean	23.86	46.60	34.33	1.37	116.16	85.08	10.04	20.95	69.01
SE	1.14	0.83	0.70	0.08	5.36	0.48	0.25	0.99	0.95

Appendix 10b (continued). Broodstock of T. philippinarum conditioned for 7 weeks with filtered sea water

Date: 10.03.92 **Ref:** 1st broodstock 1992 - spawning II (7 weeks)

Species *Tapes philippinarum* **Diet:** FSW

Clams nº	Live weight g	Length mm	Breadth mm	Meat dry weight, g	Condition index	% Afdwt	Lipid (% Afdwt)	Carbohydrate (% Afdwt)	Protein (% Afdwt)
Non-spawners									
1	21.6	45	31	0.85	87.20	77.51	7.30	9.05	83.65
2	17.0	45	31	0.61	62.31	75.05	8.93	3.32	87.75
3	27.9	45	34	0.94	66.89	73.82	7.78	7.56	84.66
4	23.0	44	35	0.76	65.68	75.38	7.72	3.93	88.35
5	18.5	45	30	0.71	75.99	72.06	8.37	4.84	86.79
6	24.6	44	34	0.86	81.74	77.87	7.87	7.92	84.21
7	19.5	43	32	0.57	56.61	74.24	8.28	3.34	88.38
8	19.8	40	32	0.70	79.15	76.74	8.12	8.49	83.39
9	18.7	45	32	0.70	61.86	78.97	8.00	8.14	83.86
10	17.7	42	31	0.62	45.44	75.36	8.98	4.76	86.26
11	20.4	47	30	0.74	59.83	78.82	7.39	8.4	84.21
12	19.6	44	35	0.78	77.75	78.07	7.33	10.64	82.03
13	19.2	47	34	0.62	60.84	78.35	8.17	6.28	85.55
14	28.1	48	34	0.94	60.65	74.41	7.98	5.23	86.79
15	23.3	46	34	0.79	62.90	74.81	7.87	5.91	86.22
Mean	21.26	44.67	32.60	0.74	66.99	76.10	8.01	6.52	85.47
SE	0.89	0.52	0.46	0.03	2.88	0.54	0.13	0.58	0.50

Appendix 10c. Broodstock of T. philippinarum conditioned for 8 weeks with a diet of dry T. suecica.

Date:	17.03.92				**Ref:** 1st broodstock 1992 - spawning III (8 weeks)				
Species	*Tapes philippinarum*				**Diet:** Dry T. suecica				
Clams nº	**Live weight g**	**Length mm**	**Breadth mm**	**Meat dry weight, g**	**Condition index**	**% Afdwt**	**Lipid (% Afdwt)**	**Carbohydrate (% Afdwt)**	**Protein (% Afdwt)**
Females									
1	17.6	44	31	0.87	96.44	85.88	9.13	20.39	70.48
Males									
1	18.7	49	35	1.09	96.85	85.98	10.82	18.77	70.41
2	20.1	45	36	0.73	75.04	89.59	9.27	16.03	74.70
3	16.6	50	38	0.92	106.94	89.88	9.47	20.78	69.75
4	18.4	47	36	0.84	72.81	90.48	10.04	19.01	70.95
5	18.4	46	33	0.89	101.98	88.13	9.52	18.89	71.59
6	15.3	47	34	1.05	110.94	91.64	9.19	24.47	66.34
7	15.2	44	34	0.67	66.70	87.27	10.10	23.06	66.84
Non-spawners									
1	19.8	47	35	1.00	123.36	89.93	9.48	19.54	70.98
2	18.9	47	34	0.95	75.83	86.33	10.21	19.63	70.16
3	19.9	45	33	1.14	101.96	87.58	9.73	21.93	68.34
4	19.5	44	34	1.12	95.88	84.46	11.29	21.61	67.10
5	17.1	48	34	1.08	127.45	87.32	11.71	14.88	73.41
6	21.3	46	34	1.20	133.73	83.23	9.03	12.29	78.68
7	17.1	42	32	0.81	132.39	85.74	9.04	20.15	70.81
Mean	18.26	46.07	34.20	0.96	101.22	87.56	9.87	19.43	70.70
SE	0.46	0.55	0.44	0.04	5.66	0.61	0.22	0.81	0.82

Appendix 10c (continued). Broodstock of T. philippinarum conditioned for 8 weeks with a diet of dry T. suecica + D. tertiolecta.

Date:	17.03.92				**Ref:** 1st broodstock 1992 - spawning III (8 weeks)				
Species	*Tapes philippinarum*				**Diet:** Dry T. suecica + D. tertiolecta				
Clams nº	**Live weight g**	**Length mm**	**Breadth mm**	**Meat dry weight, g**	**Condition index**	**% Afdwt**	**Lipid (% Afdwt)**	**Carbohydrate (% Afdwt)**	**Protein (% Afdwt)**
Females									
1	24.6	47	35	1.05	75.48	79.53	9.53	11.24	79.23
2	24.3	46	37	0.90	71.69	78.38	7.77	15.72	76.51
3	23.2	46	35	0.92	78.75	81.82	6.03	12.30	81.67
Males									
1	23.8	48	35	1.09	95.61	84.30	7.23	10.80	81.97
2	20.2	42	31	0.72	81.45	78.84	7.62	6.53	85.85
3	23.4	44	35	0.97	91.53	83.12	7.41	7.54	85.05
4	27.9	48	37	1.48	107.20	85.71	7.51	12.44	80.05
Non-spawners									
1	20.5	45	33	1.09	97.86	86.48	9.83	15.13	75.04
2	24.4	47	36	1.19	99.23	85.24	8.28	9.01	82.71
3	21.3	45	33	0.92	84.01	80.44	7.18	6.37	86.45
4	17.7	44	32	0.80	88.50	81.09	10.30	6.08	83.62
5	16.2	41	31	0.74	93.33	80.35	7.31	13.14	79.55
6	15.6	40	32	0.84	96.85	86.19	10.07	10.50	79.43
7	21.9	42	32	1.00	96.32	76.77	6.74	12.02	81.24
8	18.2	41	29	0.70	88.59	82.24	10.22	7.24	82.54
Mean	21.55	44.40	33.53	0.96	89.76	82.03	8.20	10.40	81.39
SE	0.90	0.69	0.62	0.05	2.54	0.79	0.36	0.81	0.83

Appendix 10c (continued). Broodstock of T. philippinarum conditioned for 8 weeks with a diet of dry T. suecica + S. costatum.

Date: 17.03.92 — **Ref:** 1st broodstock 1992 - spawning III (8 weeks)

Species *Tapes philippinarum* — **Diet:** Dry T. suecica + S. costatum

Clams nº	Live weight g	Length mm	Breadth mm	Meat dry weight, g	Condition index	% Afdwt	Lipid (% Afdwt)	Carbohydrate (% Afdwt)	Protein (% Afdwt)
Females									
1	17.7	42	31	0.70	76.61	83.73	11.23	15.80	72.97
Males									
1	16.0	41	29	0.82	104.33	84.88	9.83	18.30	71.87
2	19.9	44	32	0.93	108.38	85.03	10.70	15.09	74.21
3	21.2	44	31	1.18	123.41	84.73	11.13	17.79	71.08
Non-spawners									
1	20.2	44	32	1.31	115.81	75.81	12.69	13.57	73.74
2	18.3	41	30	0.92	111.28	83.10	14.35	11.25	74.40
3	30.7	48	39	1.41	107.02	85.19	11.95	17.51	70.54
4	17.9	43	31	0.89	108.26	83.66	10.76	16.06	73.18
5	22.2	45	34	1.12	97.22	86.33	13.63	13.17	73.20
6	30.8	49	36	0.96	65.91	82.28	9.53	17.08	73.39
7	14.3	42	30	0.92	112.10	88.57	9.62	16.64	73.74
8	21.8	44	32	1.13	104.26	83.11	12.36	10.08	77.56
9	19.5	43	31	1.19	119.32	84.77	12.63	14.17	73.20
10	21.7	42	32	0.97	96.32	78.63	9.41	22.18	68.41
11	24.0	46	33	1.32	122.16	82.48	10.30	15.31	74.39
Mean	21.08	43.87	32.20	1.05	104.83	83.49	11.34	15.60	73.06
SE	1.20	0.61	0.66	0.05	4.11	0.79	0.40	0.77	0.53

Appendix 10c (continued). Broodstock of T. philippinarum conditioned for 8 weeks with a diet of dry T. suecica + I. galbana.

Date: 17.03.92 **Ref:** 1st broodstock 1992 - spawning III (8 weeks)

Species *Tapes philippinarum* **Diet:** Dry T. suecica + I. galbana

Clams nº	Live weight g	Length mm	Breadth mm	Meat dry weight, g	Condition index	% Afdwt	Lipid (% Afdwt)	Carbohydrate (% Afdwt)	Protein (% Afdwt)
Females									
1	16.3	43	31	0.91	103.74	87.83	10.61	16.82	72.57
Males									
1	18.5	46	36	0.93	100.59	88.94	10.87	14.17	74.96
2	16.0	44	31	1.05	106.67	90.94	11.16	22.34	66.50
3	22.0	47	35	1.27	131.82	89.84	9.63	18.20	72.17
4	16.1	42	31	0.93	105.42	90.60	9.90	18.42	71.68
5	17.4	44	32	1.03	114.78	89.39	10.16	18.25	71.59
6	27.2	50	38	1.56	110.03	89.00	10.49	13.60	75.91
Non-spawners									
1	21.8	46	36	1.33	105.07	87.65	14.23	14.11	71.66
2	17.3	42	31	0.96	137.03	86.41	11.56	13.61	74.83
3	19.8	44	34	1.20	120.40	86.84	9.96	19.34	70.70
4	26.1	44	35	1.56	129.96	86.41	10.75	20.06	69.19
5	15.4	38	31	0.98	132.81	87.21	15.05	16.55	68.40
6	18.8	40	31	0.94	102.09	86.92	10.28	13.77	75.95
7	19.2	44	31	1.20	115.07	86.78	14.03	13.96	72.01
8	18.4	41	31	1.12	126.36	87.27	14.00	12.82	73.18
Mean	19.35	43.67	32.93	1.13	116.12	88.14	11.51	16.40	72.09
SE	0.92	0.76	0.64	0.06	3.26	0.39	0.47	0.76	0.70

Appendix 10c (continued). Broodstock of T. philippinarum conditioned for 8 weeks with filtered sea water.

Date:	17.03.92				**Ref:** 1st broodstock 1992 - spawning III (8 weeks)				
Species	*Tapes philippinarum*				**Diet:** FSW				
Clams nº	**Live weight g**	**Length mm**	**Breadth mm**	**Meat dry weight, g**	**Condition index**	**% Afdwt**	**Lipid (% Afdwt)**	**Carbohydrate (% Afdwt)**	**Protein (% Afdwt)**
Non-spawners									
1	17.3	42	32	0.66	77.48	76.73	7.90	12.53	79.57
2	21.2	46	33	0.79	67.22	77.47	10.66	4.48	84.86
3	18.5	44	31	0.60	64.19	79.84	10.67	3.21	86.12
4	18.4	43	30	0.60	70.48	80.01	9.94	9.43	80.63
5	19.5	42	32	0.58	65.19	71.44	9.43	1.80	88.77
6	20.3	44	31	0.77	77.72	79.81	8.29	15.03	76.68
7	23.6	46	35	0.94	76.61	78.17	9.39	11.45	79.16
8	23.3	47	38	0.93	73.03	77.40	8.01	13.93	78.06
9	27.5	47	35	0.90	67.87	82.14	7.76	14.87	77.37
10	19.2	45	34	0.71	72.79	81.17	9.10	9.21	81.69
11	19.0	43	31	0.74	73.18	80.76	10.27	9.48	80.25
12	17.3	44	30	0.65	65.80	81.23	10.68	6.44	82.88
13	17.7	43	30	0.57	65.85	78.82	8.01	5.61	86.38
14	18.4	43	30	0.53	56.37	77.10	7.99	4.65	87.36
15	11.6	39	28	0.40	61.88	71.81	9.94	2.63	87.43
Mean	19.52	43.87	32.00	0.69	69.04	78.26	9.20	8.32	82.48
SE	0.92	0.55	0.67	0.04	1.59	0.82	0.29	1.18	1.05

Appendix 11. Condition indices and gross biochemistry of T. philippinarum initial broodstock and conditioned for 5, 6 and 7 weeks with different diets.

Date: 16.03.92 **Ref:** 2nd broodstock conditioning 1992 - Initial

Spec.: *Tapes philippinarum*

Clams nº	Live weight g	Length mm	Breadth mm	Meat dry weight, g	Condition Index	% Afdwt	Lipid (% Afdwt)	Carbohydrate (% Afdwt)	Protein (% Afdwt)
1	20.5	43	30	0.85	100.82	82.92	11.18	21.73	67.09
2	15.1	40	30	0.75	94.51	84.20	11.62	14.01	74.37
3	18.0	44	31	1.05	118.44	71.63	9.85	17.02	73.13
4	18.2	42	32	0.87	101.76	81.06	11.51	18.16	70.33
5	18.3	42	31	0.93	110.68	79.49	18.66	18.42	62.92
6	14.4	39	29	0.70	96.88	86.52	18.68	17.90	63.42
7	20.0	42	31	0.91	98.79	84.32	10.61	25.55	63.84
8	19.1	42	32	0.94	99.35	85.81	11.86	21.36	66.78
9	17.6	44	34	0.77	89.13	84.30	9.97	17.67	72.36
10	14.0	39	28	0.60	95.63	84.88	10.20	15.61	74.19
11	13.0	39	28	0.71	117.10	86.24	10.64	24.75	64.61
12	13.0	40	28	0.74	116.38	82.04	8.52	19.87	71.61
13	16.4	41	33	0.68	81.89	79.32	11.56	10.04	78.40
14	15.5	40	30	0.74	98.63	85.77	8.40	25.14	66.46
15	17.0	41	31	0.69	89.23	78.70	10.08	9.78	80.14
16	12.0	39	29	0.55	93.41	81.92	9.32	11.61	79.07
17	16.1	39	29	0.70	101.70	86.13	11.15	16.25	72.60
18	17.5	42	32	0.82	87.09	84.02	9.87	16.10	74.03
19	17.3	41	31	1.01	106.28	72.49	11.58	17.71	70.71
20	19.2	41	31	0.83	94.98	85.03	10.03	20.44	69.53
21	13.0	40	29	0.59	95.03	78.14	9.05	16.09	74.86
22	11.4	38	26	0.49	86.56	84.22	10.30	10.23	79.47
23	13.5	38	29	0.60	94.94	79.11	9.71	16.98	73.31
24	16.9	42	31	0.77	88.71	84.86	10.21	14.67	75.12
25	13.4	40	30	0.70	95.55	82.32	10.99	12.17	76.84
26	12.7	35	27	0.58	106.70	83.47	11.49	5.13	83.38
27	13.3	37	27	0.62	117.49	79.89	11.52	11.30	77.18
28	18.0	41	31	0.84	101.34	79.72	12.71	10.67	76.62
29	14.4	40	28	0.58	62.63	79.74	10.38	8.40	81.22
30	15.5	38	29	0.69	101.90	82.26	8.73	14.44	76.83
Mean	15.81	40.30	29.90	0.74	98.12	82.02	11.01	15.97	73.01
SE	0.46	0.37	0.34	0.03	2.13	0.68	0.43	0.93	1.02

Appendix 11a. Broodstock of T. philippinarum conditioned for 5 weeks with a diet of dry T. suecica.

Date: 21.04.92 **Ref:** 2nd broodstock 1992 - spawning I (5 weeks)

Species *Tapes philippinarum* **Diet:** Dry T. suecica

Clams nº	Live weight g	Length mm	Breadth mm	Meat dry weight, g	Condition index	% Afdwt	Lipid (% Afdwt)	Carbohydrate (% Afdwt)	Protein (% Afdwt)
Non-spawners									
1	22.8	42	33	1.00	93.32	82.76	8.85	20.21	70.94
2	16.2	42	30	0.67	69.47	79.70	8.00	17.16	74.84
3	19.3	43	31	0.88	98.18	80.21	8.53	17.06	74.41
4	21.0	45	35	1.14	122.78	83.46	7.30	21.93	70.77
5	19.5	44	34	0.96	102.99	81.91	7.58	21.24	71.18
6	19.9	41	30	0.76	78.03	79.80	7.39	15.42	77.19
7	15.2	41	30	0.55	73.44	79.54	8.30	13.20	78.50
8	18.9	43	31	0.94	103.02	84.09	6.99	19.28	73.73
9	17.1	42	33	0.98	118.31	84.95	8.05	19.98	71.97
10	13.1	43	31	0.65	93.46	80.41	7.95	19.74	72.31
11	19.8	43	32	1.09	118.54	84.29	8.43	22.23	69.34
12	21.4	45	33	1.06	81.22	81.94	7.34	24.71	67.95
13	20.9	43	33	0.97	84.03	82.56	7.78	19.93	72.29
14	17.8	40	31	0.80	80.47	79.77	8.21	16.41	75.38
15	18.7	43	31	0.78	74.53	71.73	11.04	15.36	73.60
Mean	18.77	42.67	31.87	0.88	92.79	81.14	8.12	18.92	72.96
SE	0.66	0.36	0.40	0.04	4.52	0.82	0.25	0.80	0.73

Appendix 11a (continued). Broodstock of T. philippinarum conditioned for 5 weeks with a diet of dry T. suecica + D. tertiolecta.

Date:	21.04.92				**Ref:** 2nd broodstock 1992 - spawning I (5 weeks)				
Species	*Tapes philippinarum*				**Diet:** Dry T. suecica + D. tertiolecta				
Clams nº	**Live weight g**	**Length mm**	**Breadth mm**	**Meat dry weight, g**	**Condition index**	**% Afdwt**	**Lipid (% Afdwt)**	**Carbohydrate (% Afdwt)**	**Protein (% Afdwt)**
Non-spawners									
1	13.0	36	29	0.55	93.81	81.08	9.17	15.83	75.00
2	17.5	41	33	0.84	82.74	82.93	9.51	17.58	72.91
3	19.8	42	33	0.94	101.17	82.79	8.86	15.97	75.17
4	18.0	43	32	0.73	85.65	81.31	8.54	11.88	79.58
5	19.5	44	32	0.74	84.57	80.09	8.66	15.30	76.04
6	17.5	44	32	0.76	82.02	80.33	8.23	14.45	77.32
7	17.8	42	31	0.83	92.17	80.50	10.66	15.46	73.88
8	16.9	39	30	0.70	91.10	81.98	8.14	17.40	74.46
9	17.1	42	30	0.81	97.87	86.23	7.65	18.31	74.04
10	15.9	41	30	0.86	124.09	75.06	11.02	19.91	69.07
11	12.7	37	28	0.51	131.18	84.49	8.30	16.40	75.30
12	11.9	38	28	0.77	179.63	81.09	8.58	18.11	73.31
13	17.7	42	31	0.78	90.37	83.87	6.60	22.50	70.90
14	16.0	40	30	0.67	81.78	79.83	8.48	15.76	75.76
15	14.9	40	29	0.75	112.45	76.21	8.74	20.56	70.70
Mean	16.41	40.73	30.53	0.75	102.04	81.19	8.74	17.03	74.23
SE	0.61	0.62	0.42	0.03	6.78	0.74	0.28	0.68	0.69

Appendix 11a (continued). Broodstock of T. philippinarum conditioned for 5 weeks with a diet of dry T. suecica + S. costatum.

Date: 21.04.92 **Ref:** 2nd broodstock 1992 - spawning I (5 weeks)

Species *Tapes philippinarum* **Diet:** Dry T. suecica + S. costatum

Clams nº	Live weight g	Length mm	Breadth mm	Meat dry weight, g	Condition index	% Afdwt	Lipid (% Afdwt)	Carbohydrate (% Afdwt)	Protein (% Afdwt)
Non-spawners									
1	19.8	45	31	1.01	92.86	87.37	8.63	15.54	75.83
2	13.2	42	32	0.76	86.52	81.36	9.35	13.51	77.14
3	17.6	40	32	0.81	94.94	82.71	10.92	15.23	73.85
4	16.6	44	31	0.91	91.05	87.08	8.76	17.37	73.87
5	21.7	45	32	0.99	95.61	82.28	8.98	17.73	73.29
6	18.2	42	31	0.81	93.31	81.27	8.81	15.82	75.37
7	16.0	40	31	0.78	93.49	85.90	10.75	16.32	72.93
8	20.9	44	30	0.95	93.89	82.87	8.91	20.17	70.92
9	20.8	41	34	0.95	101.40	84.69	7.97	18.05	73.98
10	16.2	39	31	0.63	80.69	75.14	10.01	12.64	77.35
11	15.0	41	30	0.68	91.45	83.80	8.28	19.99	71.73
12	14.3	38	28	0.70	129.96	80.22	9.27	17.39	73.34
13	17.5	43	32	0.82	122.22	81.24	9.07	15.61	75.32
14	19.8	44	32	1.21	137.44	87.72	8.26	23.11	68.63
15	17.8	41	30	0.85	111.38	74.84	10.06	17.80	72.14
Mean	17.69	41.93	31.13	0.86	101.08	82.57	9.20	17.09	73.71
SE	0.66	0.56	0.35	0.04	4.27	1.01	0.23	0.69	0.60

Appendix 11a (continued). Broodstock of T. philippinarum conditioned for 5 weeks with a diet of dry T. suecica+I. galbana.

Date:	21.04.92				**Ref:** 2nd broodstock 1992 - spawning I (5 weeks)				
Species	*Tapes philippinarum*				**Diet:** Dry T. suecica + I. galbana				
Clams nº	**Live weight g**	**Length mm**	**Breadth mm**	**Meat dry weight, g**	**Condition index**	**% Afdwt**	**Lipid (% Afdwt)**	**Carbohydrate (% Afdwt)**	**Protein (% Afdwt)**
Non-spawners									
1	17.9	43	31	0.92	111.08	84.35	8.01	21.07	70.92
2	17.6	42	32	0.99	109.46	86.63	8.43	22.50	69.07
3	19.4	44	33	1.22	121.27	86.17	7.99	28.60	63.41
4	19.3	44	31	1.07	111.19	83.61	8.35	20.73	70.92
5	20.2	44	33	1.20	125.26	81.29	9.04	21.04	69.92
6	16.1	40	30	1.09	133.20	85.55	8.00	23.94	68.06
7	14.3	41	30	0.82	118.14	84.36	7.92	18.64	73.44
8	22.0	40	32	1.27	119.63	85.45	7.63	26.99	65.38
9	22.0	43	32	1.18	129.35	83.45	10.86	17.74	71.40
10	12.8	39	29	0.76	105.99	82.60	8.93	19.67	71.40
11	20.8	42	31	1.09	122.58	83.99	10.70	22.08	67.22
12	13.2	39	30	0.86	132.22	86.71	9.55	22.18	68.27
13	18.3	41	31	0.96	104.41	82.79	9.90	19.93	70.17
14	19.3	42	31	0.92	97.93	82.12	8.92	20.17	70.91
15	18.4	45	31	1.06	129.59	80.74	9.39	21.45	69.16
Mean	18.11	41.93	31.13	1.03	118.09	83.99	8.91	21.78	69.31
SE	0.75	0.49	0.29	0.04	2.83	0.48	0.26	0.75	0.66

Appendix 11a (continued). Broodstock of T. philippinarum conditioned for 5 weeks with filtered sea water.

Date:	21.04.92				**Ref:** 2nd broodstock 1992 - spawning I (5 weeks)				
Species	*Tapes philippinarum*				**Diet:** FSW				
Clams nº	**Live weight g**	**Length mm**	**Breadth mm**	**Meat dry weight, g**	**Condition index**	**% Afdwt**	**Lipid (% Afdwt)**	**Carbohydrate (% Afdwt)**	**Protein (% Afdwt)**
Non-spawners									
1	18.1	43	31	0.65	71.80	77.78	9.89	10.07	80.04
2	19.2	44	32	0.89	90.63	81.83	9.41	14.09	76.50
3	21.3	44	33	0.81	68.90	74.34	9.93	8.21	81.86
4	18.4	43	32	0.60	67.03	76.62	8.85	8.24	82.91
5	17.3	40	30	0.71	91.22	80.40	8.82	12.45	78.73
6	17.6	42	31	0.63	77.77	75.45	9.88	4.50	85.62
7	21.0	45	34	0.72	72.83	79.31	9.25	9.12	81.63
8	18.2	45	35	0.85	89.47	81.70	9.60	15.10	75.30
9	19.2	43	34	0.72	85.43	78.17	9.35	9.03	81.62
10	14.6	40	31	0.50	68.89	78.25	9.64	6.18	84.18
11	13.9	41	30	0.59	63.64	81.36	11.70	4.43	83.87
12	20.8	43	32	0.75	78.80	76.46	10.74	8.62	80.64
13	18.6	45	31	0.83	89.69	77.71	9.30	10.00	80.70
14	18.1	40	34	0.57	65.54	78.12	10.06	6.46	83.48
15	19.1	45	32	0.71	70.22	80.94	9.55	12.39	78.06
Mean	18.36	42.87	32.13	0.70	76.79	78.56	9.73	9.26	81.01
SE	0.53	0.49	0.40	0.03	2.59	0.60	0.19	0.83	0.75

Appendix 11b. Broodstock of T. philippinarum conditioned for 6 weeks with a diet of dry T. suecica.

Date: 28.04.92 **Ref:** 2nd broodstock 1992 - spawning II (6 weeks)

Species *Tapes philippinarum* **Diet:** Dry T. suecica

Clams nº	Live weight g	Length mm	Breadth mm	Meat dry weight, g	Condition index	% Afdwt	Lipid (% Afdwt)	Carbohydrate (% Afdwt)	Protein (% Afdwt)
Non-spawners									
1	15.1	41	30	0.70	106.55	83.34	7.36	20.54	72.10
2	13.2	39	28	0.58	78.99	84.82	10.99	15.42	73.59
3	15.0	36	27	0.70	100.72	85.57	8.93	22.61	68.46
4	18.5	44	32	0.88	94.70	83.40	8.54	16.59	74.87
5	17.7	40	30	0.79	100.78	83.64	8.40	21.35	70.25
6	13.4	39	28	0.59	79.01	82.95	8.43	12.20	79.37
7	16.2	42	33	0.85	99.13	81.02	12.07	16.66	71.27
8	14.9	38	29	0.61	91.58	91.37	9.02	12.91	78.07
9	16.3	40	30	0.62	71.20	84.21	8.41	11.45	80.14
10	15.4	41	28	0.65	87.49	76.57	10.21	14.88	74.91
11	20.3	44	32	0.87	80.57	80.25	9.87	16.87	73.26
12	17.9	41	30	0.88	126.30	83.94	9.13	13.47	77.40
13	17.8	42	31	0.83	110.83	72.47	8.75	21.74	69.51
14	17.4	41	29	0.85	82.90	80.14	9.74	12.95	77.31
15	20.0	40	32	0.87	75.25	81.90	8.69	13.18	78.13
Mean	16.61	40.53	29.93	0.75	92.40	82.37	9.24	16.19	74.58
SE	0.56	0.54	0.46	0.03	3.93	1.09	0.30	0.97	0.97

Appendix 11b (continued). Broodstock of T. philippinarum conditioned for 6 weeks with a diet of dry T. suecica + D. tertiolecta

Date:	28.04.92				**Ref:** 2nd broodstock 1992 - spawning II (6 weeks)				
Species	*Tapes philippinarum*				**Diet:** Dry T. suecica + D. tertiolecta				
Clams nº	**Live weight g**	**Length mm**	**Breadth mm**	**Meat dry weight, g**	**Condition index**	**% Afdwt**	**Lipid (% Afdwt)**	**Carbohydrate (% Afdwt)**	**Protein (% Afdwt)**
Non-spawners									
1	13.8	40	30	0.71	89.66	85.27	8.14	15.25	76.61
2	17.6	42	30	0.91	129.64	84.65	8.21	18.52	73.27
3	13.7	40	30	0.72	114.57	85.00	8.77	15.84	75.39
4	15.5	43	32	0.69	107.56	84.49	10.03	13.29	76.68
5	17.2	40	30	0.82	103.04	73.43	11.54	19.37	69.09
6	18.6	40	31	0.93	107.81	82.84	8.39	16.82	74.79
7	13.9	40	29	0.72	108.58	85.09	9.04	17.41	73.55
8	20.6	45	33	0.98	102.09	85.70	10.12	16.15	73.73
9	18.3	41	31	0.89	99.18	84.57	9.04	16.95	74.01
10	17.6	41	34	1.02	108.19	81.41	15.77	14.92	69.31
11	19.1	43	31	0.93	103.53	83.74	10.14	17.55	72.31
12	12.8	39	29	0.54	84.98	83.57	11.19	13.82	74.99
13	14.6	40	28	0.73	95.21	84.80	9.45	18.51	72.04
14	13.5	39	31	0.74	112.39	86.19	9.51	21.57	68.92
15	13.6	39	29	0.55	85.66	81.43	10.25	9.88	79.87
Mean	16.03	40.80	30.53	0.79	103.47	83.48	9.97	16.39	73.64
SE	0.65	0.45	0.41	0.04	3.02	0.80	0.49	0.72	0.79

Appendix 11b (continued). Broodstock of T. philippinarum conditioned for 6 weeks with a diet of dry T. suecica + S. costatum

Date:	28.04.92				**Ref:** 2nd broodstock 1992 - spawning II (6 weeks)				
Species	*Tapes philippinarum*				**Diet:** Dry T. suecica + S. costatum				
Clams nº	**Live weight g**	**Length mm**	**Breadth mm**	**Meat dry weight, g**	**Condition index**	**% Afdwt**	**Lipid (% Afdwt)**	**Carbohydrate (% Afdwt)**	**Protein (% Afdwt)**
Non-spawners									
1	20.8	44	32	0.90	86.39	81.37	11.70	18.02	70.28
2	17.7	41	30	1.01	116.90	83.02	11.37	14.72	73.91
3	14.8	39	29	0.74	104.11	80.52	12.61	16.69	70.70
4	16.3	41	32	0.66	84.10	79.82	8.66	13.73	77.61
5	13.6	37	29	0.63	100.48	81.48	9.59	18.50	71.91
6	17.5	44	32	0.88	127.80	84.61	9.36	19.56	71.08
7	20.1	43	33	0.98	158.03	80.31	9.01	12.05	78.94
8	11.9	39	26	0.66	104.75	84.62	10.66	14.94	74.40
9	17.3	40	31	0.78	113.43	78.07	9.46	15.70	74.84
10	16.9	44	32	0.91	71.73	84.63	8.98	23.99	67.03
11	17.1	40	31	0.70	121.43	81.55	11.53	18.85	69.62
12	13.7	37	29	0.71	90.81	83.92	9.20	20.31	70.49
13	14.2	39	28	0.68	108.44	83.06	8.66	15.56	75.78
14	20.2	44	31	1.03	143.49	81.75	8.66	15.19	76.15
15	16.4	39	29	0.84	74.43	82.87	10.68	11.68	77.64
Mean	16.57	40.73	30.27	0.81	107.09	82.11	10.01	16.63	73.36
SE	0.67	0.65	0.49	0.04	6.28	0.50	0.34	0.85	0.90

Appendix 11b (continued). Broodstock of T. philippinarum conditioned for 6 weeks with a diet of dry T. suecica + I. galbana

Date:	28.04.92				Ref:	2nd broodstock 1992 - spawning II (6 weeks)			
Species	*Tapes philippinarum*				Diet:	Dry T. suecica + I. galbana			
Clams nº	Live weight g	Length mm	Breadth mm	Meat dry weight, g	Condition index	% Afdwt	Lipid (% Afdwt)	Carbohydrate (% Afdwt)	Protein (% Afdwt)
Non-spawners									
1	12.1	36	27	0.56	90.02	81.76	12.00	19.90	68.10
2	16.4	39	28	0.81	106.59	83.37	11.60	22.51	65.89
3	18.8	42	31	1.10	122.56	85.19	9.56	23.89	66.55
4	19.7	42	32	0.90	100.79	83.55	9.17	18.55	72.28
5	22.2	41	34	1.19	122.46	74.58	11.30	22.82	65.88
6	20.9	43	31	1.20	122.53	83.95	9.32	24.19	66.49
7	23.4	47	34	1.07	93.60	79.37	10.54	15.59	73.87
8	16.8	40	31	0.89	106.43	85.68	9.51	21.29	69.20
9	14.3	39	28	0.82	117.83	85.57	10.12	18.20	71.68
10	13.6	39	28	0.74	114.26	78.16	8.75	24.24	67.01
11	18.6	42	31	0.99	110.44	71.05	13.45	24.38	62.17
12	17.0	41	30	1.03	133.39	86.67	9.71	24.04	66.25
13	15.7	38	28	0.84	113.39	84.73	9.51	21.17	69.32
14	14.4	37	28	0.90	124.78	86.42	12.81	18.90	68.29
15	13.1	38	28	0.77	111.94	84.80	10.71	18.18	71.11
Mean	17.13	40.27	29.93	0.92	112.73	82.32	10.54	21.19	68.27
SE	0.88	0.71	0.59	0.05	3.08	1.19	0.37	0.73	0.79

Appendix 11b. Broodstock of T. philippinarum conditioned for 6 weeks in filtered sea water

Date: 28.04.92 **Ref:** 2nd broodstock 1992 - spawning II (6 weeks)

Species *Tapes philippinarum* **Diet:** FSW

Clams nº	Live weight g	Length mm	Breadth mm	Meat dry weight, g	Condition index	% Afdwt	Lipid (% Afdwt)	Carbohydrate (% Afdwt)	Protein (% Afdwt)
Non-spawners									
1	16.5	40	30	0.64	83.34	81.91	6.75	14.63	78.62
2	20.9	41	32	0.75	83.17	79.52	8.64	16.26	75.10
3	20.0	43	32	0.69	68.05	72.05	7.63	9.13	83.24
4	13.9	40	28	0.54	74.07	74.72	7.92	8.56	83.52
5	19.3	43	33	0.64	71.11	77.61	10.46	6.61	82.93
6	19.2	41	30	0.61	72.98	77.43	9.74	8.09	82.17
7	18.7	42	32	0.63	64.15	80.42	9.58	6.71	83.71
8	13.3	36	27	0.33	43.55	77.44	9.63	4.71	85.66
9	16.2	40	28	0.60	66.93	75.79	9.72	8.02	82.26
10	18.1	44	32	0.74	65.34	79.60	10.57	12.62	76.81
11	14.4	40	28	0.59	94.87	81.24	8.16	10.56	81.28
12	16.4	41	31	0.53	66.08	75.28	9.43	5.48	85.09
13	18.3	45	32	0.62	109.49	79.04	8.27	14.06	77.67
14	12.2	38	27	0.46	61.43	77.81	7.81	10.20	81.99
15	15.6	41	32	0.62	67.90	76.93	9.08	6.87	84.05
Mean	16.87	41.00	30.27	0.60	72.83	77.79	8.89	9.50	81.61
SE	0.68	0.59	0.55	0.03	3.96	0.68	0.29	0.90	0.81

Appendix 11c. Broodstock of T. philippinarum conditioned for 7 weeks with a diet of dry T. suecica.

Date:	05.05.92				**Ref:** 2nd broodstock 1992 - spawning III (7 weeks)				
Species	*Tapes philippinarum*				**Diet:** Dry T. suecica				
Clams nº	**Live weight g**	**Length mm**	**Breadth mm**	**Meat dry weight, g**	**Condition index**	**% Afdwt**	**Lipid (% Afdwt)**	**Carbohydrate (% Afdwt)**	**Protein (% Afdwt)**
Females									
1	13.7	38	28	0.71	99.12	86.16	8.96	27.02	64.02
2	14.9	39	29	0.61	83.55	79.87	9.37	18.24	72.39
3	14.4	40	30	0.62	92.48	77.99	9.53	20.69	69.78
4	15.6	40	30	0.70	86.99	82.70	9.67	18.78	71.55
5	17.2	41	32	0.83	93.39	78.91	10.88	20.55	68.57
6	16.1	41	29	0.67	81.52	77.17	9.37	20.18	70.45
7	19.6	45	32	1.00	91.61	84.56	14.55	22.35	63.10
8	20.0	45	33	0.88	82.82	82.49	11.05	16.30	72.65
9	13.2	39	30	0.72	153.77	87.75	9.75	15.75	74.50
Males									
1	13.3	40	31	0.67	81.24	84.24	9.42	18.28	72.30
2	19.7	43	31	0.78	83.62	79.38	9.19	16.47	74.34
Non-spawners									
1	13.2	41	30	0.67	121.44	84.07	8.15	25.47	63.98
2	15.4	41	29	0.72	89.71	81.46	8.83	21.29	70.06
3	13.0	39	29	0.51	81.10	78.45	10.55	18.56	81.44
4	19.7	44	31	0.87	96.02	81.47	8.65	21.92	68.22
Mean	15.93	41.07	30.27	0.73	94.56	81.78	9.86	20.12	70.49
SE	0.69	0.57	0.36	0.03	5.00	0.82	0.39	0.83	1.22

Appendix 11c (continued). Broodstock of T. philippinarum conditioned for 7 weeks with a diet of dry T. suecica + D. tertiolecta.

Date: 05.05.92 **Ref:** 2nd broodstock 1992 - spawning III (7 weeks)

Species *Tapes philippinarum* **Diet:** Dry T. suecica + D. tertiolecta

Clams nº	Live weight g	Length mm	Breadth mm	Meat dry weight, g	Condition index	% Afdwt	Lipid (% Afdwt)	Carbohydrate (% Afdwt)	Protein (% Afdwt)
Females									
1	18.5	43	32	0.74	79.42	83.49	10.50	15.43	74.07
2	21.1	44	34	0.76	71.57	76.95	9.52	15.90	74.58
3	11.9	35	29	0.61	91.89	79.75	9.94	16.35	73.71
4	16.1	41	31	0.67	90.97	78.72	9.26	16.06	74.68
5	12.1	41	29	0.67	79.56	82.98	9.51	13.38	77.11
6	18.1	42	30	0.74	93.87	78.9	9.85	12.84	77.31
7	12.8	38	29	0.67	98.96	84.04	8.96	21.01	70.03
8	15.8	42	31	0.85	106.59	84	8.47	22.76	68.77
Males									
1	19.1	43	32	0.86	98.10	77.73	8.40	15.03	76.93
2	19.1	43	32	0.89	103.22	79.33	9.12	13.65	76.38
3	18.4	42	34	0.60	81.38	80.46	8.04	12.63	78.47
4	14.5	38	30	0.84	93.98	83.32	9.97	14.43	76.07
Non-spawners									
1	22.7	42	32	0.88	76.86	79.02	8.90	10.13	80.65
2	22.3	47	34	0.77	67.65	71.9	9.50	8.47	91.35
3	14.1	39	27	0.51	81.87	77.1	8.40	9.58	90.42
Mean	17.11	41.33	31.07	0.74	87.73	79.85	9.22	14.51	77.37
SE	0.92	0.75	0.54	0.03	3.03	0.86	0.18	0.99	1.62

Appendix 11c (continued). Broodstock of T. philippinarum conditioned for 7 weeks with a diet of dry T. suecica + S. costatum.

Date: 05.05.92 | **Ref:** 2nd broodstock 1992 - spawning III (7 weeks)

Species *Tapes philippinarum* | **Diet:** Dry T. suecica + S. costatum

Clams nº	Live weight g	Length mm	Breadth mm	Meat dry weight, g	Condition index	% Afdwt	Lipid (% Afdwt)	Carbohydrate (% Afdwt)	Protein (% Afdwt)
Females									
1	14.7	39	31	0.75	88.36	84.19	11.37	17.99	70.64
2	15.3	39	30	0.77	111.00	80.64	10.84	13.50	75.66
3	16.8	41	33	0.87	97.47	80.78	10.12	18.24	71.64
4	15.5	40	32	0.72	86.01	80.28	10.33	16.36	73.31
5	15.3	40	31	0.79	106.24	83.22	10.66	19.64	69.70
6	14.5	40	31	0.70	74.98	82.36	10.43	15.43	74.14
7	16.7	40	31	0.84	98.59	81.8	9.56	17.99	72.45
8	15.1	39	29	0.77	95.66	79.87	9.11	18.96	71.93
Males									
1	18.9	45	32	1.08	107.53	84.87	8.85	12.74	78.41
2	20.8	45	33	1.06	90.81	80.4	8.38	15.13	76.49
3	16.1	40	30	0.87	111.99	81.26	9.27	17.07	73.66
4	18.0	41	30	0.79	92.12	76.74	8.76	11.91	79.33
5	15.5	38	30	0.80	103.44	83.82	9.49	14.54	75.97
Non-spawners									
1	18.3	44	33	0.92	121.24	82.96	8.28	17.27	74.45
2	14.8	41	28	0.71	99.63	79.92	8.47	19.13	72.40
Mean	16.42	40.80	30.93	0.83	99.01	81.54	9.59	16.39	74.01
SE	0.47	0.56	0.38	0.03	3.04	0.54	0.25	0.63	0.71

Appendix 11c (continued). Broodstock of T. philippinarum conditioned for 7 weeks with a diet of dry T. suecica + I. galbana .

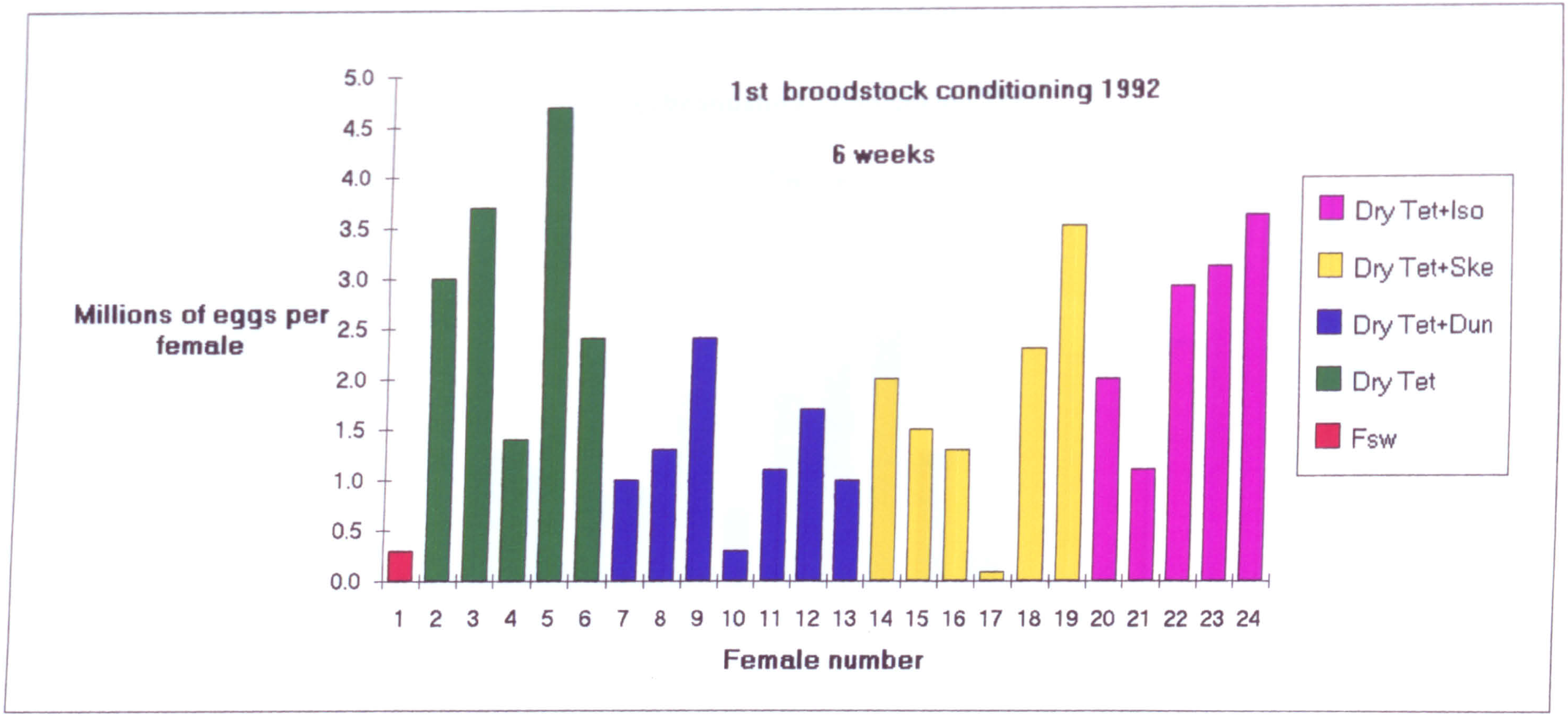
Date:	05.05.92				Ref: 2nd broodstock 1992 - spawning III (7 weeks)				
Species	*Tapes philippinarum*				Diet: Dry T. suecica + I. galbana				
Clams nº	Live weight g	Length mm	Breadth mm	Meat dry weight, g	Condition index	% Afdwt	Lipid (% Afdwt)	Carbohydrate (% Afdwt)	Protein (% Afdwt)
Females									
1	16.0	40	31	0.75	90.90	83.61	11.64	20.83	67.53
2	14.6	39	30	0.74	108.69	83.76	11.90	16.89	71.21
3	15.3	42	31	0.89	141.83	87.13	10.55	17.23	72.22
4	19.4	46	32	0.99	148.03	86.8	10.08	21.06	68.86
5	14.8	40	31	0.78	97.69	84.26	9.74	21.81	68.45
6	14.9	40	31	0.84	110.96	83.88	10.76	22.56	66.68
Males									
1	19.8	43	31	1.16	127.88	84.33	10.02	21.68	70.49
2	21.6	43	31	1.14	105.14	84.53	10.80	15.13	76.21
3	16.0	41	29	0.84	114.67	81.14	9.88	19.49	69.27
4	12.8	39	29	0.62	96.03	84.06	9.00	12.99	78.01
5	13.9	39	27	1.04	150.06	75.88	10.61	20.85	68.54
Non-spawners									
1	17.6	41	30	1.00	129.97	84.23	10.26	24.37	67.44
2	14.1	38	29	0.77	105.56	83.51	9.73	21.92	69.92
3	14.6	41	28	0.70	102.37	81.68	8.19	22.17	91.81
4	14.7	39	29	0.69	99.70	84.51	8.16	21.66	71.80
Mean	16.01	40.73	29.93	0.86	115.30	83.55	10.09	20.04	71.90
SE	0.64	0.54	0.36	0.04	5.03	0.67	0.28	0.80	1.64

Appendix 11c. Broodstock of T. philippinarum conditioned for 7 weeks with filtered sea water.

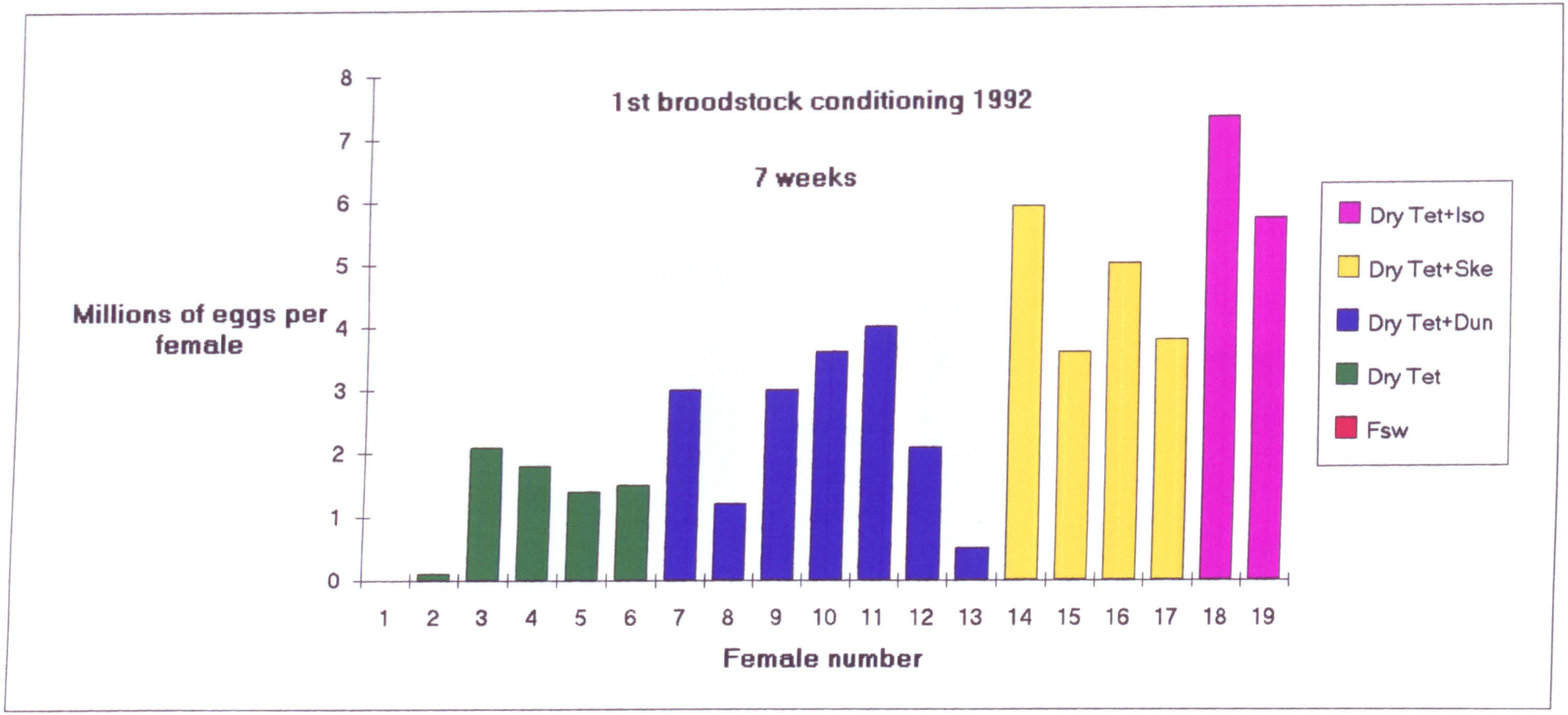
Date: 05.05.92 **Ref:** 2nd broodstock 1992 - spawning III (7 weeks)

Species *Tapes philippinarum* **Diet:** FSW

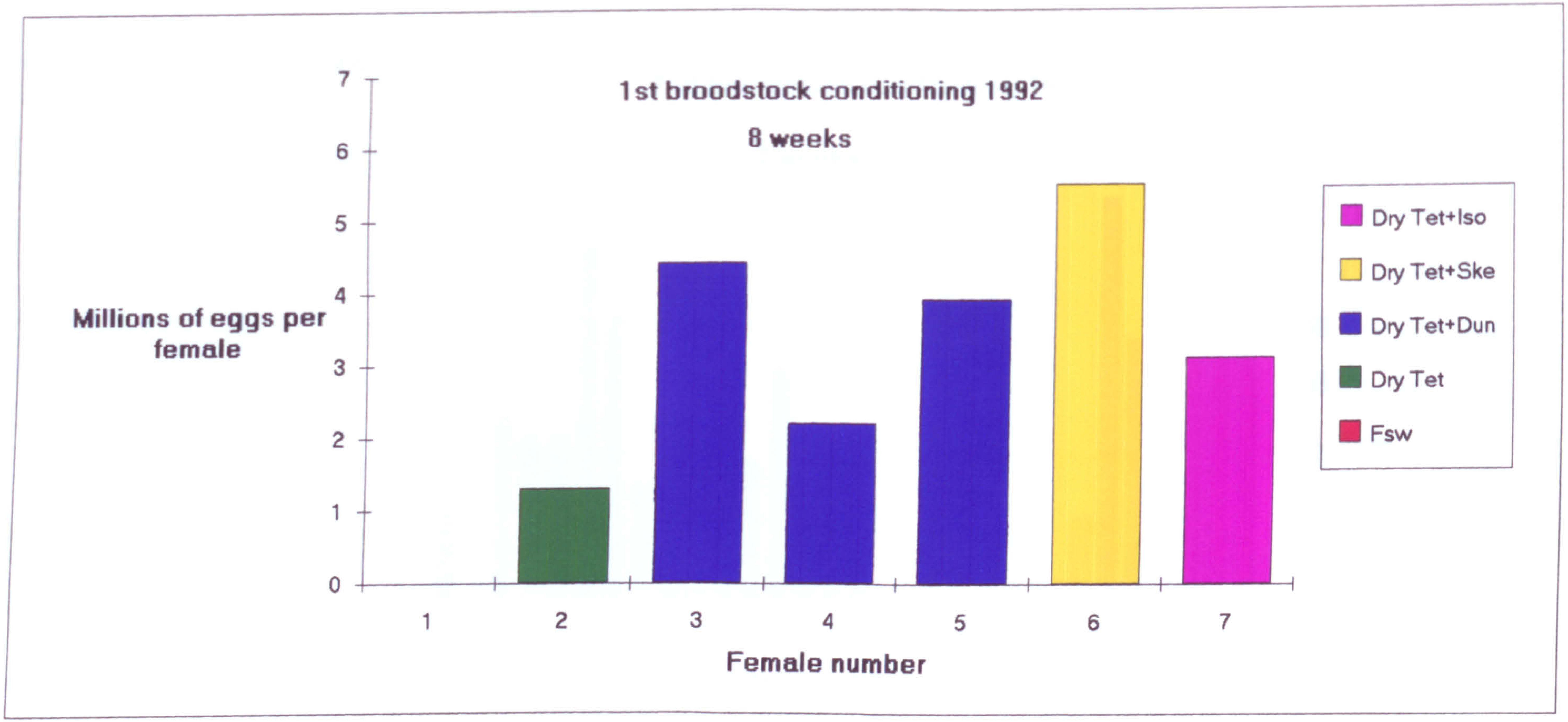
Clams nº	Live weight g	Length mm	Breadth mm	Meat dry weight, g	Condition index	% Afdwt	Lipid (% Afdwt)	Carbohydrate (% Afdwt)	Protein (% Afdwt)
Females									
1	16.2	41	30	0.56	70.30	77.69	10.64	7.66	81.70
Non-spawners									
1	12.0	37	26	0.36	79.00	73.19	10.54	3.69	85.77
2	19.3	43	32	0.63	57.68	76.98	9.36	10.91	79.73
3	14.6	37	27	0.63	90.94	78.67	9.17	14.58	76.25
4	12.3	39	27	0.38	73.94	77.92	9.69	5.46	84.85
5	14.1	39	29	0.51	73.65	76.60	9.64	10.88	79.48
6	15.4	38	30	0.52	69.85	70.84	9.59	5.87	84.54
7	15.7	40	29	0.62	76.53	80.51	11.60	6.86	81.54
8	15.7	40	30	0.54	67.95	76.19	9.48	7.67	82.85
9	19.8	41	31	0.71	77.57	73.59	9.66	13.96	76.38
10	18.1	39	31	0.52	63.54	72.23	9.45	7.50	83.05
11	18.8	43	30	0.59	63.20	75.73	11.04	7.01	81.95
12	14.1	39	30	0.49	67.27	73.50	10.61	9.13	80.26
12	13.3	36	27	0.47	72.78	80.26	9.14	12.26	78.60
14	17.8	42	30	0.52	66.69	65.02	11.50	4.82	83.68
Mean	15.81	39.60	29.27	0.54	71.39	75.26	10.07	8.55	81.38
SE	0.64	0.55	0.45	0.02	2.06	1.04	0.22	0.85	0.75



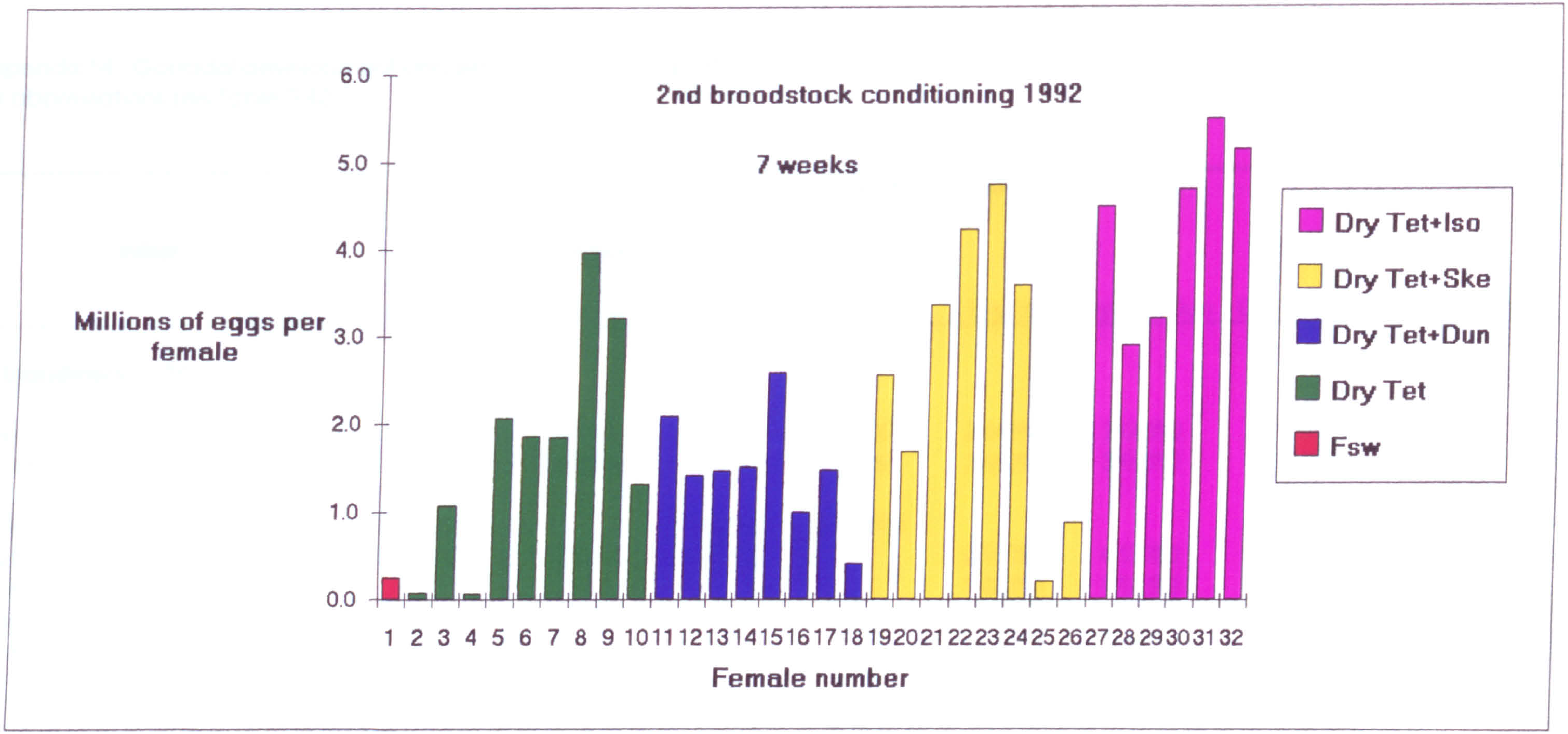
Appendix 12. Number of eggs produced per T. philippinarum female in relation to diet after being conditioned for 6 weeks, spawning I (1st broodstock 1992). For abbreviations see Table 3.42.



Appendix 12a. Number of eggs produced per <u>T. philippinarum</u> female in relation to diet after being conditioned for 7 weeks, spawning II (1st broodstock 1992). For abbreviations see Table 3.42.



Appendix 12b. Number of eggs produced per <u>T. philippinarum</u> female in relation to diet after being conditioned for 8 weeks, spawning III (1st broodstock 1992). For abbreviations see Table 3.42.



Appendix 13. Number of eggs produced per T. philippinarum female in relation to diet after being conditioned for 7 weeks, spawning III (2nd broodstock 1992).

Appendix 14. Gonadal development and sex ratio of _T. philippinarum_ conditioning with different diets in the 1st broodstock 1992.
For abbreviations see Table 3.42.

		Conditioning period									
	Initial	2 weeks					5 weeks				
		Dry Tet	Dry Tet+Dun	Dry Tet+Ske	Dry Tet+Iso	Fsw	Dry Tet	Dry Tet+Dun	Dry Tet+Ske	Dry Tet+Iso	Fsw
1st broodstock 1992											
Male	7 (70%)	1 (20%)	2 (40%)	2 (40%)	2 (40%)	1 (20%)	2 (40%)	2 (40%)	4 (80%)	1 (20%)	2 (40%)
Female	3 (30%)	4 (80%)	3 (60%)	3 (60%)	3 (60%)	4 (80%)	3 (60%)	3 (60%)	1 (20%)	4 (80%)	3 (60%)
Stage I	10 (100%)	2 (40%)	3 (60%)	-	1 (20%)	10 (100%)	-	-	-	-	4 (80%)
Stage II	-	3 (60%)	2 (40%)	5 (100%)	4 (80%)	-	2 (40%)	4 (80%)	2 (40%)	3 (60%)	1 (20%)
Stage III	-	-	-	-	-	-	1 (20%)	1 (20%)	3 (60%)	1 (20%)	-
Stage IV	-	-	-	-	-	-	2 (40%)	-	-	1 (20%)	-
Stage V	-	-	-	-	-	-	-	-	-	-	-
Stage VI	-	-	-	-	-	-	-	-	-	-	-

Appendix 14a. Gonadal development and sex ratio of T. philippinarum conditioning with different diets in the 2nd broodstock 1992. For abbreviations see Table 3.42.

	Conditioning period										
	Initial	2 weeks					4 weeks				
		Dry Tet	Dry Tet+Dun	Dry Tet+Ske	Dry Tet+Iso	Fsw	Dry Tet	Dry Tet+Dun	Dry Tet+Ske	Dry Tet+Iso	Fsw
2nd broodstock 1992											
Male	2 (20%)	3 (60%)	–	2 (40%)	–	2 (40%)	1 (20%)	2 (40%)	1 (20%)	3 (60%)	1 (20%)
Female	8 (80%)	2 (40%)	5 (100%)	3 (60%)	5 (100%)	3 (60%)	4 (80%)	3 (60%)	4 (80%)	2 (40%)	4 (80%)
Stage I	10 (100%)	1 (20%)	–	1 (20%)	2 (40%)	5 (100%)	–	–	–	–	–
Stage II	–	4 (80%)	5 (100%)	2 (40%)	3 (60%)	–	–	–	–	–	–
Stage III	–	–	–	2 (40%)	–	–	–	1 (20%)	–	–	2 (40%)
Stage IV	–	–	–	–	–	–	1 (20%)	1 (20%)	2 (40%)	1 (20%)	2 (40%)
Stage V	–	–	–	–	–	–	4 (80%)	3 (60%)	3 (60%)	4 (80%)	1 (20%)
Stage VI	–	–	–	–	–	–	–	–	–	–	–

Appendix 15. Fatty acid composition of T. philippinarum eggs and larvae from broodstock fed on different diets. 1st broodstock 1992, spawning I. Values are percentages of total fatty acid. For abbreviations see Table 3.45.

1st broodstock 1992 - Spawning I	**Eggs**				**D-larvae**			
Algae	Dry Tet	Dry Tet+Dun	Dry Tet+Ske	Dry Tet+Iso	Dry Tet	Dry Tet+Dun	Dry Tet+Ske	Dry Tet+Iso
Fatty acid								
14:0	27.38	17.94	26.84	22.43	29.13	24.76	30.74	22.67
16:0	23.90	24.35	24.80	24.88	25.51	23.49	26.17	25.75
18:0	3.35	4.21	3.74	3.43	4.49	3.37	4.37	3.72
16:1w9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16:1w7	6.85	7.85	9.21	8.07	5.67	6.47	7.60	7.23
18:1w9	25.40	20.88	20.86	23.27	22.58	23.60	22.03	21.23
18:1w7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20:1w9	5.02	5.58	3.22	4.64	3.60	4.98	2.27	5.40
18:2w6	1.38	2.64	1.33	2.38	2.03	2.62	1.76	2.27
18:3w3	0.19	5.23	0.22	1.34	0.74	3.87	0.00	1.30
18:4w3	4.49	2.74	1.66	3.85	1.57	2.94	0.00	2.81
20:2w6	0.00	1.44	0.00	0.00	0.00	0.00	0.00	0.00
20:3w3	0.00	1.36	0.00	0.00	0.00	0.00	0.00	0.00
20:4w6	0.00	1.31	0.00	0.00	0.00	0.00	0.00	0.00
20:5w3	0.91	2.45	6.76	1.41	1.29	1.93	5.06	1.88
22:5w3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
22:6w3	1.13	2.02	1.36	4.30	3.39	1.97	0.00	5.74
Saturated	54.63	46.50	55.38	50.74	59.13	51.62	61.28	52.14
Monoeno	37.27	34.31	33.29	35.98	31.85	35.05	31.90	33.86
Polyenoic	8.10	19.19	11.33	13.28	9.02	13.33	6.82	14.00

_ Contaminated sample

(continued)

Appendix 15 - (continued)

14 day larvae				17 day larvae			
Dry Tet	Dry Tet+Dun	Dry Tet+Ske	Dry Tet+Iso	Dry Tet	Dry Tet+Dun	Dry Tet+Ske	Dry Tet+Iso
19.10	24.17	22.78	20.23	21.68	20.25	-	21.19
19.02	19.18	19.55	19.41	19.27	19.53	-	19.44
4.71	5.04	5.29	4.83	5.11	5.07	-	5.14
0.00	0.00	0.00	0.00	0.00	0.00	-	0.00
6.87	5.61	5.05	5.99	5.63	6.42	-	5.05
13.34	15.01	15.40	14.18	14.36	13.71	-	15.14
3.55	2.89	2.66	3.03	3.39	3.84	-	3.27
1.73	1.92	1.54	1.44	1.87	1.79	-	1.79
3.24	2.95	2.79	3.01	2.78	2.72	-	2.82
1.76	1.63	1.45	1.90	1.25	1.66	-	1.55
6.40	5.42	4.87	7.00	4.88	5.68	-	6.02
5.34	6.53	6.03	5.59	6.12	5.41	-	5.80
0.00	0.00	0.00	0.00	0.00	0.00	-	0.00
0.77	0.68	0.67	0.70	0.91	0.90	-	0.86
5.94	4.71	4.83	4.91	5.27	6.12	-	4.81
0.11	0.17	0.23	0.13	0.17	0.13	-	0.13
8.12	4.09	6.86	7.65	7.31	6.77	-	6.99
42.83	48.39	47.62	44.47	46.06	44.85	-	45.77
25.49	25.43	24.65	24.64	25.25	25.76	-	25.25
31.68	26.18	27.73	30.89	28.69	29.39	-	28.98

Appendix 15a. Fatty acid composition of _T. philippinarum_ eggs and D-larvae from broodstock fed on different diets, 1st broodstock 1992, spawning II. Values are percentages of total fatty acid. For abbreviations see Table 3.45.

1st broodstock 1992 - Spawning II	Eggs				D-larvae			
Algae	Dry Tet	Dry Tet+Dun	Dry Tet+Ske	Dry Tet+Iso	Dry Tet	Dry Tet+Dun	Dry Tet+Ske	Dry Tet+Iso
Fatty acid								
14:0	26.04	15.05	14.14	15.89	30.43	33.27	24.54	26.94
16:0	25.03	28.44	26.49	25.31	24.23	24.03	24.17	23.08
18:0	6.77	6.02	5.43	4.70	5.81	6.13	5.62	5.68
16:1w9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16:1w7	3.52	8.27	10.31	7.04	3.94	2.57	4.96	4.40
18:1w9	20.33	13.35	11.19	15.44	21.78	21.86	17.45	18.07
18:1w7	2.00	3.43	4.76	3.48	1.16	0.00	2.64	2.20
20:1w9	3.46	4.58	4.58	5.59	2.86	1.33	3.34	2.95
18:2w6	1.49	2.27	0.98	2.09	1.74	2.08	1.75	1.74
18:3w3	0.21	5.57	0.36	1.37	0.00	1.38	0.43	0.37
18:4w3	2.49	1.05	1.63	4.01	0.00	0.00	1.36	1.07
20:2w6	5.98	4.89	3.11	4.72	6.29	7.35	5.75	6.17
20:3w3	0.00	1.40	0.00	0.42	0.00	0.00	0.00	0.00
20:4w6	0.00	1.00	1.55	1.24	0.57	0.00	1.02	0.90
20:5w3	1.50	2.43	11.74	2.61	1.19	0.00	3.91	3.30
22:5w3	0.00	0.11	0.30	0.11	0.00	0.00	0.16	0.19
22:6w3	1.18	2.14	3.43	5.98	0.00	0.00	2.90	2.94
Saturated	57.84	49.51	46.06	45.90	60.47	63.43	54.33	55.70
Monoenoic	29.31	29.63	30.84	31.55	29.74	25.76	28.39	27.62
Polyenoic	12.85	20.86	23.10	22.55	9.79	10.81	17.28	16.68

Appendix 15b. Fatty acid composition of T. philippinarum eggs and larvae from broodstock fed on different diets. 1st broodstock 1992, spawning III.
Values are percentages of total fatty acid. For abbreviations see Table 3.45.

1st broodstock 1992 - Spawning III	Eggs				D-larvae				14 day larvae				17 day larvae			
Algae	Dry Tet	Dry Tet+Dun	Dry Tet+Ske	Dry Tet+Iso	Dry Tet	Dry Tet+Dun	Dry Tet+Ske	Dry Tet+Iso	Dry Tet	Dry Tet+Dun	Dry Tet+Ske	Dry Tet+Iso	Dry Tet	Dry Tet+Dun	Dry Tet+Ske	Dry Tet+Iso
Fatty acid																
14:0	21.28	13.57	18.08	23.77	15.79	20.48	25.98	*	-	21.21	20.62	-	-	23.39	27.25	-
16:0	23.69	26.38	21.27	17.83	22.10	22.58	24.12	*	-	17.59	18.77	-	-	19.12	20.16	-
18:0	5.15	5.31	5.24	4.21	4.89	5.23	5.86	*	-	4.21	3.92	-	-	4.75	5.04	-
16:1w7/9	9.01	9.29	9.42	2.13	12.89	3.88	4.87	*	-	5.33	6.58	-	-	5.15	4.12	-
18:1w9	22.60	13.73	19.58	17.93	22.75	20.96	17.60	*	-	12.87	12.93	-	-	14.54	16.93	-
18:1w7	2.08	3.81	2.55	0.71	2.44	2.44	2.39	*	-	3.21	3.31	-	-	2.98	2.54	-
20:1w9	5.18	4.83	4.88	3.62	4.93	6.60	3.62	*	-	1.49	1.47	-	-	1.53	0.82	-
18:2w6	1.72	2.52	1.66	1.72	2.52	2.23	1.59	*	-	2.32	2.56	-	-	2.54	2.32	-
18:3w3	1.10	6.58	2.65	2.49	2.27	4.97	0.86	*	-	1.61	1.91	-	-	1.54	0.94	-
18:4w3	0.00	1.34	0.84	1.07	0.90	0.00	0.00	*	-	6.45	8.25	-	-	5.68	4.59	-
20:2w6	4.97	4.99	3.96	5.14	2.91	5.68	5.11	*	-	5.35	5.35	-	-	6.26	6.14	-
20:3w3	1.67	2.19	0.00	0.00	0.00	1.33	0.00	*	-	0.00	0.00	-	-	0.00	0.00	-
20:4w6	0.00	0.98	0.58	0.00	0.34	0.00	0.86	*	-	0.63	0.63	-	-	0.82	0.00	-
20:5w3	0.00	2.32	5.64	17.82	2.10	1.54	5.06	*	-	8.70	6.05	-	-	5.03	3.88	-
22:5w3	0.00	0.09	0.08	0.00	0.05	0.11	0.00	*	-	2.60	0.20	-	-	0.24	0.14	-
22:6w3	1.55	2.07	3.56	1.56	3.13	1.98	2.08	*	-	6.43	7.45	-	-	6.43	5.13	-
Saturated	50.12	45.26	44.59	45.81	42.78	48.29	55.96	*	-	43.01	43.31	-	-	47.26	52.45	-
Monoenoic	38.87	31.66	36.43	24.39	43.01	33.88	28.48	*	-	22.90	24.29	-	-	24.20	24.41	-
Polyenoic	11.01	23.08	18.97	29.80	14.22	17.84	15.56	*	-	34.09	32.40	-	-	28.54	23.14	-

* Contaminated sample
\- No sample

Appendix 16. Fatty acid composition of _T. philippinarum_ eggs and larvae from broodstock fed on different diets. 2nd broodstock 1992, spawning III
Values are percentages of total fatty acid. For abbreviations see Table 3.45.

2nd broodstock 1992 - Spawning III	Eggs				D-larvae				14 day larvae			
Algae	Dry Tet	Dry Tet+Dun	Dry Tet+Ske	Dry Tet+Iso	Dry Tet	Dry Tet+Dun	Dry Tet+Ske	Dry Tet+Iso	Dry Tet	Dry Tet+Dun	Dry Tet+Ske	Dry Tet+Iso
Fatty acid												
14:0	27.04	17.32	31.20	12.95	38.29	18.64	15.67	27.78	15.74	20.00	19.04	15.36
16:0	27.45	25.48	27.74	25.64	24.67	27.10	27.32	24.78	18.37	19.28	20.32	19.05
18:0	5.79	5.21	5.95	4.96	5.59	6.19	6.44	5.30	3.92	4.61	4.19	4.16
16:1w7/9	4.52	8.93	5.32	5.89	1.33	12.19	13.52	3.08	10.12	6.96	9.37	10.36
18:1w9	19.33	14.02	17.27	14.14	23.37	21.02	19.44	18.17	10.11	13.64	11.87	10.46
18:1w7	2.00	3.39	2.45	4.33	0.00	0.26	1.88	2.27	4.44	3.34	4.07	4.57
20:1w9	2.46	3.72	1.73	4.99	1.01	1.63	1.96	2.57	1.37	1.45	1.45	1.53
18:2w6	1.79	2.14	1.51	2.34	2.02	5.62	6.23	2.13	2.92	2.93	2.57	3.13
18:3w3	0.56	7.51	0.00	1.76	0.00	1.37	0.26	0.71	1.98	1.74	1.67	1.97
18:4w3	1.49	0.74	0.00	4.88	0.00	0.00	0.26	1.92	7.50	6.35	6.10	7.23
20:2w6	2.50	4.04	2.73	3.28	2.31	2.37	1.45	4.43	4.66	3.25	3.06	2.47
20:3w3	0.00	1.89	0.00	0.50	0.00	0.28	0.00	0.00	0.00	0.00	0.00	0.18
20:4w6	0.00	1.10	0.00	1.47	0.74	0.38	0.51	0.69	0.73	0.77	0.64	0.86
20:5w3	1.58	2.51	4.10	3.66	0.00	1.18	2.59	1.59	9.57	6.40	8.22	9.66
22:5w3	0.20	0.17	0.00	0.60	0.00	0.53	0.59	0.21	0.19	0.75	0.64	0.62
22:6w3	3.29	1.83	0.00	8.61	0.67	1.24	1.88	4.37	8.38	8.53	6.79	8.39
Saturated	60.28	48.01	64.89	43.55	68.55	51.93	49.43	57.86	38.03	43.89	43.55	38.57
Monoenoic	28.31	30.06	26.77	29.35	25.71	35.10	36.80	26.09	26.04	25.39	26.76	26.92
Polyenoic	11.41	21.93	8.34	27.10	5.74	12.97	13.77	16.05	35.93	30.72	29.69	34.51

Appendix 17. Sand-filtered sea water quality in the hatchery. 1st and 2nd broodstock 1992
(Afdwt = Ash-free dry weight ; Dwt = Dry weight ; Chl = Chlorophyll)

Date	Particulates							
	2.5 - 5.0 µm (µl/l)	5.0 - 10 µm (µl/l)	Dwt (> 1 µm (mg/l)	Afdwt (mg/l)	% Afdwt	Lipid (% Afdwt)	Dwt (0.22 - 1 µm) (mg/l)	CHL (µg/l)
1st broodstock 1992								
(22.01.92)	13.75	2.44	5.69	1.23	21.54	5.15	22.0	0.81
(27.01.92)	60.22	6.48	8.76	1.40	15.96	4.14	9.5	0.81
(03.02.92)	41.16	10.89	9.13	1.26	13.76	7.62	11.0	1.26
(10.02.92)	17.67	4.83	8.07	1.67	20.67	3.71	26.0	0.19
(17.02.92)	72.63	12.40	12.45	3.19	25.59	2.93	12.0	1.88
(24.02.92)	83.04	14.46	12.42	2.31	18.62	3.65	16.5	1.29
(02.03.92)	27.50	1.65	10.36	1.96	18.94	3.34	14.0	1.17
(09.03.92)	25.30	3.18	7.77	1.75	22.56	7.24	14.0	1.67
Mean	42.66	7.04	9.33	1.84	19.71	4.72	15.63	1.14
SE	9.28	1.74	0.82	0.23	1.32	0.63	2.01	0.19
2nd broodstock 1992								
(16.03.92)	18.40	3.01	9.60	1.6740	17.44	4.32	11.50	1.05
(23.03.92)	34.24	4.30	7.15	1.5210	21.28	6.68	12.00	1.33
(01.04.92)	97.11	17.25	18.23	3.1150	17.08	3.96	15.00	3.63
(06.04.92)	53.96	9.70	11.03	1.9565	17.73	3.45	14.50	2.05
(13.04.92)	45.56	7.61	10.47	2.4410	23.31	4.99	26.00	1.98
(20.04.92)	27.28	3.92	5.80	1.5550	26.81	6.34	15.00	1.73
(27.04.92)	93.22	5.22	7.02	2.3950	34.12	3.27	22.00	1.90
(04.05.92)	82.23	17.38	18.45	3.9030	21.16	3.41	19.50	3.76
Mean	56.50	8.55	10.97	2.32	22.37	4.55	16.94	2.18
SE	10.84	2.06	1.73	0.30	2.05	0.47	1.80	0.35