Energy Reserves in Male Three-spined Stickleback, *Gasterosteus aculeatus* L. (Pisces, Gasterosteidae): Annual Variation and Relation to Reproductive Aggression.

By

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I declare that the work recorded in this thesis is entirely my own, unless otherwise stated, and that it is of my own composition. No part of this work has been submitted for any other degrees.

S. Chellappa

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ABSTRACT

The aims of this project were to investigate the annual variation in energy reserves in males of a Scottish population of three-spined stickleback, Gasterosteus aculeatus and to relate this to their body condition and major life history events such as gonad maturation and breeding. Another aim of this study was to examine the association between energy reserves and reproductive aggression in the male sticklebacks. The work concentrated on estimations of energy sources (viz. glycogen, lipids and protein) in the different body compartments (viz. liver, gonad and carcass) of the fish over one complete year. The resulting observations were related to changes in body size, condition factor, somatic condition factor, hepatosomatic and gonadosomatic indices.

The study involved regular monthly collection of sticklebacks from November 1985 to October 1986 from the River Kelvin in Glasgow. Samples collected during the months of August to December consisted fish of two different age categories namely the newly hatched young of the year and adult fish which had failed to breed. The 1^{+} adults survived until their second winter after which no fish of this age category were encountered in the sample. Fish were killed by immersing in liquid nitrogen and stored at -70^{o} C prior to analyses to avoid breakdown of biochemical components.

There were marked annual variations in glycogen reserves of the different body compartments in the male stickleback. Liver glycogen stores in the young fish were low but increased sharply by October. They levelled off during the winter months, presumably due to lack of food or low rates of food consumption due to low winter temperatures. From February onwards liver glycogen reserves accumulated and reached a peak in April. From May to July the liver glycogen reserves dropped dramatically as they were mobilised during the breeding season. The overall patterns of annual variation in gonad and carcass glycogen reserves were similar to that of liver glycogen reserves, apart from the fact that gonad and carcass glycogen levels were consistently much lower than that of the liver. Glycogen reserves in the different body compartments were positively correlated during the non-breeding season, but this relationship changed during the breeding season due to differential depletion of glycogen reserves in different body compartments. Body weights and glycogen reserves were positively correlated outside the breeding season, but this relationship gradually reversed to a negative one during the breeding season as larger fish bred and depleted their glycogen reserves.

There was clear parallelism in the trends of accumulation and depletion of lipid and glycogen reserves, particularly in the liver and gonad of the male stickleback. There was a positive correlation between body weight and lipid reserves during the non-breeding season but this was reversed to a negative correlation during the breeding season as the lipid reserves accumulated during the non-breeding season were mobilized in larger fish during the breeding season.

Protein concentration in the liver, gonad and carcass of the male stickleback varied inversely with the lipid reserves during the non-breeding season. The initial growth of young of the year was mainly due to an increase in protein concentration but as the body size increased there was a shift from protein growth to lipid accumulation. Glycogen and lipid stores as well as carcass protein were depleted during the breeding season.

The relationships between glycogen and lipid reserves in the liver, gonad and carcass were strongly positive during the non-breeding season. This relationship disappeared altogether during the breeding season either due to overall depletion of energy reserves (as in the liver and gonad) or due to differential depletion (as in the carcass). Liver glycogen, liver lipid and carcass protein were the main energy sources of the male stickleback during the breeding season. Carcass glycogen and carcass lipid play a less important role.

The condition factor and the somatic condition factor varied markedly across the year. The relationship between hepatosomatic and gonadosomatic indices was strongly positive during the non-breeding season indicating that gonad development was prominant in male sticklebacks with good energy reserves. This relationship disappeared during the breeding season as the size of the liver declined which was most marked in fish with high gonadosomatic index. Stepwise multiple regression analyses suggest that condition factor is not a good measure of energy reserves of the body, but hepatosomatic index is a good measure of liver energy reserves.

Size-and colour-matched breeding male sticklebacks were allowed to participate in brief territorial disputes. In each case, a clear winner emerged rapidly. The winning fish had significantly higher levels of liver glycogen than the loser. There were no significant differences in the protein and lipid levels of winners and losers. This is a consequence and not a cause of the outcome of the fight. The longer and more intense the encounter the greater the differential depletion of liver glycogen reserves in the loser suggesting that natural variation in energy reserves among breeding male stickleback is not reflected in agonistic behaviour during territorial disputes.

CHAPTER 1

INTRODUCTION

CHAPTER 1. INTRODUCTION

1.1. ALLOCATION OF ENERGY RESERVES TO GROWTH AND REPRODUCTION

General principles

All animals gain matter and energy from ingested food and these are used as building blocks in the synthesis of tissues and as fuel in the metabolic processes. If body mass is to be maintained, absorbed dietary energy must equal energy lost to maintenance and activity. When absorbed dietary energy exceeds these requirements, growth can occur by the deposition of matter. Energy may also be stored as chemical energy in the macromolecules of carbohydrates, lipids and proteins. If dietary energy is insufficient to cover catabolism, then growth of some body components may occur at the expense of such previously stored endogenous sources. In the absence of any dietary input all energy for maintenance and activity must be provided from endogenous sources (as reviewed by Love, 1970; 1980).

An animal without an energy storage capacity is constantly dependent upon energy flow from its food resource for its survival; any significant interruption in this flow will result in death. When energy can be stored, the animal attains increased stability which allows it to survive periods of interruption in energy flow from its food supply. This stability would be of considerable value to animals in any temperate environment where the food supply varies seasonally to such an extent that during winter months availability may attain

levels equal to or less than maintenance requirements. Under these circumstances, a storage capacity would be necessary to survive periods of several months when the mean level of food availability is below maintenance requirements. Many animals are well adapted for mobilizing their stored energy reserves as fuel for survival during starvation and periods of natural depletion such as spawning.

One of the aims of this project is to investigate the annual variation in energy reserves in a fish, the male three-spined stickleback (Gasterosteus aculeatus), in relation to periods of energetic stress such as the winter when feeding levels are low and the breeding season when energy expenditure is high.

1.2 ENERGY RESERVES

When the supply of food to an animal exceeds the rate of utilization, the surplus energy is stored in the macromolecules. For example, excess carbohydrates are converted into storage polysaccharides, glycogen being the main storage polysaccharide of animals. Lipids such as fats and oils represent the chief form in which excess energy is stored in the animal body; they arise from ingested lipid and from the metabolism of proteins and carbohydrates. Amino acids arise from two main sources, viz. from food and from catabolism of existing body proteins. Excess aminoacids are either rapidly deaminated (as there are no known body stores of amino acids) or converted to glycogen or lipid. The concentrations of energy

* (Bradfield and Llewellyn, 1982)

reserves vary enormously in different parts of the body of the animal depending on the species, time of the year, environmental conditions, stage of maturity of the gonads, state of nutrition and age.

Oxidation of carbohydrates, fats and proteins yield energy. The average amount of energy made available by oxidation of carbohydrate, fat and protein are 17.20, 39.50 and 23.60 Kilo joules per gramme respectively.* During fasting, glucose is derived from body reserves, which include glycogen stored in liver and muscle, fat stored in various parts of the body, and the protein of muscle and other tissues. Fat is broken down to glycerol and fatty acids, and the glycerol is converted to glucose in the liver or oxidized by various tissues. Protein is broken down into amino acids, and these are metabolized by the liver to produce some glucose. The sources of energy available to a mammal during fasting are summarised in Figure 1.1

1.2.1 Glycogen

The advantage of storing carbohydrates as polysaccharides (eg glycogen) rather than monosaccharides (eg glucose) lies in the physical properties of the molecules. Large quantities of free sugars such as glucose would produce a high osmotic pressure within the cells of the animal and would increase the uptake of water, whereas glycogen is osmotically inactive and causes no such problem. The glycogen macromolecule is very large and has many loose ends which are available for enzyme action to permit rapid breakdown in response to energy

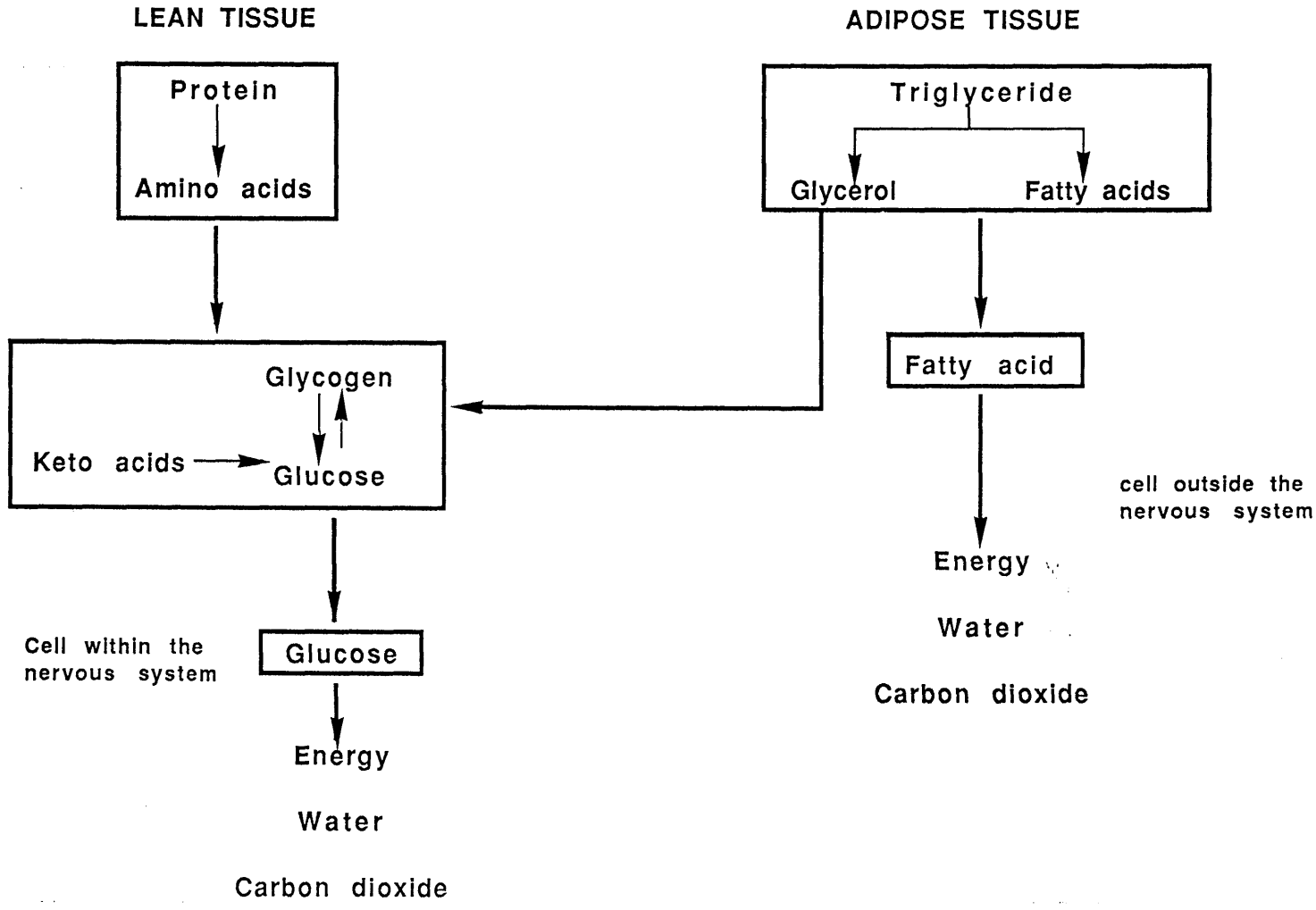


FIG. 1.1 THE MAIN SOURCES OF ENERGY AVAILABLE DURING STARVATION

requirements of the animal. The liver, white muscle and gonads contain up to 95 percent of the total glycogen in the body of most animals. The glycogen of working muscles decreases in amount when it is used to supply energy for contraction. Liver glycogen serves as a major source of blood glucose and supplies glucose to other organs especially to the brain.

The total amount of glycogen varies during the different periods of life cycle of an animal. In mammals, liver glycogen constitutes a reserve from which glucose can be rapidly formed during starvation (Freedland, 1967). There appears to be considerable variation in the ability of fish to utilize liver glycogen. Carp (Cyprinus carpio) (Wittenberger and Vitca, 1966; Nagai and Ikeda, 1971) and eel (Anguilla spp) (Larsson and Lewander, 1973; Hayashi and Ooshiro, 1975) maintain liver glycogen at pre-fasting levels for periods of 20 days of fasting. However, goldfish (Carassius auratus) (Stimpson, 1965), rainbow trout (Salmo gairdnerii) (Black et al., 1966) and Tilapia (Tilapia mossambica) (Swallow and Fleming, 1969) show a marked decline in both liver glycogen and liver weight during comparatively shorter starvation periods. The explanation for these species differences is not apparent, but may be related to variations in the nutrient composition of the pre-starvation diet.

1.2.2 Lipid

Free fatty acids are important metabolic fuels especially in the red muscle of actively swimming fish. Some fatty fish store their

reserve lipid in extra hepatic tissues such as muscle or viscera. For example the rainbow trout (Salmo gairdneri) possess perivisceral adipose tissue which is composed of distinct fat cells or adipocytes. Other pelagic species including Atlantic herring (Clupea harengus) capelin (Mallotus villosus) and mackeral (Scomber scombrus) have more obvious subcutaneous lipid depots sometimes penetrating far into muscle which is often assumed to be a muscle adipose store. Demersal non-fatty fish species like the dogfish (Squalus acanthus) and the cod (Gadus morhua) with lean muscle generally store lipid in their liver (for a review see Henderson and Sargent, 1985).

The Arctic capelin (Mallotus villosus) is an example of a species in which the metabolism of lipid is linked strongly to the life cycle of fish. During the summer the capelin feeds heavily on lipid-rich zooplankton and deposits large reserves of lipids in its flesh. They feed very little during over-wintering and rely largely on their own body reserves for the production of gonads, which occurs during winter. Both males and females lose up to 76 percent of their lipid reserves; the males catabolise all the mobilised lipid to meet energy demands during the breeding season, whereas the female deposit up to 38 percent of the lipid in the roe (Henderson et al., 1984 a).

1.2.3 Protein

In mammals both carbohydrate and lipid are important energy stores (Love, 1980). Mammals are adapted to accept a considerable proportion of carbohydrate in their diet, and they may suffer a

slight retardation in growth when restricted to a high protein diet because of the energy needed to eliminate the excess nitrogen. In fish, the carbohydrates seem to play a less important role and the main sources of energy appear to be protein and lipid. Fish can take high protein diet because they have the added ability to eliminate nitrogen waste through their gills (Ashley, 1972). Fish can excrete most of their ammonia and urea continuously and rapidly.

During standard metabolism in fish energy is derived entirely from amino acid catabolism, but as the metabolic rate increases the lipid catabolism becomes increasingly important (Brett and Zala, 1975). In common carp (Cyprinus carpio), the absence of the enzyme glycogen phosphorylase impairs glucose utilization and makes protein the main energy source (Murat, 1976 b). Specific activities of lysosomal enzymes which are involved in the breakdown of protein are found to be higher in fish than in mammals (Love, 1970).

1.2.4 Corticosteroids and catabolism

Energy expended by fish essentially comes from the oxidation of lipids or from glucose derived from amino acids by gluconeogenesis. Adrenocortical steroids appear to accelerate this process in fish (Butler, 1968). Cortisol and cortisone are the major glucocorticoids present in fish and are produced by the interrenal tissue. The protein catabolic property of these steroids becomes useful to fish during the non-breeding phase, when mobilization of protein from muscle provides energy and raw material for the developement of gonads. In salmon

(Oncorhynchus sp.) a six fold increase in the plasma corticoids during spawning migration brings about catabolism of 60 percent of the body protein (Fontaine, 1975 as reviewed by Love, 1980).

1.3 THE ENERGY BUDGET

The way energy is partitioned between metabolic demands will have a profound effect on both survival and breeding. For example, investment in activity may influence survival by facilitating escape from predators, investment in somatic component may influence the time it takes for somatic structure to become sufficiently developed to allow reproduction, and investment in gonadal tissue may influence fecundity (Calow,1985). One theoretical framework in which these issues can be considered is that of the energy budget (Warren and Davies,1967; Brett and Groves,1979 and Calow,1985). The energy budget of an animal summarises the way the total available energy is allocated to the various requirements for survival and reproduction. Thus, for a defined time period the energy budget is described as follows :

$$C = F + U + P + R$$

where C = energy content of the food consumed

F = energy content of the faeces

U = energy content of nitrogenous wastes

P = growth: change in the total energy content of the body, including any reproductive products released during the defined time period

R = total energy of metabolism

The latter term can be subdivided into three components: the standard metabolism or the rate of energy expenditure of a resting unfed animal (Rs), the additional rate of energy expenditure of an active animal(Ra) and the additional rate of energy expenditure associated with the ingestion and digestion of food . The latter is known as the specific dynamic action (SDA= Rd). Thus

$$C = F + U + P + Rs + Ra + Rd$$

A convenient framework for the discussion of energy partitioning is the bioenergetics model illustrated for a fish in Fig 1.2

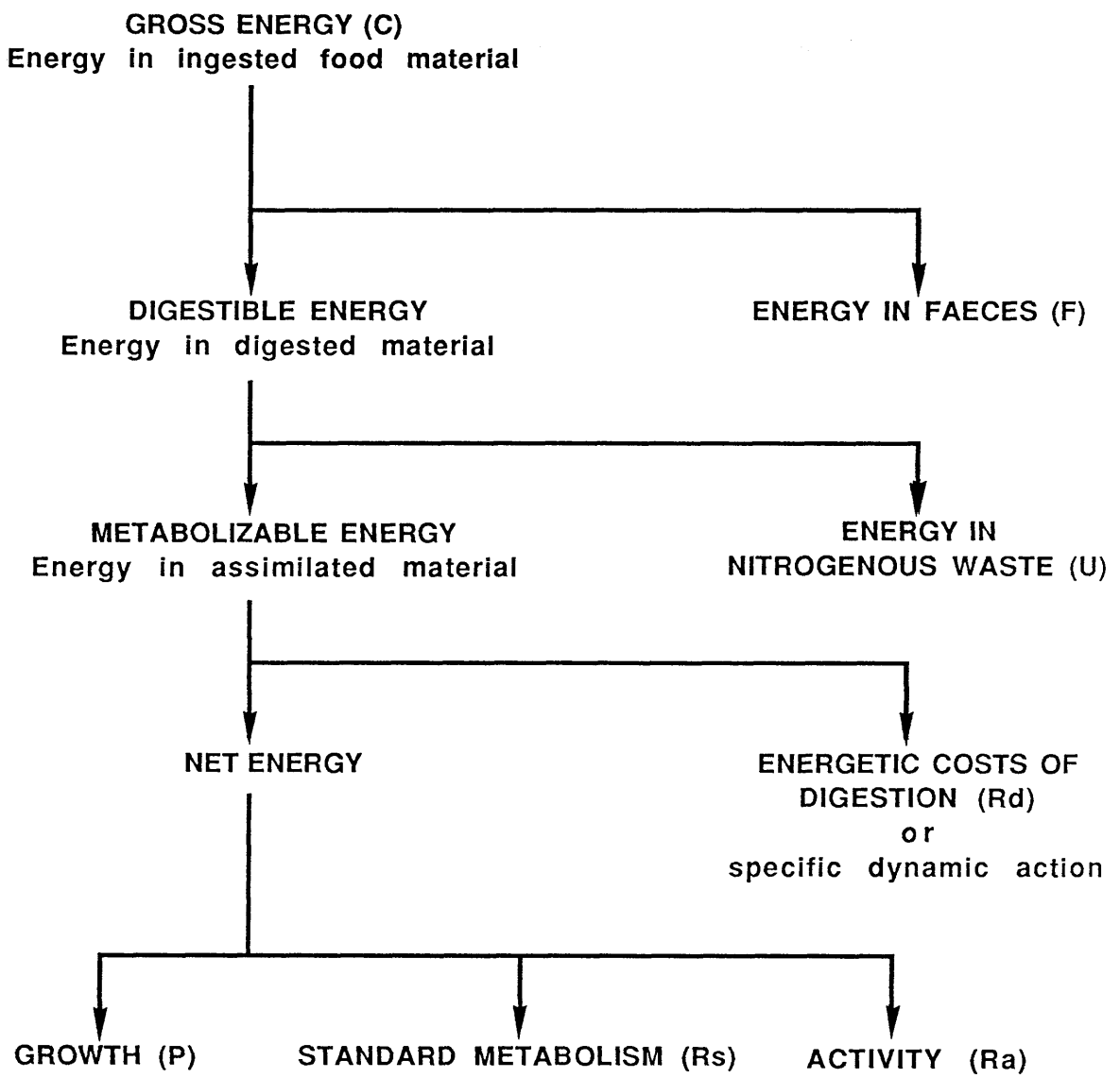


FIG. 1.2 MODEL OF ENERGY PARTITIONING IN A FISH (AFTER WARREN & DAVIES, 1967)

Accurate measurement of the energy content of food i.e. heat of combustion, is vital for calculating the components of the energy budget. The energy content of the food is generally measured by bomb calorimetry. A dried sample of food is weighed and placed in a thick-walled and air-tight steel chamber and is completely burned by igniting it in a high pressure of oxygen. The heat released by the combustion is measured and compared with that produced by burning a substance of known energy content. The energy in the food (C) eaten over a period, the energy retained as growth (P) and the energy content of faeces produced (F) can all be estimated by means of bomb calorimetry. Energy lost in urine (U) is estimated by measuring the amounts of ammonia excreted and multiplying this by the known energy content of ammonia. Respiratory heat loss (R) is obtained by monitoring oxygen consumption and multiplying this by an oxycalorific coefficient to arrive at an estimate of the amount of energy lost as heat. The oxycalorific coefficient varies according to the respiratory substrate. For example, the respiration of one mole of glucose to carbon dioxide and water requires 6 moles of oxygen (192 g) and releases 2833 kJ as heat, so the appropriate oxycalorific coefficient for carbohydrate is 2833/192 or 14.76 joules per mg of oxygen consumed. This technique is known as indirect calorimetry, whereas direct calorimetric methods measure the heat loss by an animal. The unit for energy is the joule and one thermochemical calorie is equal to 4.184 joules.

A sample energy budget has been calculated for the perch (Perca fluviatilis) over a 28 day period (Solomon and Brafield, 1972). The components of the energy budget show the following values.

$$C = F + U + P + R$$

$$58.9 = 9.6 + 6.4 + 11.9 + 32.0$$

The sum of the values for F, U, P and R is 59.9 kilojoules (kJ), whereas the estimate for energy of the food is 58.9 kJ. Probably the discrepancy is due to error in the measurement of one or more of the components. The balance of this energy budget is satisfactory when considering the variety of techniques and the potential sources of error involved. The component P of the energy budget which represents the change in the total energy content of the body involved in growth and reproduction is discussed in the next section.

1.3.1 The partitioning of energy between growth and reproduction

Somatic growth and reproduction are both production processes and are competing components for the limited net energy resources. The balance between growth and reproduction is dealt within the theoretical framework now designated as Life History Theory. It seeks to understand the selective forces that mould life history characteristics such as growth, life span, age maturity and fecundity (Cole, 1954; Bryant, 1971; Charnov and Schaffer, 1973).

Two fundamental questions involved in the partitioning of energy between growth and reproduction are the following: at what stage of somatic development should energy be directed from somatic to gametic processes and how much energy should be devoted to reproduction as opposed to somatic production. As an attempt to answer the first question, the following explanation is rendered. There is an important trade-off between fecundity and developmental time, with size acting as a 'hidden intermediary' (Calow, 1985); this depends on the relationships between size and fecundity and the time it takes to reach a particular size. Under poor environmental conditions, if an animal waits to achieve a large size before it begins to reproduce (thus gaining higher fecundities) it loses time and is also exposed to increased chances of mortality. On the other hand if it starts to reproduce early it has a reduced chance of dying without reproducing but suffers a low fecundity associated with a smaller adult size. Mortality risk is therefore one factor which determines either early breeding for the sake of saving time or late spawning for the sake of high fecundity (Stearns and Crandall, 1984).

A second question regarding how much energy should be invested in reproduction as opposed to somatic production has been considered in detail (Leon, 1976; Alexander, 1982; Calow, 1985). In the case of animals such as some insects and polychaete worms that reproduce on one occasion only and then die (semelparity), the answer is simple. Once reproduction is initiated, all energy reserves should be utilized for this purpose. However, issues are more complex in the case of

those animals which reproduce successively (iteroparity), since investment in the current breeding episode has implications both for future survival and future reproduction. Negative correlations have been recorded between fecundity and subsequent survival of the parent in a variety of species (Calow, 1979) and it has been possible to extend the longevities of some fishes by artificially preventing a reproductive drain, for example in freshwater river lampreys (Larsen, 1973).

Starvation of animals which reproduce in successive breeding seasons may lead to reduced fecundity, wherein short term reproduction is sacrific ed for long term survival of the parent. For example, the winter flounder (Pseudopleuronectes americanus) responds to food shortage by favouring investment in the somatic component of the body at the expense of gonads (Tyler and Dunn, 1976). This strategy is in contrast to that of the smaller fishes which have relatively short life expectancies and give priority to the gonadal development. e.g. the cyprinodont medaka, Oryzias latipes (Hirshfield, 1980).

1.4 ENERGETIC COSTS OF REPRODUCTION IN FISH

Reproduction usually requires more than the production of gametes; it may involve the development of secondary sexual characters such as breeding colours and other morphological features; release of pheromones and other secretions such as mucus for attaching the eggs to the substrate or for the nest building. All these will require the

expenditure of energy in addition to the energy spent on the production of gametes. Reproduction frequently depends on complex behaviour which can include migration to the spawning areas as in the Atlantic salmon (Salmo salar), territorial defence of spawning areas, complex courtship sequences and parental care as in the cichlid fish Sarotherodon mossambicus. These behavioural activities also demand an expenditure of energy. Thus the energy costs of reproduction are three fold: first, those of the primary sex products, the eggs or sperm; secondly, those of the secondary sexual characteristics, and thirdly, those of reproductive behaviour (Wootton, 1985).

1.4.1 Energetic cost of gamete production

Possible ways of estimating the cost of gamete production are: (a) number of gametes produced per parent (b) biomass of gametes produced (c) biomass of gametes produced per biomass of parent (d) energy invested in gonadal development as a proportion of energy taken in (Hirshfield and Tinkle, 1975; Calow, 1979). Method d expresses reproductive output in terms of nutrient input but does not make allowance for the utilization of energy reserves. Studies on the energetics of ovarian maturation indicate the ability of fish to maintain ovarian growth even during periods of low energy intake by adopting an extremely low routine metabolism. For example, in a population of pike (Esox lucius) in Alberta, spawning take place in April and early May. Ovarian growth starts in August but 81 per cent

of the total increase is achieved between October and March. Ovarian growth during winter is not at the expense of depletion of the soma but is supported by winter feeding (Diana, 1979; Diana and Mac Kay, 1979). However, in the largemouth bass (Micropterus salmoides) and perch (Perca fluviatilis) ovarian growth is maintained during periods of low energy intake by transferring resources from soma to the ovaries (Adams et al., 1982; Craig, 1977). This partitioning of energy ensures that the ovaries are ripe at the most suitable time of the year for the production of young.

The energetic cost involved in the maturation of the testes may be lower compared with the cost of ovarian maturation. For example, in the pike (Esox lucius) growth of the testes occur in August. The energy for this growth seems to come from the liver, which shows a loss in energy content equivalent to the testicular gain during the same period (Diana and Mac Kay, 1979). However, low energetic cost for the maturation of testes compared to ovaries is not a rule. In the Arctic cod (Boreogadus saida) the testes of mature fish constitutes 10 to 27 percent of the body weight (Craig et al., 1982).

1.4.2 Energetic cost of secondary sexual charateristics

Male lionhead cichlids (Steatocranus casuarius) have an enormous fatty lump on the forehead. The male Mexican swordtail (Xiphophorus helleri) has the lower part of its tail prolonged into a sword. In bristle-nosed catfish (Ancistrus sp.) males have branched fleshy tentacles on their heads. These secondary sexual morphological

features appear to be expensive to make and may in some cases increase the vulnerability of the fish to predators. In some fish species the colours are short lasting and may indicate sexual maturity as in the case of the three-spined stickleback (Gasterosteus aculeatus) (for a review see Turner, 1986).

Studies on the energetic cost of the development of secondary sexual morphological characteristics in fish are very limited. One of the indirect studies is the investigation on the effect of food ration on the development of the kidney into an effective mucus producing organ in the three-spined stickleback (Gasterosteus aculeatus). In similar sized mature male stickleback maintained on different food rations (viz. 2%, 6% and 18% of total body weight ration per day) it was found that males on low rations had significantly smaller kidneys than those on higher rations, indicating some inhibition of nest building at low food levels (Stanley, 1983).

1.4.3 Energetic cost of reproductive behaviour

As mentioned earlier in section 1.3 reproductive activities involved in spawning migration, territorial defence, courtship and parental care demand expenditure of energy. For example, during spawning migration the Atlantic salmon (Salmo salar) suffers a massive depletion of energy reserves, losing up to 99% of its lipid, 72% of its protein and 63% of its glycogen (Tilik, 1932). Territoriality plays an important role in reproduction in many species of fish and this can involve considerable energetic costs. Aggression and

territorial defence in fish, particularly reproductive aggression and territoriality, is therefore considered in detail in the next section.

1.5 REPRODUCTIVE AGGRESSION AND TERRITORIALITY IN FISH

One of the aims of this study is to examine whether natural variation in energy reserves among breeding male sticklebacks is reflected in behaviour during territorial disputes. From this point of view, it is of interest to review literature relating to reproductive aggression and territoriality in fish.

1.5.1 Aggression in fish

Aggressive behaviour may be treated as one extreme of a spectrum of behaviour in which an animal may attack, threaten or flee. The term agonistic behaviour (Scott and Fredericson, 1951) is often used to cover a whole range of behaviour and has the advantage that it avoids drawing arbitary distinctions between aggression, threat and flight. It thus gives a better picture of the complexity of conflict between animals than does the term aggression alone (Huntingford and Turner, 1987).

The fact that male fish often aggressively defend discrete breeding territories is well established with studies on the three-spined stickleback (Gasterosteus aculeatus) (Tinbergen, 1951; 1953; Huntingford, 1976 a ; Assem, 1967), green sunfish (Lepomis cyanellus)

(Greenberg, 1947), pup fish (Cyprinodon macularius) (Barlow, 1961; Brown, 1978), pomacentrid fish (Hypsypops rubicunada) (Clarke, 1970), pygmy sunfish (Elassoma evergladei) (Rubenstein, 1981), Reef fishes (Forsterygion varium) and (Pseudolabrus celidotus) (Thomson and Jones, 1983), Cichlid fishes (Tilapia nilotica) (Mishrigi and Kubo, 1979), and dusky damselfish (Eupomacentrus dorsopunicans) (Mahoney, 1981).

Most studies on aggressive behaviour are based on fish displays, attack and escape. In Atlantic salmon (Salmo salar) and rainbow trout (S.gairdneri) agonistic interactions between young fish by way of chasing and nipping becomes less common with age and are replaced by stereotyped fin displays and head-down postures (Dill, 1977; Cole and Noakes, 1980). At the end of the encounter between two Siamese fighting fish (Betta splendens) the gill cover erections of the winner are of longer duration than those of the loser (Simpson, 1968). Cichlid males suddenly seize one another by the mouth and remain more or less motionless for a while with their jaws interlocked and this is known as mouth fighting.

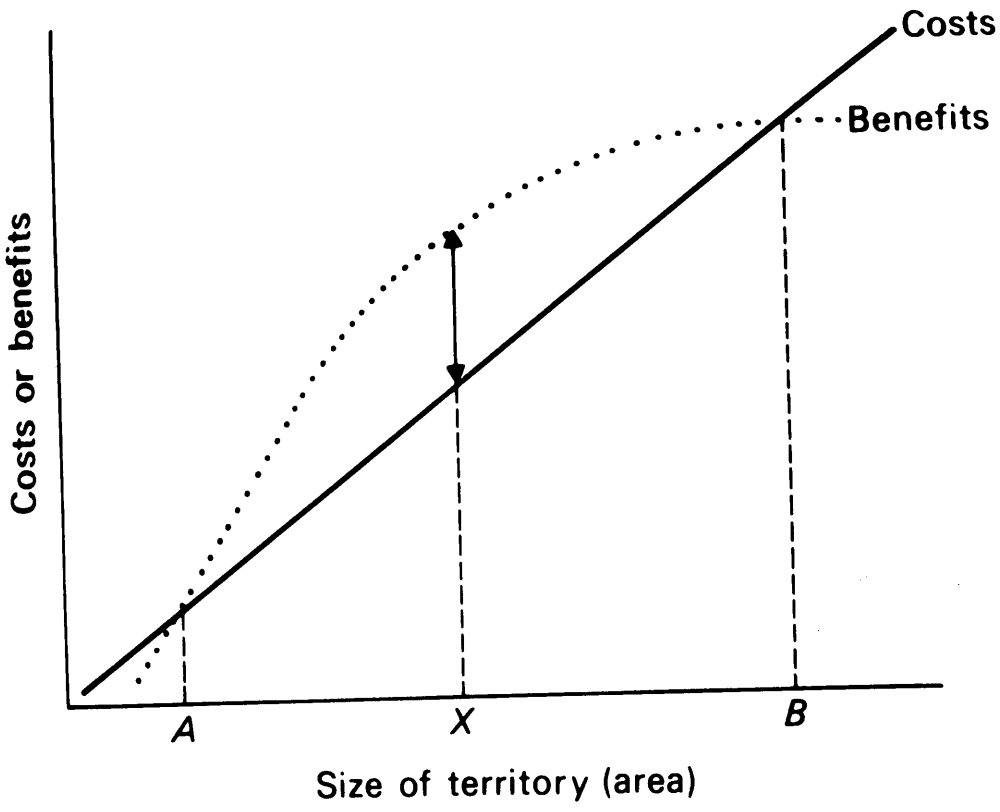
Encounters between breeding adult male three-spined stickleback, Gasterosteus aculeatus usually lead to aggressive interactions. There is an interesting form of fighting in stickleback described as spine fighting or roundabout fighting in which two males circle round each other rapidly, often with open mouth and erected spines (Iersel, 1953; Wootton, 1976). When two nest-owning males are introduced at the same time into a neutral area which is not large enough to accommodate two

territories, one male will become dominant and eventually build a nest, the other will give up fighting and become the subordinate, hiding somewhere so as not to elicit attacks from the dominant fish (Bakker and Sevenster, 1983). Dyadic encounters between two deliberately mismatched males suggest that larger or more brightly coloured males have a significant advantage over smaller and duller rivals (Whoriskey and Wootton, 1986).

1.5.2 Territoriality in fish

The behaviour of despotic animals, maintaining an exclusive area by aggressive means, results in the phenomenon of territoriality. Territorial behaviour is a conspicuous activity of many animals who defend resources such as mating sites, feeding areas and nests against competitors. The area defended is usually fixed with clearly defined boundaries and ownership is proclaimed with distinctive displays and fighting.

Early attempts to list the functions of territories were valuable in emphasising the diversity of kinds of territory (Hinde, 1956; Timbergen, 1957). Since the introduction of the idea of an economically defensible resource (Brown, 1964), both empirical and theoretical studies have explored the conditions under which territories are established and the sizes of the territories that result. In fish the males generally care for the young and territorial behaviour is common (eg the three-spotted Damselfish, Eupomacentrus planifrons, Thresher, 1976). Breeding territories are defended for



A graphical model of optimum territory size (After Krebs and Davies, 1981)

* The model assumes that as the area of a territory increase so do the costs of defence. The benefits increase at first but level off if the resource becomes superabundant for the animal. The area is economically defendable between A and B; within this range maximum net gain is at X which therefore represents the optimum territory size. As the richness of the territory increases the point at which benefits level off is reached at a smaller size and optimum territory is therefore smaller. The second assumption is valid for male pomacentrid fish due to limited capability for sperm production but for females the assumption is violated because the benefit curve continues to rise with territory quality as more eggs are produced.

several weeks in stickleback (see details in latter part of this chapter).

1.5.3 Energetics of territorial behaviour

Most studies of consequences of territoriality have been carried out on birds but not fish (Tinbergen, 1953; Gill and Wolf, 1975). Recently, most such studies have involved estimating the costs and benefits of territorial behaviour and predicting the territory size that maximises net gains or some other currency. Such analyses have helped scientists to explain natural variability both in the occurrence of territoriality and in the size of the defended area (Krebs and Davies, 1984; Huntingford and Turner, 1987). For example, considerations of the probable relationship between territory size and benefits in male and female pomacentrid fish (Eupomacentrus planifrons) suggest that in males (where the curve of benefits and territory size levels off) territory size will fall as territory quality increases. In females, on the other hand (where the benefit curve continues to rise with territory quality), the reverse is predicted.* Experimental manipulations of territory quality supported these predictions, suggesting that territorial behaviour is indeed adapted to the consequent costs and benefits (Ebersole, 1980). However, most studies in fish have concentrated on the behaviour of territorial defence rather than its energetic costs. Attempts are currently being made to estimate the metabolic costs involved in aggressive activities and to assess the energetics of territorial

behaviour. Costs of aggression in Rainbow trout (Salmo gairdneri) and pup fish (Cyprinodon macularius) have been studied (Feldmeth, 1983).

1.5.4 Hormones and aggression

In adult animals, hormones may influence peripheral structures used during conflicts, such as body size (in male squirrel monkeys), weapons (in soldier termites), colour patterns (in lizards) and scent production (in mice). Hormones may also influence the sense organs which detect agonistic cues (in toads giving release calls), the muscles (in stags) and neuromuscular junctions (in lobsters) to respond to them. On the other hand, in many species hormones act directly on the brain during battles over food, mates or in defence of young, as in lizards and mice (Huntingford and Turner, 1987).

Aggressive encounters between two male animals and the subsequent fighting activities rapidly modulate hormonal levels, increasing testosterone levels in mammals (Wingfield, 1985). Short-term increases in testosterone levels are observed at the start of a fight, probably contributing to its escalation. On a longer time scale, levels stay high in the victor and fall in the loser.

There is an evidence that experience in agonistic situations can alter pituitary-adrenocortical and gonadal activity and if such changes last sufficiently long they may change the animal's baseline hormonal state and hence its agonistic responding. For example, after fierce fights for rank order position between pairs of male swordtails

(Xiphophorus helleri) corticoid levels in the blood and the body extracts of both winners and losers increase at times ranging from one hour to 14 days after the end of the fight, if the rivals were kept in the same aquarium. Androgen levels determined by radioimmunoassay (RIA) suggest that shortly after the fight the losers had significantly lower androgen levels than winners (Hannes et al., 1984). Studies on sunfish (Lepomis gibbosus) based on the quantity of interrenal tissue show that the least aggressive fish may have been under the most severe stress (Erickson, 1967). There is an inverse relationship between dominance status and nuclear size of the interrenal tissue in male blue-gouramis (Trichogaster trichopterus) (Pollak and Christian, 1977). In seven out of twelve groups of swordtails (Xiphophorus helleri) the top ranking male had lower adrenocortical activity, assessed by measuring the mean nuclear diameter of adrenocotical cells (Scott and Currie, 1980).

PLATE 1.1 Study species - the three-spined stickleback (Gasterosteus aculeatus L.)

A mature male with breeding colouration from the River Kelvin, Glasgow.

PLATE 1.2 A female three-spined stickleback (Gasterosteus aculeatus L.) from the River Kelvin, Glasgow.

1.6 GENERAL BIOLOGY OF THE THREE-SPINED STICKLEBACK (GASTEROSTEUS ACULEATUS)

The male three-spined stickleback is the study species of this project and was selected because of its elaborate reproductive behaviour and availability in abundance in the Scottish aquatic systems. In addition, their small size facilitates care and maintenance in laboratory.

It is difficult to distinguish male and female sticklebacks during the non-reproductive phase, but during the breeding season there are external signs of sexual maturity such as the red nuptial colouration of the male stickleback (see Plate 1.1) and swollen abdomen of the female stickleback (see Plate 1.2). An account of their general biology is given in this section.

1.6.1 Distribution and morphology

The three-spined stickleback is one of the smallest fish of northern waters, with a maximum adult length of 10 cm and belongs to the family Gasterosteridae. It does not support an economically important commercial or sport fishery, but has made important contributions to ethology, evolutionary biology, physiology, ecology and reproductive biology. Its geographical distribution is entirely in the Northern Hemisphere between latititudes 35^{o} and 74^{o}N.

The three-spined stickleback is one of the few freshwater fish species native to Scotland (Campbell, 1984) (see Appendix 1 for

species list). Stickleback populations ~~occur in many rivers, estuaries~~ occur in many river systems, estuaries and lochs both in the mainland and Islands. For example, the River Kelvin (situated in Glasgow), Luggie and Aurs Burn (situated in the suburb of Glasgow), Mar burn stream (upland), Lennox Castle reservoir (situated in the Campsie Hills, north of Glasgow), Loch Lomond (the largest loch), ponds in Victoria park, Springburn park and Lochs in the Isle of North Uist-outer Hebrides (Giles, 1981; Campbell, 1984; Ukegbu, 1986). The commonly occurring stickleback morph in freshwaters is leiurus, though a range of morphological variation may occur from the large, heavily armoured and spined specimens to individuals with no plates or spines. The body of the stickleback is laterally compressed and is spindle shaped, tapering to a slender caudal peduncle which has a truncate caudal fin. The dorsal and anal fins are found farback along the body. The pectoral fins are broad and rounded and the pelvic fins consist of a spine and a soft fin ray. In front of the dorsal fin are the three spines from which the fish takes its common name.

The stickleback lacks scales and can usually be assigned to one of the three morphological forms or morphs depending on the number and arrangement of lateral plates. The three forms are named as trachurus, semiarmatus and leiurus (Munzing, 1959; Hagen, 1967). The Scottish fresh water populations of stickleback are leiurus which have few lateral plates in the anterior region of the body between one and nine plates in a row, and lack a caudal keel.

1.6.2 General biology

The trunk musculature of the stickleback lacks red muscle fibres (which are rich in lipid, glycogen and myoglobin) but consist only of white muscle fibres which have low lipid and high glycolytic enzyme activity which induces a predominantly anaerobic type of metabolism (Kronnie et al., 1983). The stickleback is not a very active swimmer and the white muscle is used for short rapid bursts of swimming. In laboratory conditions they spend most of their time remaining motionless in the water column. Adjustments in the quantity of gas in the swim bladder allows the fish to maintain neutral buoyancy as it moves up and down in the water column (Fange, 1953). The normal form of locomotion in the stickleback is a leisurely sculling with the pectoral fins which are aided by a well developed pectoral skeleton (labriform locomotion), but at times they adopt rapid burst of swimming (carangiform locomotion) when escaping from a predator, chasing a rival or approaching a gravid female.

A swimbladder which is divided into anterior and posterior portions is situated in the dorsal region of the abdominal cavity. In the stickleback, the swimbladder loses its connection with the alimentary canal in a few days after hatching and hence the stickleback is a physoclist fish. Neutral buoyancy in the water is achieved by the secretion or reabsorption of gas through specially adapted areas in the wall of the swimbladder (Wootton, 1984).

The alimentary canal consists of an oesophagus, stomach and a short straight intestine and a rectum. The liver, a gall bladder, pancreatic tissue and a spleen are associated with the alimentary canal. The liver has two lobes, of which the right lobe is larger than the left and it curves around the stomach reaching the pyloric region. The liver cells are polyhedral and they surround the blood sinusoids (Wootton, 1984).

The kidneys are paired but they fuse posteriorly. They are found under the vertebral column and the interrenal cells occur in the head kidney. The trunk portion of the kidney is concerned with production of urine and a glue used for sticking the nest together in the sexually mature male.

The gonads lie in the abdominal cavity, paired testes in the male and paired ovaries in the female fish. Fish less than 9 mm in length have gonads that cannot be differentiated as male or female. The thin visceral peritonium that covers the testes contains melanophores which produce black pigments and in mature males the testes appear dark whereas the ovaries are whitish in colour. Found dorsally in each testes is the vas deferens and the vasa deferentia from each testis fuse and the common duct opens into the cloaca between the anus and the opening of the urinary bladder (Wootton, 1976).

1.6.3 Age structure

In sticklebacks the age structure is highly variable. Under favourable conditions of long day lengths, high temperatures of about 20 ^{o} C and adequate supplies of food, stickleback usually mature at an age of one year. The maximum reported life span in natural populations ranges from 1 year to 4 years. For example, sticklebacks from River Birket (England) breed in their second summer of life when about one year old and live up to four years (Jones and Hynes, 1950). Sticklebacks from lakes in Alaska first breed when one or two years old and have a life span of over two years (Greenbank and Nelson, 1959). In contrast, populations from two streams in southern England and from Llyn Frongoch in North Wales are annual (Mann, 1971; Allen and Wootton, 1982) and few survive to an age of more than one year.

Age structure studies based on small scale sampling from several sites in Scotland suggest that sticklebacks from the more northerly sites are annual (Giles, 1981). Further age structure studies based on length fequency histogrammes and otolith analysis together with an assessment of gonadal state of three Scottish populations of stickleback show that the life span is just over a year, with only a few females of some sites surviving to two years. However, survival to a second breeding season is rare in females and never occurs in males. In contrast, sticklebacks from several sites in North Uist, Outer Hebrides may be biannual (Ukegbu, 1986; Ukegbu and Huntingford, in press).

1.6.4 Annual growth rates

Growth rates in stickleback are variable, even between populations with a broadly similar age structure; for example, by their first April sticklebacks from two annual populations in southern England grow to about 44 mm (Mann, 1971) while at the same age those from Llyn Frongoch in Wales only measure about 30 mm (Wootton, 1985). In the first year of life the southerly populations of stickleback have a growth curve that contains two periods when growth is checked (Jones and Hynes, 1950; Mann, 1971; Wootton et al., 1978). After hatching, there is a period of rapid growth which is checked for several months during the winter, while a period of renewed growth in spring is checked by the onset of the breeding season in late spring or summer.

Studies based on length frequency histograms for three Scottish stickleback populations (River Kelvin, Aurs Burn and River Luggie) suggest that growth is rapid immediately after hatching but levels off to a steady rate between the months of December and March. On an average the fish become 40 mm, 41 mm and 31 mm in standard length by the April of their first spring with average weights of 1.2 g, 0.99g and 0.78g. By the end of August they measure 46 mm, 52 mm and 43 mm in standard length and 1.27g, 1.32g and 1.20g in weight. The largest fish in the three populations are all females with standard lengths of 68 mm, 65 mm and 59 mm weighing 4.38g, 2.78g and 2.50g respectively. Sticklebacks generally feed on tubificid worms (Tubifex), benthic species such as the cladoceran Daphnia, copepod

Cyclops, other crustaceans like Asellus, and the larvae of the dipteran insect chironomid. Differences in growth rate within and between populations may reflect differences in diet. Growth may depend on the relative availability of profitable food items like copepods and less profitable items like the crustacean Asellus (Ibrahim, 1988; Ukegbu, 1986).

1.6.5 Seasonal changes in energy reserves

One of the earlier studies to examine seasonal changes in energy reserves (viz. glycogen) in the stickleback is that of Immers (1953). In this study, glycogen estimations were based on the techniques that were then available (see chapter 3). A more recent study which examines the annual changes in energy reserves (viz. glycogen and lipids) in female three-spined sticklebacks from two Welsh populations is that of Wootton et al., (1978).

In Russia, a Latvian population of sticklebacks showed variations in glycogen reserve during the different periods of the sexual cycle. The changes in total glycogen in both sexes of sticklebacks are given in Table 1.1 (After Immers, 1953). The maximum amount of glycogen (180 mg of glycogen per 100 gram of wet body weight) was found in both sexes during the period from August to December, and the minimum amount of glycogen (30 mg of glycogen per 100 gm of wet body weight in males and 50 mg of glycogen per 100 gm of wet body weight in females) was found during the spawning period from May to July. Male sticklebacks have their largest glycogen reserves

TABLE 1.1 Total mean glycogen in stickleback from Dangava (in Latvia, Russia) during the different periods of the sexual cycle - in mg per 100 g of body wet weight (After Immers, 1953)

Period	Female	Male
Early pre-spawning period (Jan- March)	80 ± 5.3	50 ± 4.1
Late pre-spawning period (April- June)	63 ± 4.5	43 ± 4.3
Spawning season (June-July)	70 ± 2.6	35 ± 1.3
Post-spawning period (July-Aug.)	80 ± 4.4	115 ± 4.8
Period of regeneration (Aug-Dec)	155 ± 5.3	150 ± 4.6
Annual mean	90	70

in the liver (about 42.5% to 89.0% of the total glycogen reserve) and the amount of glycogen in the gonads did not exceed 35% of the total glycogen reserve (Immers, 1953).

The check to the growth of females in the breeding season (in terms of dryweight of liver and carcass) occurs as a result of the heavy investment the females make in egg production at this time (Wootton, 1973; Wootton and Evans, 1976). The winter and the breeding season are periods of relative depletion from the body and liver, but the ovaries in female sticklebacks are insulated from depletion during winter (Wootton et al., 1978). The seasonal fluctuations in lipid and glycogen content of the different body components from female sticklebacks are given in Table 1.2 (After Wootton et al., 1978).

So far no systematic monthly sampling programme has been carried out to estimate the annual variation in energy reserves in the male three-spined stickleback. This is of considerable interest because of its elaborate reproductive behaviour (viz. territorial defence, courtship and parental care) which demand expenditure of energy. Therefore energy reserves in male stickleback are important for successful breeding. One of the aims of this study is therefore to estimate the energy reserves in the male three-spined stickleback, on a monthly basis over one complete year and to relate these to changes in body size and breeding.

TABLE 1.2 Seasonal fluctuations in lipid and glycogen content of carcass, liver and ovaries (mg g^{-1} dry wt) from female sticklebacks (Llyn Frongoch and the Rheidol). Means and standard errors of determinations from pooled samples (After Wootton, Evans and Mills, 1978)

	FRONGOCH			RHEIDOL		
Season	Carcass	Liver	Ovaries	Carcass	Liver	Ovaries
Lipid						
Autumn	126 ± 4.4	NM	NM	197 ± 10	250 ± 9.1	NM
Winter	110 ± 7.2	176 ± 20	124 ± 13.8	150 ± 12.1	201 ± 30	158 ± 26.8
Spring	133 ± 20	217 ± 54.2	140 ± 7.4	140 ± 6.5	165 ± 23.7	172 ± 10.6
Summer	105 ± 35.6	137 ± 6.3	147 ± 3.3	114 ± 10.2	118 ± 11.9	176 ± 21.9
Glycogen						
Autumn	1.1 ± 0.2	NM	NM	1.4 ± 0.1	163 ± 29.3	NM
Winter	1.4 ± 0.1	52.6 ± 16.9	2.4 ± 0.5	0.7 ± 0.1	136 ± 29.3	1.5 ± 0.2
Spring	1.7 ± 0.1	47.3 ± 10	7.6 ± 1.0	1.0 ± 0.1	58.1 ± 1.23	7.1 ± 0.1
Summer	2.0 ± 0.5	15.6 ± 1.9	7.5 ± 1.1	2.6 ± 0.8	17.1 ± 1.6	11.2 ± 0.7

NM = No material for analysis

Autumn = Aug – Oct; Winter = Nov – Jan; Spring = Feb – Apr; Summer = May – Jul

1.6.6 Reproduction

The age at maturity is typically one year in the stickleback and breeding takes place at some time in the period between late March and early August (Wootton, 1976; Mullem, 1967). In the south, sticklebacks breed between March and May but they breed later in the northerly populations (Vrat, 1949).

Sticklebacks live in environments where the photoperiod and temperature show strong seasonal patterns. Normally, sticklebacks become sexually mature during spring, at a time of increasing daylengths and higher water temperature. Maturation at long photoperiods of 16 L - 8 D is accelerated at high temperature of about 20^{o} C; however, maturation does occur at the lower temperature of 10^{o} C (Baggerman, 1980). The result of the interaction of the seasonally varying abiotic factors of light and temperature in conjunction with neuroendocrine system of the stickleback may be a precise timing of the breeding season at an ecologically appropriate time (Wootton, 1984).

Sexual maturation in the male stickleback falls into two phases. The first is the process of spermatogenesis by which mature spermatozoa are produced that are capable of fertilizing the eggs of female. The second is the maturation of cells in the testes which can synthesise steroid sex hormones, particularly androgens (testosterone). Most of the secondary sexual characteristics of the male are androgen dependent.

Timing of maturation events in the male stickleback is variable. In sticklebacks from the Scottish populations, spermatids are most abundant between January and June as the males reach maturity and come into breeding condition. In the River Kelvin males, occurrence of spermatozoa is high between April and August (Ukegbu, 1986).

During the second phase of sexual maturation the interstitial cells of testes increase their activity, secreting androgen which stimulates a number of secondary sexual characteristics of the male stickleback. The breeding colours are most striking among the secondary sexual characters. The first sign of the appearance of the nuptial colour is the development of a patch of blue in the upper part of the iris and eventually the entire iris of the eye becomes blue. The blue colour of the iris is due to reduction in the amount of guanin crystals in the guanophores. As the iris becomes blue, a red colouration develops in the opercular region due to the expansion of erythrophores. At the maximum expression of the nuptial colours, the underside of the mouth, the fore-belly and the opercular region are all red. Due to the contraction of the melanophores which contain a black pigment, the general colour of the body lightens considerably (for a review see Wootton, 1976).

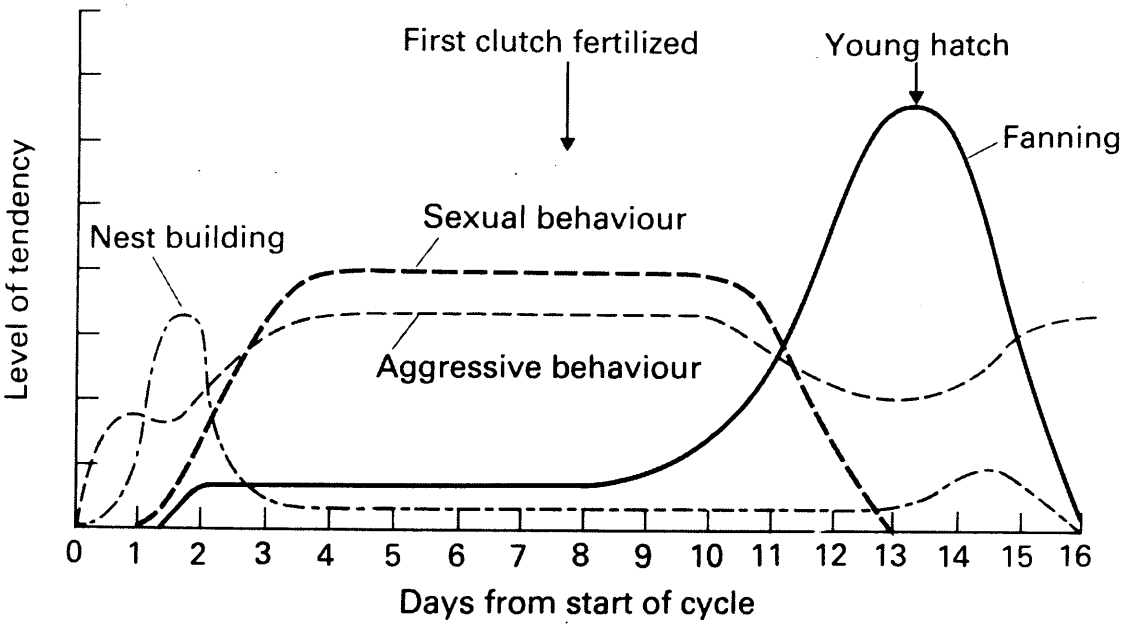
As the nuptial colouration develops the tubules in the kidneys become modified for the production of the glue used in nest building (Mourier, 1970). The transformation of the ducts and tubules is accompanied by an increase in the relative size of the kidneys. Once the glue (a mucopolysaccharide) has been synthesised by the kidneys it is stored in the urinary bladder before use.

PLATE 1.3 Nest built by a male-three spined stickleback (Gasterosteus aculeatus L.)

Accompanying these physiological changes of the reproductive cycle, are major changes in the behaviour of male sticklebacks. The reproductive cycle of the male stickleback can be divided into different behavioural phases such as selection of a breeding site, establishment of territory, nest building, courtship and mating, parental care with fanning (Wootton, 1984).

Reproduction in the male stickleback starts with a phase of nest building (Plate 1.3 shows a nest built by a male stickleback) which then declines to a low level whilst the aggressive and sexual tendencies rise rapidly and both remain high for a period. The male defends his territory actively. Although the defence of his territory tends to be directed most strongly towards conspecifics, other species of sticklebacks and intruders are also attacked. After one or more fertilizations, sexual activity declines and the parental tendency rises with the male spending long periods fanning at the nest. During fanning the male positions himself in front of the nest entrance and stays there, beating vigorously with his pectoral fins and tail. The net result is that the fish stays still and a current of water is propelled through the nest and over the developing eggs, which keeps them well oxygenated. Plate 1.4 shows the fluctuations in the tendencies to perform nest building, sexual behaviour, aggressive behaviour and nest ventilation during the reproductive cycle of the male stickleback (After Sevenster, 1961).

PLATE 1.4 Fluctuation in the tendencies to perform nest building, sexual behaviour, aggressive behaviour and nest ventillation during the reproductive cycle of the male stickleback (From Sevenster, 1961)


First clutch fertilized
Young hatch
Fanning
Sexual behaviour
Nest building
Aggressive behaviour
Level of tendency
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Days from start of cycle

These are all energetically expensive activities which are likely to deplete energy reserves. The full sequence of territorial defence, courtship and parental care must be completed if the young are to be reared successfully. Stanley and Wootton (1986) suggest that the probability that a male stickleback will contest a territory will depend on his current energy reserves. Males will only gain a fitness advantage from having a territory if they can use it to rear young. Experimental manipulation of diet in the weeks before the start of the breeding season give support to this suggestion. Individual male stickleback show great natural variation in aggressiveness (Huntingford, 1982) and it is possible that this variability is caused by differences in energy reserves. A final aim of this project is to test this possibility by relating energy reserves to the outcome of territorial fights.

1.6 SUMMARY OF AIMS

1) To estimate the energy reserves of the different body compartments (viz. liver, gonad and carcass) in males of a Scottish population of three-spined stickleback (*Gasterosteus aculeatus*) on a monthly basis over one year.

2) To relate the annual variation in energy reserves to changes in (a) body size (b) condition factor of the body (c) somatic condition factor (d) hepatosomatic index and (e) gonadosomatic index.

3) To correlate all these changes to major life cycle events such as maturation and breeding.

4) To examine the relationship between energy reserves and the outcome of territorial fights ie. to examine whether natural variation in energy reserves among breeding male stickleback is reflected in behaviour during territorial disputes.

CHAPTER 2

GENERAL METHODS

CHAPTER. 2 GENERAL METHODS

2.1 COLLECTION SITES: LOCATION AND DESCRIPTION

The River Kelvin is situated in Glasgow and is located 4 degrees 18 minutes West longitude and 53 degrees 0 minutes North latitude. The River Kelvin rises is an area of marshy ground 55 metres above sea level near the village of Kelvinhead. It then flows for 30 km to join the Clyde estuary in the west end of Glasgow. In its lower reaches it is the central feature of the Kelvin walkway, an area of recreation. The River Kelvin catchment has eight flow gauging stations sited on it and from this network the flow rates of the main river and the major tributaries are monitored by the Clyde River Purification Board.

The River Kelvin is polluted close to its point of origin by discharges of iron-bearing ground water draining from the abandoned coal mines. However, in its upper reaches this effect diminishes with the flow of the river. The river passes through the west end of Glasgow down a series of short falls which improve the oxygen concentration in the river before it flows into the Clyde estuary. The River Kelvin itself is an extensive and a fast flowing urban river, but the sampling was carried out in a slow flowing outlet of the river.

The other fish species which were commonly encountered during sampling were mainly minnows, Phoxinus phoxinus, and few nine-spined stickleback, Pungitius pungitius.

2.2 COLLECTION METHODS

Samples were collected using a 3 mm mesh hand net of 40 cm. diameter. Sticklebacks were usually found underneath aquatic vegetation in depths less than 1 metre and were seined by hand net. Live fish were carefully transported back to the laboratory in opaque plastic containers.

In order to avoid depletion of the stickleback population with frequent sampling in the River Kelvin, it was necessary to find the minimum sample size that gives an adequate measure of the body parameters of the fish in the population. The mean and variance of length, weight and biochemical parameters of different sample sizes were plotted, and this revealed that the estimates stabilise with a sample size of twenty fish.

Regular collections of 20-30 male stickle~~back~~backs were made in the middle of each month from November 1985 to October 1986 for analyses of energy reserves. Table 2.1 gives the sample size, mean length, mean weights and standard errors of the male three-spined stickleback collected from the River Kelvin. Sexually mature male sticklebacks with breeding colouration were collected for behavioural experiments during the breeding seasons of 1985 and 1986. During the breeding season the male sticklebacks could be easily distinguished by their characteristic red colouration. During the non-breeding season it was difficult to distinguish between the male and female sticklebacks since there is no marked sexual dimorphism. Hence at least forty

Table 2.1 Sample size, mean lengths, mean weights and standard errors of the male three-spined stickleback collected from the River Kelvin from November 1985 to October 1986.

Month and year	Sample size			Mean length ± S.E. (mm)		Mean wet weight ± S.E. (gms)	
	0	1	Total	0	1	0	1
November 85	08	14	22	35.60 ± 2.09	41.93 ± 1.79	0.602 ± 0.1107	1.067 ± 0.1595
December 85	14	08	22	35.86 ± 1.60	42.62 ± 0.66	0.577 ± 0.0962	1.224 ± 0.1338
January 86	20	00	20	35.95 ± 1.34	NA	0.680 ± 0.0968	NA
February 86	24	00	24	36.91 ± 1.24	NA	0.758 ± 0.1032	NA
March 86	26	00	26	37.96 ± 1.46	NA	0.697 ± 0.0639	NA
April 86	30	00	30	38.56 ± 1.03	NA	0.753 ± 0.0759	NA
May 86	21	00	21	38.88 ± 1.88	NA	0.842 ± 0.1827	NA
June 86	22	00	22	42.00 ± 1.79	NA	1.103 ± 0.128	NA
July 86	02	18	20	30.00 ± 1.01	44.16 ± 1.020	0.300 ± 0.0137	1.160 ± 0.1591
August 86	03	23	26	31.00 ± 1.02	38.78 ± 0.81	0.303 ± 0.0583	0.679 ± 0.1020
September 86	13	07	20	31.31 ± 1.07	39.85 ± 0.96	0.360 ± 0.0299	0.961 ± 0.1578
October 86	12	18	30	35.00 ± 1.02	39.77 ± 1.02	0.588 ± 0.0402	0.827 ± 0.0531

0 = young of the year

1 = adult fish

NA = Not available

sticklebacks had to be collected in order to get about twenty male fish for analysis. Sticklebacks that were parasitised with plerocercoids of the cestode, Schistocephalus solidus were not used for energy reserve analyses and in behavioural experiments. Fish less than about 30 mm of length could not be sexed after dissection without histological analyses of the gonads and such small fish were excluded.

Mass mortality of spawned males was observed in the field soon after the breeding season. June and July samples consisted only of breeding male sticklebacks as they were selectively chosen for ease of sex identification. Though newly hatched fish fry were encountered in the July sample they were not used for the analyses of energy reserves as it was difficult to identify the sex of young fish below 30 mm length.

2.3 ASSIGNING FISH TO AGE CLASSES

A picture of the age structure for the River Kelvin stickleback population has been obtained from studies based on length frequency histogrammes and otolith analysis where age and length agreed well. Body weight proved to be an accurate discriminator between one year old and zero year old fish present in a given month (Ukegbu, 1986). In the present study, weight was used as a criterion for distinguishing young of the year from adult fish.

2.4 STRESS DUE TO CAPTURE

Capture of fish by net and handling involves various degrees of struggle and asphyxiation which cause behavioural and physiological changes collectively referred to as stress. There are a number of characteristic external signs of stress in fish which include ataxia, tachyventilation and marked colour change. Most of the components of any general stress response are the result of the following two physiological changes.

a) Release of catecholamines: The chromaffin cells contain the active catecholamines viz., epinephrine (adrenaline) and norepinephrine (noradrenaline), which are released as a stress response. These in turn produce a series of significant changes, such as glycogenolysis in the liver and muscle (by which extra energy is made available to the stressed fish), increased blood glucose and lactate, changes in free fatty acids, tachycardia, tachyventilation, vasodilation or vasoconstriction and increased peristalysis. These changes may begin in less than a second and may last up to a few hours (Ross and Ross, 1984).

b) Release of corticosteroids: Cortisol, which is the major corticosteroid in teleost fish, is released as a stress response from the interrenal tissue in the kidney. This results in a series of changes such as protein mobilisation, increased glucose production from tissue protein, increased activity Na^+/K^+ (ATP-ase) (Yaron (Yaron, et al., 1983).

Due to the above mentioned reasons, the newly caught exhausted fish could not be used for meaningful determinations of biochemical components and the fish were allowed to rest in aquaria for two days. Fish were housed in 1 m long aquaria at a density of not more than 10 fish per tank.

2.5 CARE AND MAINTENANCE OF THE FISH

Breeding male sticklebacks were collected during the breeding season for behavioural experiments. Each male fish was housed singly in tanks measuring 500 X 280 X 360 mm and allowed to settle down to commence nest construction. The tank floors were provided with a layer of gravel, fine sand and aquatic vegetation. All fish were kept in a light regime of 16 light hours and 8 dark hours. The fish were fed daily ad libitum with live Tubifex worms and occasionally with live Daphnia.

As the male fish settled down in their tanks, most of them started building nests. The water in the tanks was always kept clean and was changed frequently in order to prevent fish diseases and to eliminate the accumulation of waste products in aquaria. A constant aeration system was employed for the fish. The air temperature in the laboratory varied in summer from 18 ^{o} C to 20 ^{o} C. All possible precautions were taken to minimise disturbance to the fish.

2.6 HANDLING AND PROCESSING OF FISH PRIOR TO ANALYSIS

Fish for analysis were lifted out of the aquaria, quickly frozen by immersing in liquid nitrogen (-196^{o} C) to avoid glycogen breakdown. The frozen fish were labelled and stored at -70^{o} C in a deep freezer immediately in order to arrest the enzyme reactions which are involved in the conversion of glycogen to glucose (Glycogenolysis).

At the time of analysis fish were removed from the deep freezer and their lengths and weights were recorded. The lengths were measured using finely pointed dividers which were then transferred to vernier calipers to the nearest 0.1 mm. The fish were dissected, sexed and in males the liver, gonad and carcass were separated. The wet weights of these body compartments were recorded. The body compartments were labelled and were kept in a freezer at -40^{o} C for at least one hour prior to freeze drying. Then they were loaded in the freeze-dryer (Edwards EF03) in order to dehydrate the body compartments by a process of lyophilization which avoids destruction of glycogen. The pirani-gauge of the freeze-dryer was checked to read the degree of dryness and after about 24 hours the samples were removed and transferred to a desiccator and kept in a fridge. The dry weights of the samples were recorded.

Estimations were carried out to assess the glycogen, lipids and protein contents of the samples. The details are given in chapters 3, 4 and 5 respectively. Details about the behavioural experiments are given in chapter 8.

2.7 STATISTICAL ANALYSIS

The data were checked for normal distribution with the help of the Mainframe Computer System of the Glasgow University and logarithmically transformed when necessary. Parametric tests were carried out where appropriate using the Minitab package. Graphs were drawn using the Laser Writer Apple Macintosch.

In the behavioural tests, the values obtained for energy reserves in winners and losers were analysed statistically using parametric tests and where appropriate non-parametric test (the Wilcoxon matched-pair signed-rank test) was used. This test was chosen because the study employed two related samples and yielded scores which were ranked in order of absolute magnitude.

2.8 LITERATURE SEARCH

Literature survey was done by Biosis database Microcomputer system with the help of the Glasgow University library.

CHAPTER 3

SEASONAL VARIATION IN GLYCOGEN RESERVES

CHAPTER 3

SEASONAL VARIATION IN GLYCOGEN RESERVES

3.1 INTRODUCTION

Glycogen is the form in which carbohydrate is stored in animals; it is found in the liver, muscles and other tissues, but is very low in the brain. Glycogen is a homopolysaccharide of high molecular weight. In the glycogen molecule the component glucose residues are linked in chains by both 1,4 and 1,6 alpha glycoside bonds to give a highly branched structure. This open tree-like structure has many ends available for enzyme action (Oser,1965).

Major precursors of liver glycogen within the animal body include: glucose, fructose and galactose produced by carbohydrate digestion in the intestinal tract, blood glucose and blood lactic acid, the glycerol portion of the fats and glucogenic aminoacids of the protein. Fats and proteins are derived from either the diet or the body which are capable of being converted into glucose and then into glycogen. Precursors of muscle glycogen include the glucose of the blood and the lactic acid produced from glycogen during muscle contraction.

Excess blood glucose is converted to glycogen in the liver and muscles. The formation of liver glycogen is a mechanism whereby the excess glucose derived from the diet may be stored until it is reconverted to blood glucose to meet the demands of energy

requirement. These various interrelationships with regard to liver and muscle glycogen are summarized in Figure 3.1.

3.2 GLYCOGEN METABOLISM

Glycogen is synthesised from glucose 6-phosphate wherein the latter is converted to glucose 1-phosphate by the enzyme phosphoglucomutase, which is then combined with uridine triphosphate (UTP) by the enzyme UDPG-phosphorylase to form uridine diphosphate glucose (UDPG). The glucose moiety is then transferred from the uridine diphosphate (UDP) carrier to the uncompleted end of a glycogen molecule by the enzyme UDPG - glycosyltransferase to elongate the glycogen chain.

Glycogen can easily be reconverted to glucose 1-phosphate by the action of glycogen phosphorylase. Glucose 1-phosphate can be converted to glucose 6-phosphate ~~(by the enzyme phosphoglucomutase)~~ which in turn can be converted to glucose.

In muscle tissue the glycogen is converted to carbon dioxide, water and energy, which occurs in two steps: the first is an anaerobic conversion of glycogen to pyruvic acid and lactic acid and is known as glycolysis or the glycolytic path; the second is the aerobic oxidation of pyruvic acid to carbon dioxide, water and energy which is known as the tricarboxylic acid or TCA cycle. The anaerobic glycolytic phase is more rapid than the aerobic phase and lactic acid tends to accumulate. This lactic acid is carried back to the liver by the blood stream

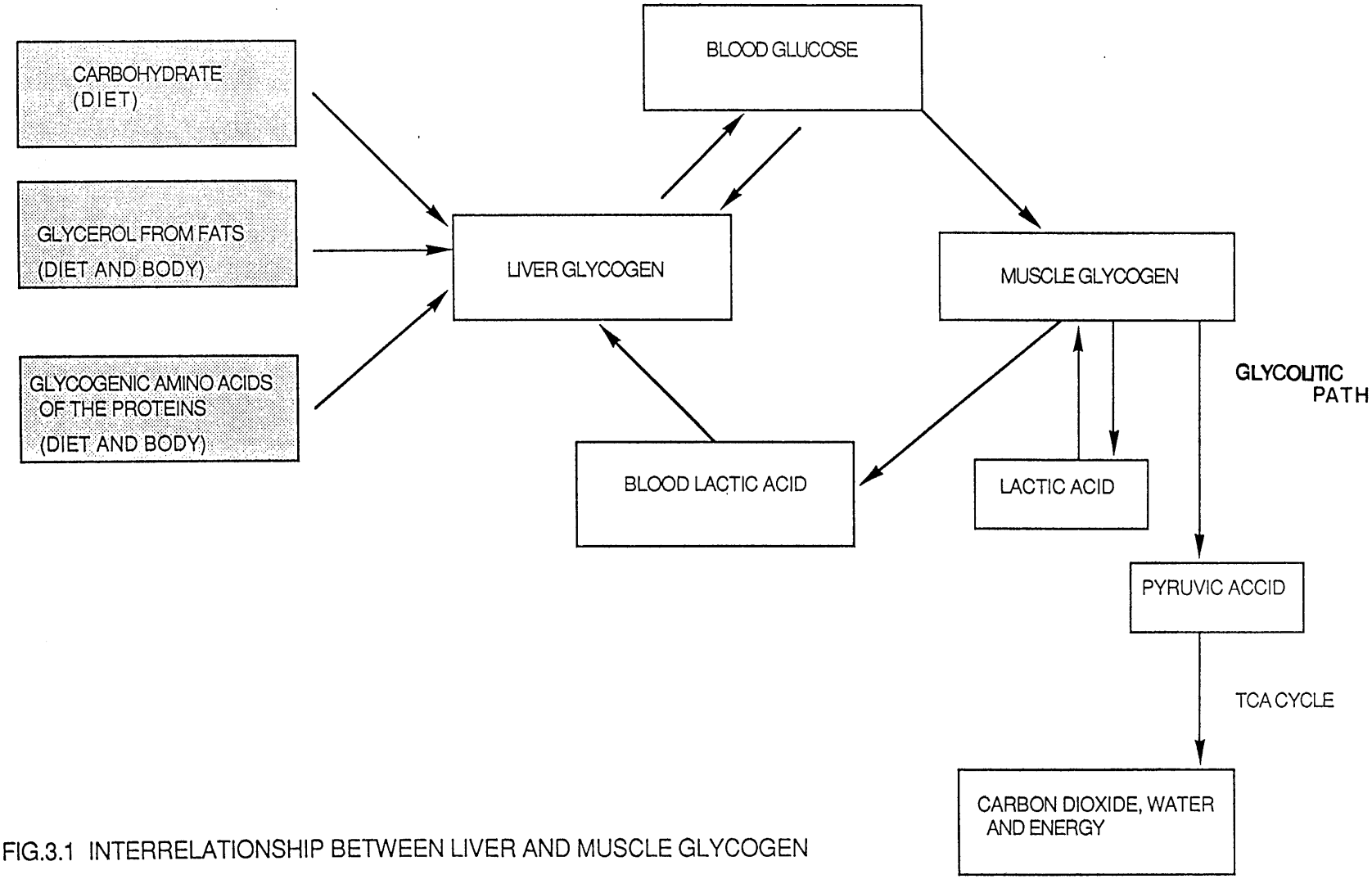


FIG.3.1 INTERRELATIONSHIP BETWEEN LIVER AND MUSCLE GLYCOGEN

(ADAPTED FROM OSER, 1965)

where it is reconverted to glycogen or glucose. Figure 3.2 illustrate the pathways of glycogen metabolism. The conversion of glycogen to glucose and its phosphates is referred to as glycogenolysis. The reverse process, that is, the conversion of glucose to glycogen is known as glycogenesis (Oser, 1965).

Key to Fig 3.2 Pathways of glycogen metabolism

1. hexokinase
2. phosphoglucose isomerase
3. phosphofructokinase
4. aldolase
5. glycogen synthesising enzymes
6. glycogen cleavage enzymes
7. glucose-6-phosphate dehydrogenase
8. 6-phosphogluconate dehydragenase
9. other enzymes of pentose phosphate pathway
10. enzymes of nucleic acid and nucleotide synthesis
11. glycerol 3-phosphate dehydragenase
12. other enzymes of glycolysis
13. pyruvate kinase
14. lactate dehydragenase
15. pyruvate dehydrogenase

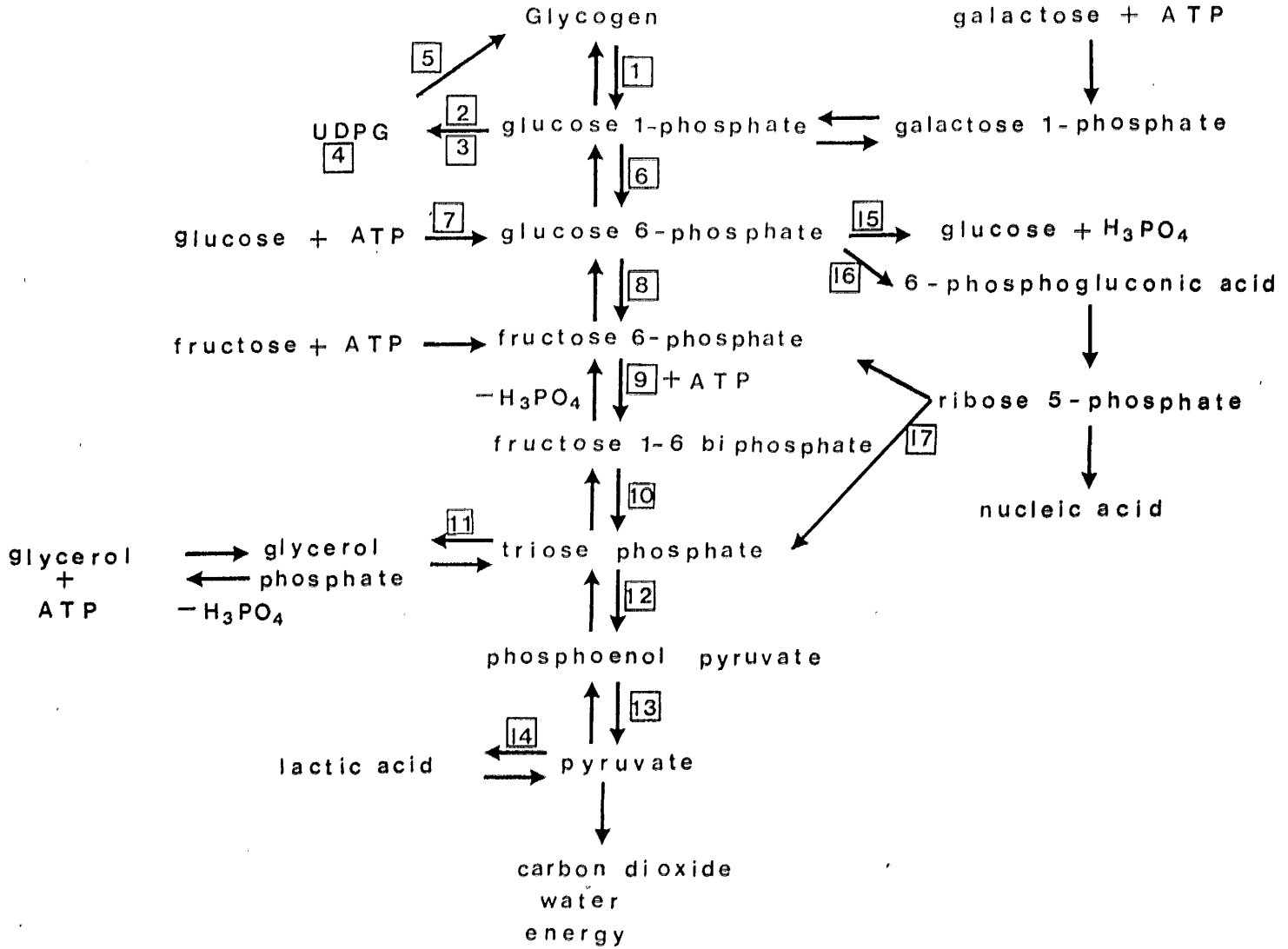


FIG 3.2 PATHWAYS OF GLYCOGEN METABOLISM

3.3. METHODS OF GLYCOGEN DETERMINATION

Microcolorimetric techniques for the determination of sugars have replaced the earlier volumetric reducing procedures and have also permitted the direct estimation of non-reducing oligosaccharides and polysaccharides. This procedural simplification is a consequence of the combined conditions of high acidity and temperature required for the development of the coloured compounds when concomitant hydrolysis of glycosidic bond occurs (Montgomery, 1957).

The original Pfluger procedure, 1905 (as reviewed by Montgomery, 1957) involves boiling the tissue in 30% KOH solution, alcohol precipitation, acid hydrolysis of the precipitate and copper reduction of the neutralized hydrolysate. Good, Kramer and Somogyi (1933) concluded that the original Pfluger procedure is the only adequate method for the determination of glycogen and these authors introduced improvements into the Pfluger procedure which speeded the method up considerably.

In currently used methods the tissue is extracted either by boiling with 30% potassium hydroxide solution (KOH) or by homogenizing with 5% trichloroacetic acid solution (TCA). The procedure is to boil the tissue with KOH and then to determine the glycogen in the alkali-treated mixture with anthrone reagent (Seifter et al., 1950; Bloom et al., 1951). A method based upon the use of anthrone reagent gave results of a high degree of specificity and precision (Carroll, Longley and Roe, 1956). Montgomery (1957)

considers that the most specific quantative method for glycogen determination is the turbimetric procedure involving the use of globulin, and Concanavalin A (extracted from jackbean meal according to the method of Cifonelli and Smith, 1955).

Lipid-free tissues have been used for glycogen assays (Wootton et al., 1978). The tissue was heated with 4 ml of 80% Ethyl alcohol and then centrifuged at 3000 rpm. The supernatant was discarded and the precipitate was heated at 100 ^{o} C in 4 ml trichloroacetic acid for 15 minutes to extract the glycogen. A 2 ml aliquot of the supernatant resulting from the extraction was analysed by the anthrone method (Seifter et al., 1950).

3.4 SEASONAL VARIATION IN GLYCOGEN RESERVES IN STICKLEBACKS

As discussed in chapter one, total glycogen reserves vary during the different periods of life cycle of many animals. Glycogen estimations have been carried out on Latvian populations of male and female stickleback in Russia (Immers, 1953). The samples were collected at key times of the year (a total number of 105 male and 103 female sticklebacks) and were pooled to analyse seasonal glycogen values. The liver, gonad and skin (removed from the body proper) were used to estimate the total glycogen reserves. The carcass was not used in the analysis (see Table 1.1 for glycogen values). The glycogen assays were based on the techniques that were then available viz. Pfluger's modified method (see above). Glycogen estimations have also

been carried out using female sticklebacks from two Welsh populations (Wootton et al., 1978). In this case the samples were collected monthly for one year, but the monthly sample of each body compartment from each population was pooled to estimate seasonal glycogen values (see Table 1.2). Glycogen was determined by methods described by Lambert and Dehnel, 1974. In this method, the different compartments of the fish (viz. the liver, gonad and carcass), were dried to a constant weight at 70 ^{o} C and then lipid was extracted prior to glycogen assays (see above).

So far no systematic annual sampling has been carried out on different age groups of male three-spined sticklebacks in order to estimate their energy reserves. This is of considerable interest as territorial defence, courtship and parental care in male stickleback demand expenditure of energy, hence good body condition of the male is important for successful breeding. One of the aims of this study is to examine the annual variation in glycogen reserves in males of a Scottish population of sticklebacks at different stages of the life cycle, using samples collected systematically on a monthly basis over one complete year and employing appropriate processing and assay techniques.

3.5 MATERIAL AND METHODS

Sticklebacks were collected from the River Kelvin each month between November 1985 and October 1986 and processed as described in chapter 2. The method used to estimate glycogen in this project was based on

tissue extraction with 30% potassium hydroxide solution and the estimation of glycogen with anthrone reagent (Seifter et.al.,1950). Dr.R.H.C.Strang of the Biochemistry Department of Glasgow University, designed the processing techniques for handling fish samples prior to glycogen assays to avoid glycogen breakdown due to enzyme reactions. The fish used for analyses in this project were killed in liquid nitrogen at -196 ^{o} C and were immediately placed in a deep freezer of -70 ^{o} C to avoid glycogenolysis. The fish were dehydrated by a process of lyophilization to avoid high temperatures that could result in glycogen break down.

Glycogen has a high resistence to attack by alkali, but Potassium hydroxide (KOH) acts on fats and destroys the peptide bonds of the proteins. After alkali treatment, the supernatant contained the glycogen, amino acids and glycolipids. The supernatant was decanted and treated with two volumes of 95% ethanol. This precipitated the glycogen leaving the other water soluble compounds like amino acids in the solution. Further purification of glycogen was obtained by subsequent precipitation with ethanol. Glycogen pellets were washed with chloroform-methanol 1:4 mixture to extract any glycolipids present. The glycogen pellet when redissolved in water was used to estimate the glycogen as glucose using anthrone reagent.

The anthrone reaction was the basis of a rapid and convenient method for the determination of glucose. Concentrated sulphuric acid hydrolysed the glycosidic bonds to give monosaccharides which were then dehydrated to furfural and its derivatives (from six carbon to

five carbon). Furfural reacts with anthrone (10 keto-9,10-dihydro-anthracene) to give a blue-green complex, with an absorption maximum at 620 nm. A digital spectrophotometer (model SP-6-550) was used to obtain the optical density readings which were matched with the standard glucose curve and the corresponding values were taken.

The procedure is depicted below in the form of a flow chart.

Fish killed in liquid nitrogen (-196 ^{o} C) and transferred to an ultra-cold freezer (-70 ^{o} C)

↓

Wet weight and standard length recorded and fish dissected on melting ice. Wet weights of body compartments recorded and specimens kept at -30 ^{o} C for one hour. Fish tissue dehydrated by freeze-drying for 24 hours in Edwards EF03 freeze drier

↓

Dry weight of tissues recorded and tissues ground into powder

↓

Known weight of the tissue introduced into an Eppendorf tube, 0.5 ml of 30% KOH added and heated on a water bath for 20 minutes

↓

Contents in the Eppendorf tube mixed well using vortex mixer and centrifuged for 10 minutes

↓

1 ml of 95% ethyl alcohol added to the supernatant

↓

Supernatant kept in a refrigerator for 2 hours to help precipitation of glycogen

↓

Centrifuged for 10 minutes at 10,000 g

↓

Supernatant discarded and to the precipitate added 1 ml of 65% ethyl alcohol

↓

Centrifuged for 10 minutes at 10,000 g

↓

Glycogen pellets washed with chloroform/methanol 1:4 mixture to remove glycolipids, and then washed with 65% ethanol until washings were of neutral pH

↓

Glycogen pellets dissolved in 0.5 ml of water and heated on a boiling water bath for 5 minutes

↓

Centrifuged for 10 minutes at 10,000 g

↓

1 ml of anthrone reagent added to 100 µl of the glycogen solution (1:10 ratio) and mixed well using a whirlimixer

↓

Mixture heated on a water bath for exactly 20 minutes and a bluish-green colour developed

↓

Mixture cooled on an ice bath

↓

The optical density read in the spectrophotometer at 620nm and matched with a standard glucose calibration curve.

For every batch of test solutions freshly-prepared glucose standards were used to plot a glucose calibration curve. Glucose standards of different range were prepared by diluting the 1 mM glucose stock solution keeping the total volume constant at 1 ml. Variable volume micropipettes with disposable ejector tips were used to prepare the glucose standard solutions. The molecular weight of glucose moieties was assumed to be 162 (180 - 18 ie molecular weight of glucose moieties minus molecular weight of water) when calculating the weight of glycogen standard (Strang, personal communication). The glycogen standards were taken through the same procedure as the test solutions. These were used for comparison and were matched with glucose calibration curve. Glycogen values expressed as μ mol g^{-1} d.w in this thesis refer to μ mol glycosol unit per gram dry weight of the tissue.

3.6 DATA ANALYSIS

Mean monthly levels of glycogen reserves were calculated for fish of each age class (viz. the young fish of the year and the adult fish. The data were checked for normal distribution and transformed when necessary. Product-moment correlation coefficients were calculated between the body weight and the glycogen reserves in the liver, gonad and carcass. One way analysis of variance (ANOVA) was used to check for a seasonal variation in glycogen reserves. The relationship between body weight and glycogen reserves was investigated by regression analysis for each month separately over a period of one year, considering the young of the year and adult fish separately.

3.7 RESULTS

Table 3.1 summarize the results of oneway analysis of variance (ANOVA) on glycogen reserves for the entire year. The results described in this section are based on the regular monthly samples taken from November 1985 to October 1986. Figure 3.3 A-C shows the annual changes in liver, gonad and carcass glycogen reserves respectively. The mean values and 95% confidence intervals of the means have been plotted against the months of the year. The months have been arranged on the X-axis to depict the complete life cycle of male sticklebacks from this population.

TABLE 3.1 ONEWAY ANALYSIS OF VARIANCE BY MONTH ON GLYCOGEN RESERVES

VARIABLE	SOURCE	D.F.	SUM OF SQUARES	MEAN OF SQUARES	F RATIO	F PROBABILITY
LIVER GLYCOGEN						
	Between groups	11	3691434.48	335584.95	40.27	0.0000
	Within groups	271	2258039.17	8332.24	--	--
GONAD GLYCOGEN						
	Between groups	11	17925.74	1629.61	34.26	0.0000
	Within groups	271	12887.36	47.55	--	--
CARCASS GLYCOGEN						
	Between groups	11	8340.66	758.24	40.24	0.0000
	Within groups	271	5106.08	18.84	--	--

FIGURE 3.3 ANNUAL CHANGES IN GLYCOGEN RESERVES

A) Mean and 95% confidence intervals of liver glycogen

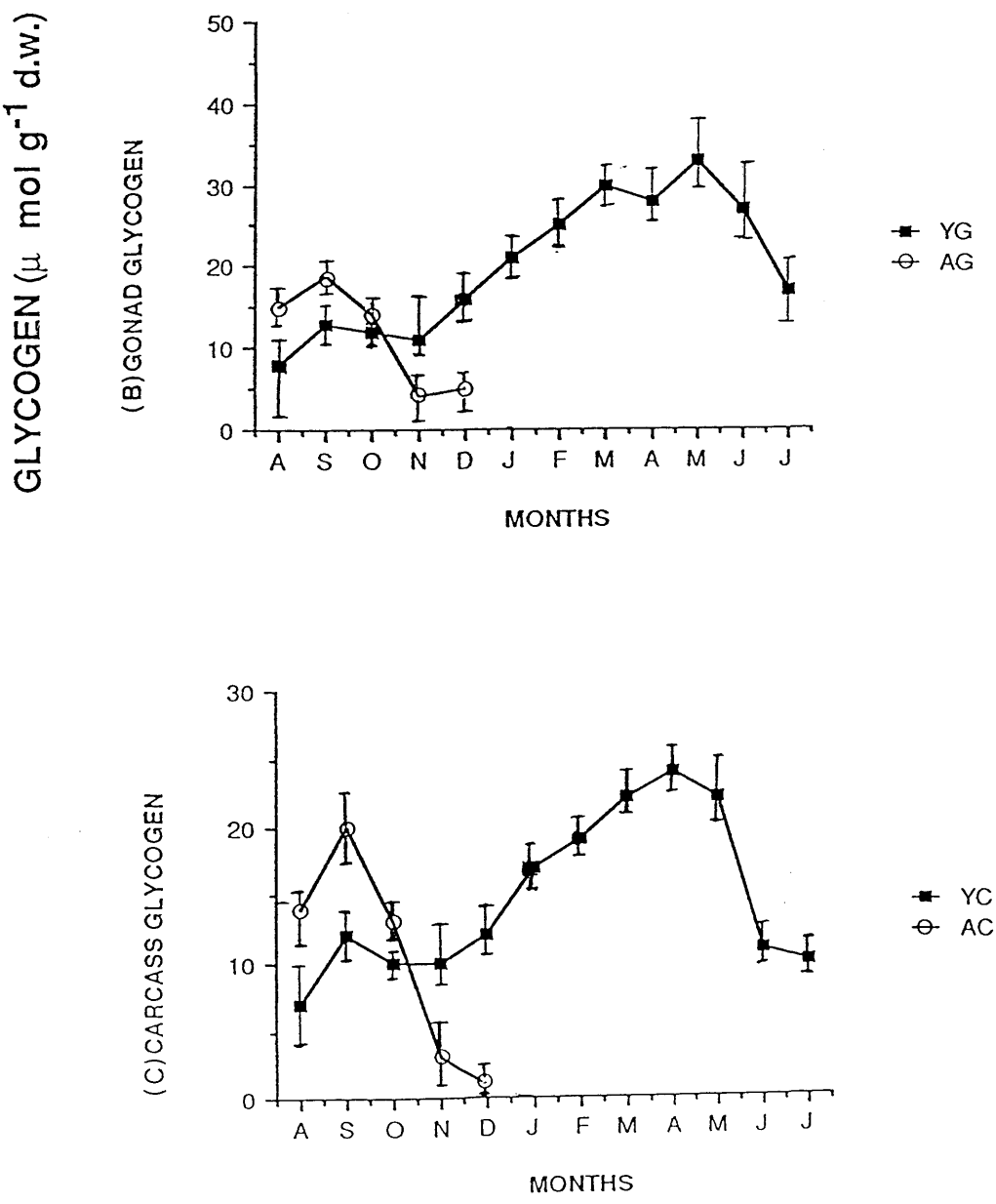
B) Mean and 95% confidence intervals of gonad glycogen

C) Mean and 95% confidence intervals of carcass glycogen

(The months are arranged on the X-axis to depict the complete life cycle of the male three-spined stickleback from the River Kelvin)

Y = Young of the year (males)

A = Non-breeder adult males


GLYCOGEN (μ mol g-1 d.w.)
(A)LIVER GLYCOGEN
600
500
400
300
200
100
0
A S O N D J F M A M J J
MONTHS
YL
AL
(B)GONAD GLYCOGEN
50
40
30
20
10
0
A S O N D J F M A M J J
MONTHS
YG
AG
(C)CARCASS GLYCOGEN
30
20
10
0
A S O N D J F M A M J J
MONTHS
YC
AC

3.7.1 Annual variation in liver glycogen reserves

Figure 3.3 A shows that young of the year had low liver glycogen reserves in August. There was a sharp increase in glycogen reserves by October but this levelled off during the winter months of November, December and January. There was another sharp rise in the liver glycogen level from February to April followed by a dramatic fall from the month of May. This coincided with the start of the breeding season as males with breeding colours were found for the first time in the sample collected during May. By July the liver glycogen reserves were virtually depleted as a result of reproductive activity.

The adults caught from August onwards showed no signs of having bred as they were small and did not have any breeding colouration at all. These adult males were probably those that hatched later in the previous year, which failed to reproduce in their first summer (Ukegbu, 1986). These fish had relatively high glycogen reserves ~~and~~ compared to the young of the year and adults that had bred. Their glycogen levels dropped dramatically between September and December, after which no further fish of this age class was caught.

3.7.2 Annual variation in gonad glycogen reserves

Fig.3.3 B shows that the overall pattern of the annual variation in gonad glycogen was similar to that of liver glycogen, apart from the fact that the gonad glycogen levels were consistently much lower than the liver glycogen levels. Until November the gonad

glycogen levels in young of the year were consistently low. A steep and sustained increase started in December and continued up to May; gonad glycogen levels then dropped dramatically between May and July. In July, the newly hatched males had lower gonad glycogen levels than the breeding males. Adult fish caught between August and October had slightly higher gonad glycogen levels than the young of the year but the levels dropped drastically in November and December.

3.7.3 Annual variation in carcass glycogen reserves

Fig 3.3 C shows that between August and November the carcass glycogen reserves in the young of the year were at a uniformly low level. From December to April there was a sharp increase in glycogen reserves. Once again, from the start of the breeding season a dramatic decrease in glycogen level occurred. In July the young of the year had slightly higher carcass glycogen levels than the breeding male fish. From August to October the adult male fish which had not bred had higher carcass glycogen reserves than young of the year but the position was reversed from November, as the glycogen reserves of the adult fish became totally depleted and the glycogen level fell to practically zero.

TABLE 3.2 Correlation coefficients and levels of significance between glycogen reserves in the liver, gonad and carcass.

A - during the non-breeding season of August to May

B - during the breeding season - month of June

C - during the breeding season - month of July

Abbreviations:

R = Product-moment correlation coefficient

P = Levels of significance

*** = $P < 0.001$

** = $P < 0.01$

* = $P < 0.05$

+ = $P < 0.1$

NS = Non-significant

N = Number of samples

Table 3.2 A

GLYCOGEN

LIVER	GONAD	CARCASS	
	R = 0.811 P = ***	P = 0.797 P = ***	LIVER
N = 241		R = 0.863 P = ***	GONAD
			CARCASS

GLYCOGEN

Table 3.2 B

GLYCOGEN

LIVER	GONAD	CARCASS	
	R =-0.301 P = NS	P =-0.266 P = NS	LIVER
N = 22		R =-0.146 P = NS	GONAD
			CARCASS

GLYCOGEN

Table 3.2 C

GLYCOGEN

LIVER	GONAD	CARCASS	
	R = 0.383 P = +	P = 0.397 P = +	LIVER
N = 18		R = 0.511 P = *	GONAD
			CARCASS

GLYCOGEN

3.7.4 The relationship between glycogen reserves in liver, gonad and carcass

Just prior to the breeding season, when glycogen reserves were at their peak, liver, gonad and carcass glycogen stores constituted 48.4%, 1.5% and 50.1% respectively of the total glycogen reserves. Table 3.3 A to C gives the Product-moment correlations between glycogen reserves in the different body compartments (viz. liver, gonad and carcass) during the non-breeding season (August to May) and in June and July - the two months of the breeding season. There were positive relationships (highly significant) between glycogen reserves in the liver, gonad and carcass during the non-breeding season of August to May. The relationships were negative (non-significant) in June, but in July the relationships were positive (marginally significant).

3.7.5 The relationship between body weight and glycogen reserves

3.7.5.1 Body weight and liver glycogen reserves

Figure 3.4 shows the scatter plots of liver glycogen against body wet weight for each month over a period of one year. In the young of the year there was a significant and strong relationship between body weight and liver glycogen reserves from August up until May when this cohort started breeding. This indicates that growth was not at the expense of liver energy reserves. In June and July this relation was reversed and replaced by a non-significant negative relationship. This change in the relationship between body weight and liver glycogen was due to depletion of liver glycogen reserves in the large fish, presumably as a result of breeding.

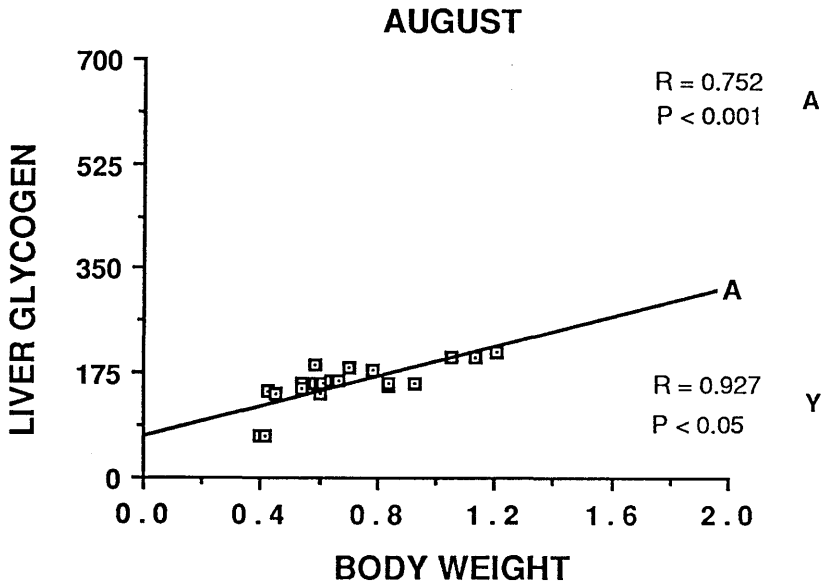
The adult fish caught during August were the ones which had failed to spawn, as post-breeding male fish died and August was the end of the breeding season. The relationship between body weight and liver glycogen in these adult fish remained positive from August to October, this supports the above suggestion that the reversal of the relationship in 0^+ age category is the result of breeding. However, by November and December there was no relationship betwen body weight and liver glycogen reserves. This is because all adult fish regardless of size had very low glycogen levels in their second winter.

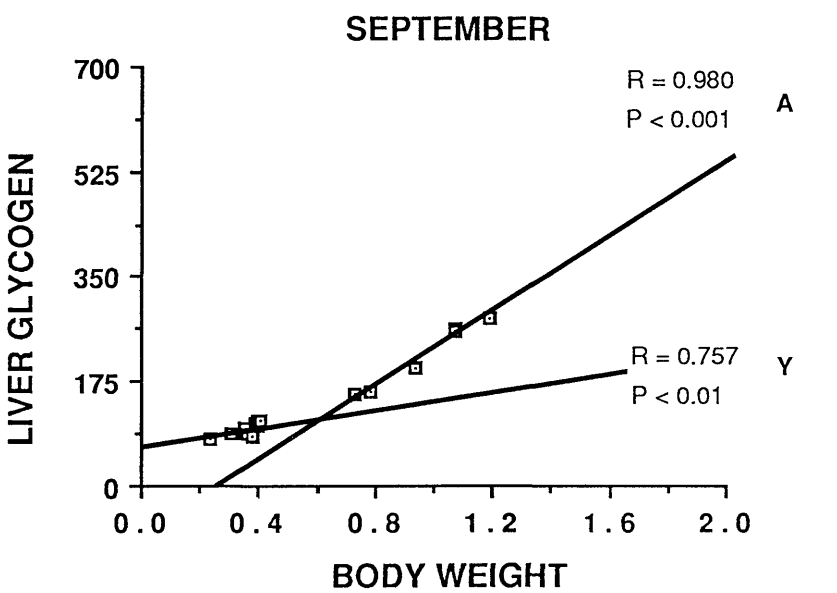
FIGURE 3.4 Scatter plots of liver glycogen against body weight for each month over a period of one year. Glycogen values are given in μ mol g^{-1} d.w and body weight in gms.

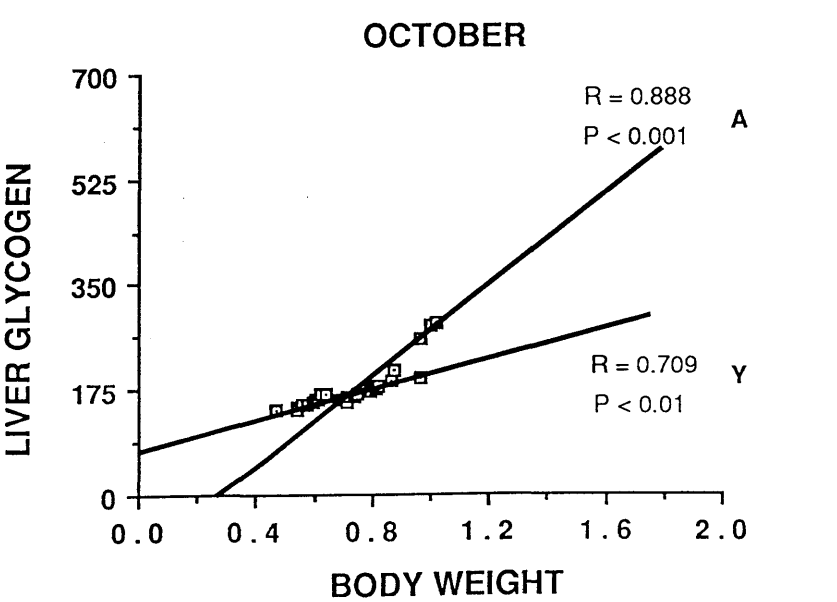
A = non-breeding adult males

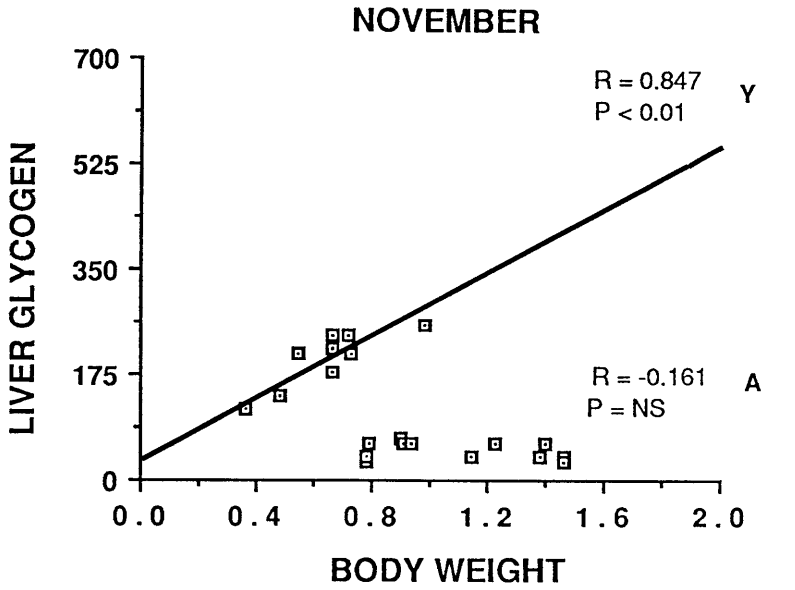
Y = young of the year

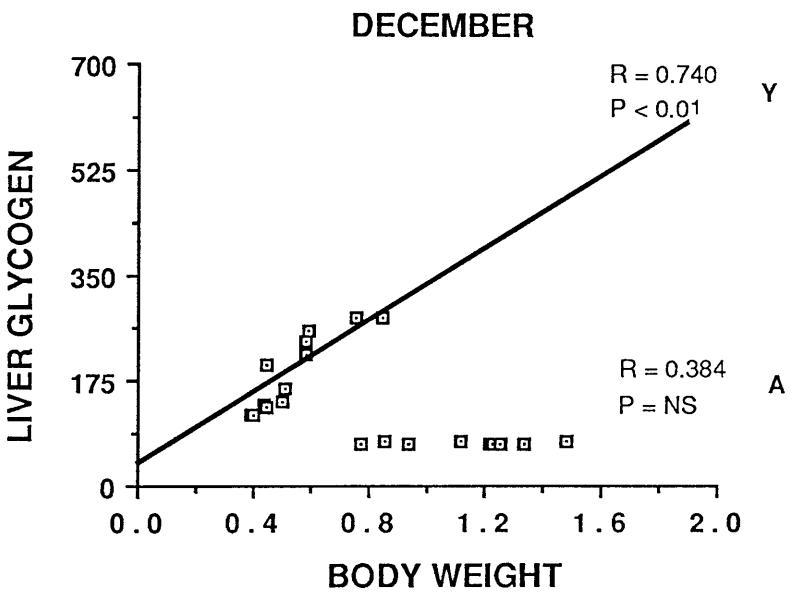
A′= breeding adult males

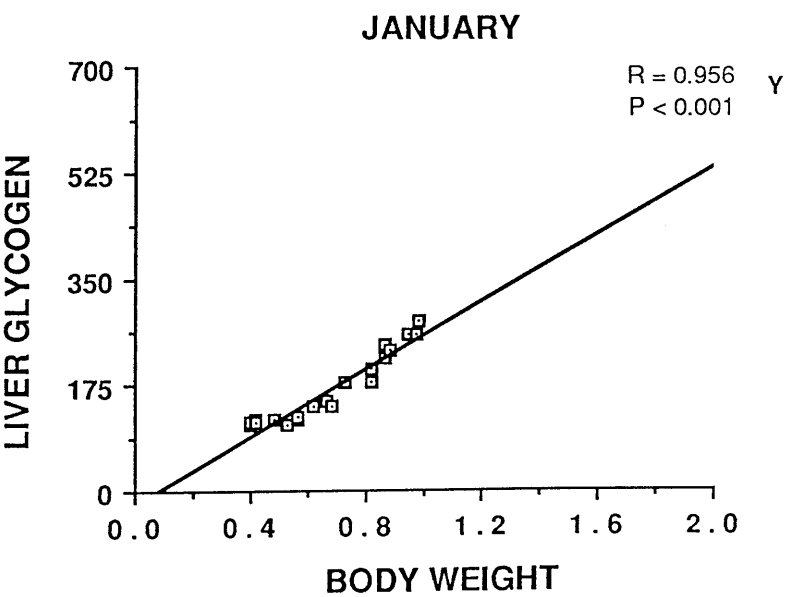

AUGUST
700
525
350
175
0
LIVER GLYCOGEN
R = 0.752
P < 0.001
A
A
R = 0.927
P < 0.05
Y
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT

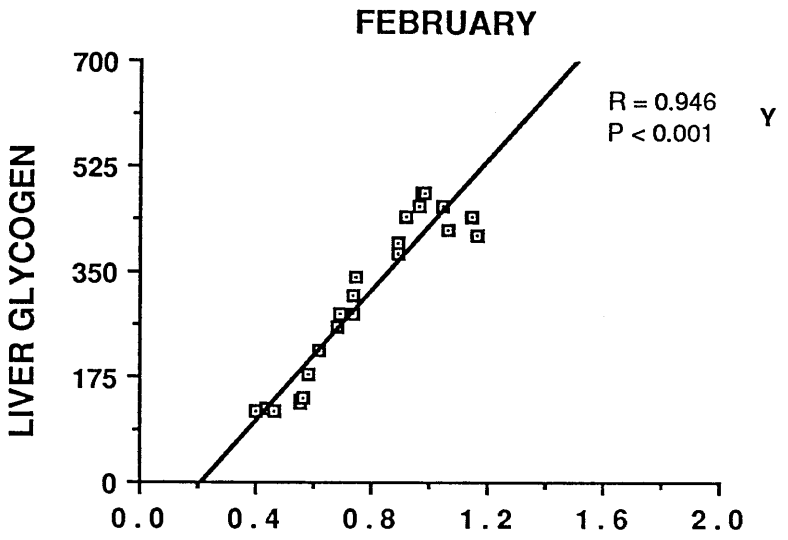

SEPTEMBER
700
525
350
175
0
LIVER GLYCOGEN
R = 0.980
P < 0.001
A
R = 0.757
P < 0.01
Y
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT

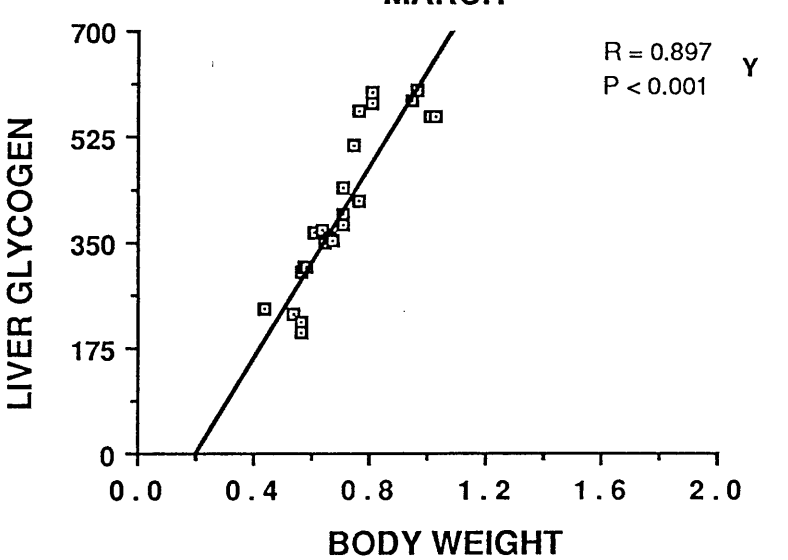

OCTOBER
700
525
350
175
0
LIVER GLYCOGEN
R = 0.888
P < 0.001
A
R = 0.709
P < 0.01
Y
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT

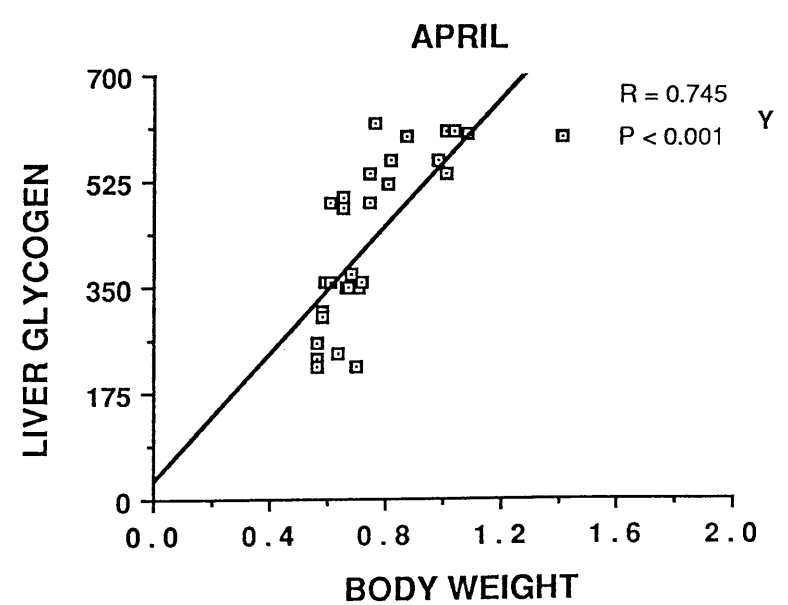

NOVEMBER
700
525
350
175
0
LIVER GLYCOGEN
R = 0.847
P < 0.01
Y
R = -0.161
P = NS
A
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT

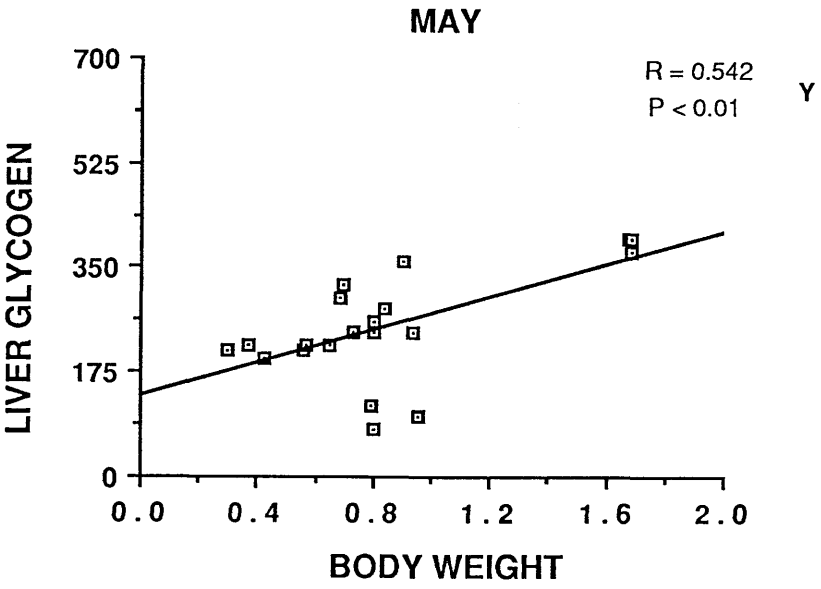

DECEMBER
700
525
350
175
0
LIVER GLYCOGEN
R = 0.740
P < 0.01
Y
R = 0.384
P = NS
A
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT

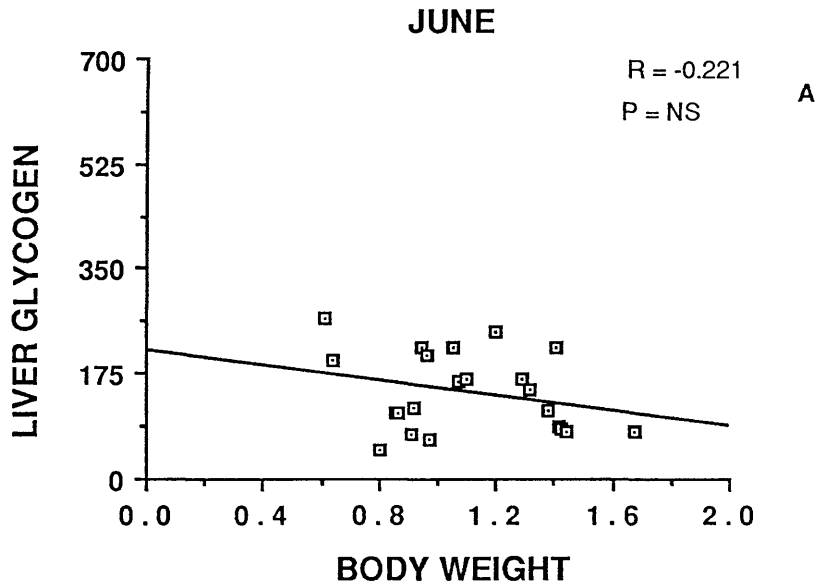

JANUARY
700
525
350
175
0
LIVER GLYCOGEN
R = 0.956
P < 0.001
Y
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT

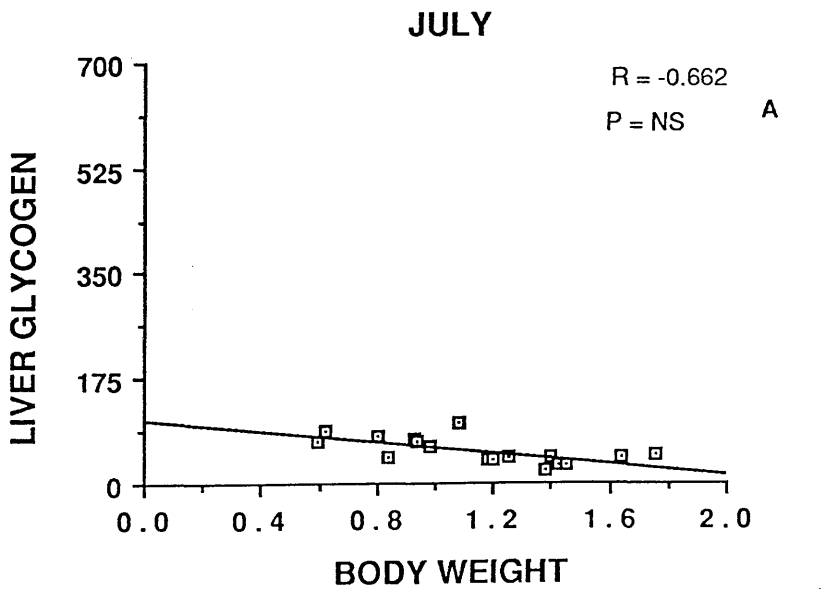

FEBRUARY
LIVER GLYCOGEN
700
525
350
175
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT
R = 0.946
P < 0.001
Y


MARCH
LIVER GLYCOGEN
700
525
350
175
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT
R = 0.897
P < 0.001
Y


APRIL
LIVER GLYCOGEN
700
525
350
175
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT
R = 0.745
P < 0.001
Y


MAY
R = 0.542
P < 0.01
Y
LIVER GLYCOGEN
700
525
350
175
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT


JUNE
R = -0.221
P = NS
A
LIVER GLYCOGEN
700
525
350
175
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT


JULY
R = -0.662
P = NS
A
LIVER GLYCOGEN
700
525
350
175
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT

3.7.5.2 Body weight and gonad glycogen reserves

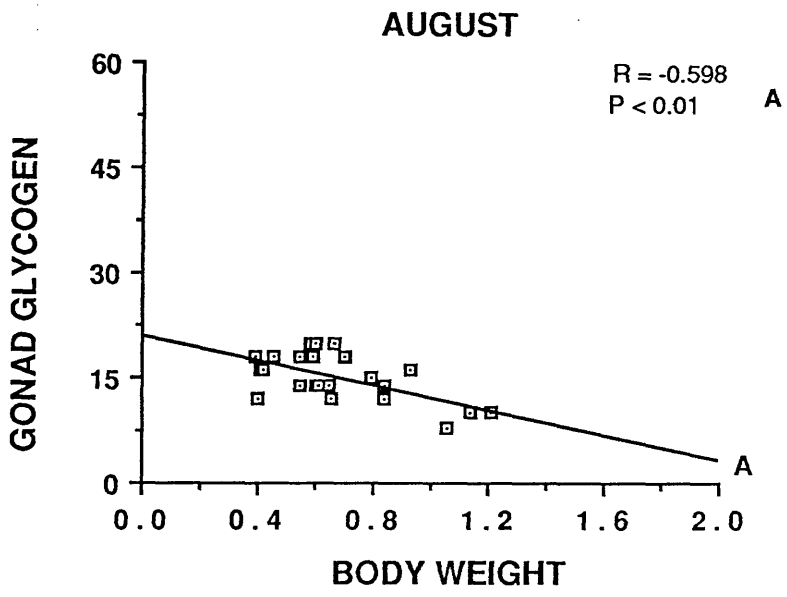
Figure 3.5 show the scatter plots of gonad glycogen against body weight for each month over a period of one year. In the young of the year during August, September and October there was a positive but a non-significant relationship between gonad glycogen and body weight, but from November through to April their relationship was strongly positive and highly significant. Between May and July the relationship reversed to a non-significant negative one. In the adult non-breeding fish caught during August, there was a negative relationship between body weight and gonad glycogen which suggests that some gonadal maturation had taken place and gonad glycogen reserves were depleted in the larger of them. However, this relationship became positive in September. The relationship between body weight and gonad glycogen in adult fish was not significant from October to December when gonad glycogen levels dropped to uniformly low levels in fish of all sizes.

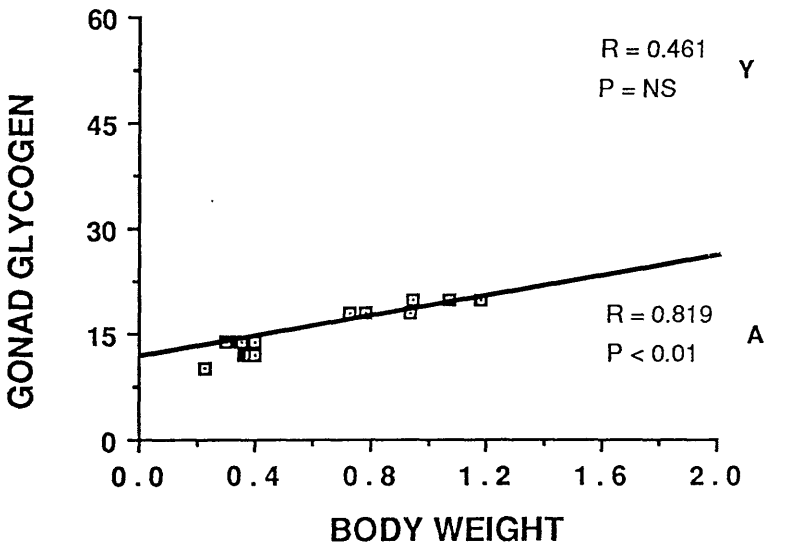
FIGURE 3.5 Scatter plots of gonad glycogen against body weight for each month over a period of one year. Glycogen values are given in μ mol g^{-1} d.w and body weight in gms.

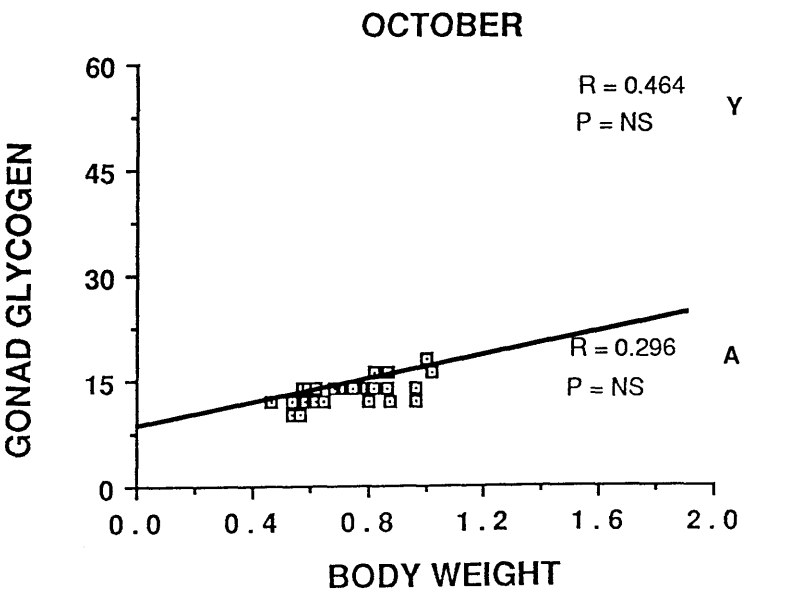
A = non-breeding adult males

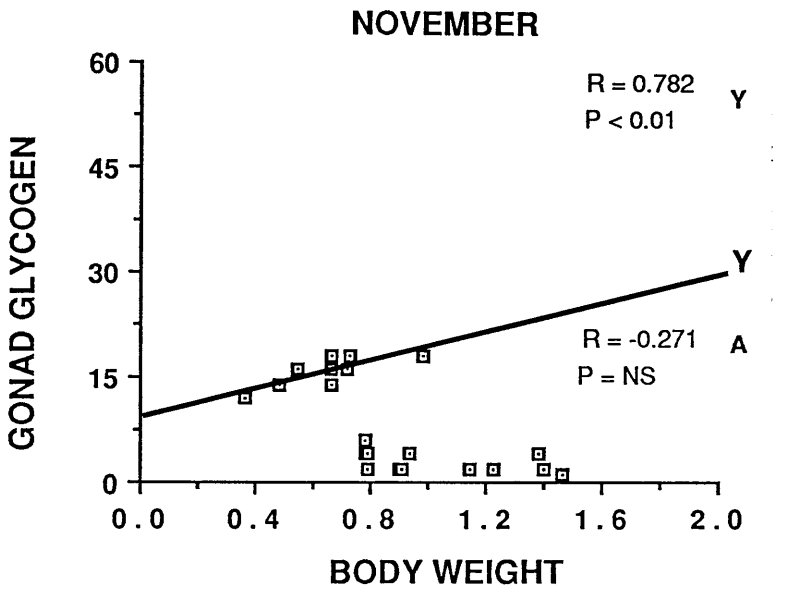
Y = young of the year

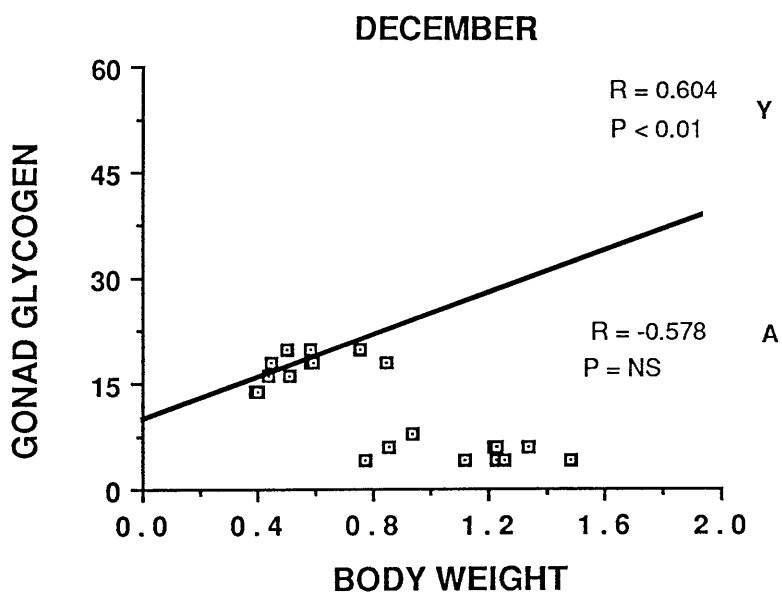
A′= breeding adult males

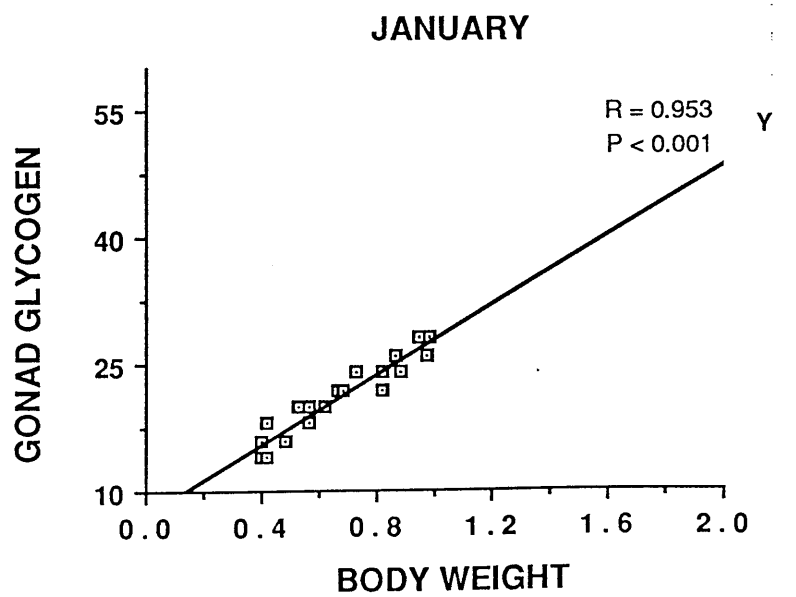

AUGUST
R = -0.598
P < 0.01
A
A
GONAD GLYCOGEN
60
45
30
15
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT

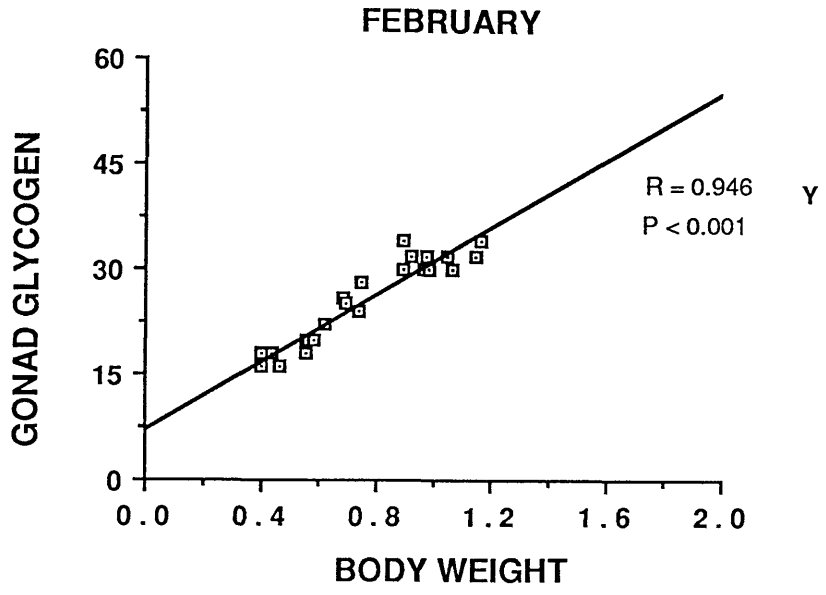

SEPTEMBER
R = 0.461
P = NS
Y
R = 0.819
P < 0.01
A
GONAD GLYCOGEN
60
45
30
15
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT

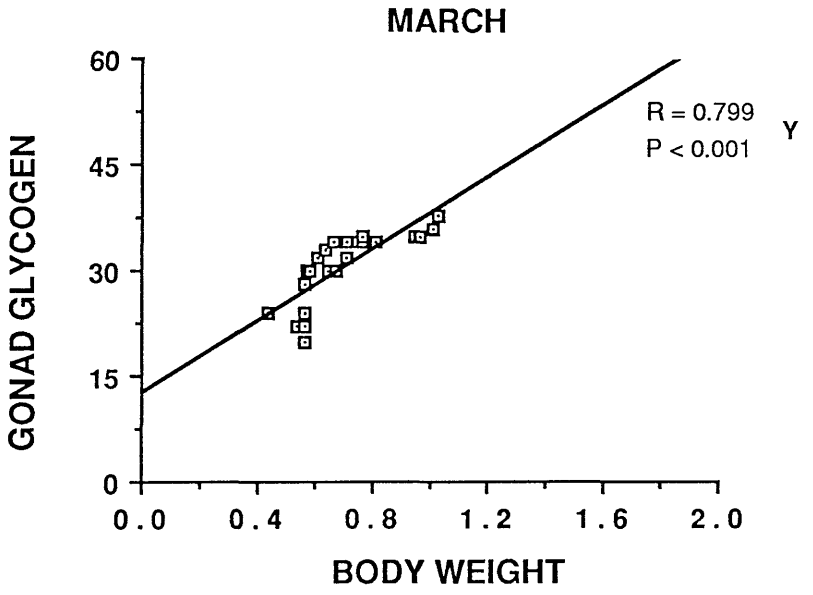

OCTOBER
R = 0.464
P = NS
Y
R = 0.296
P = NS
A
GONAD GLYCOGEN
60
45
30
15
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT

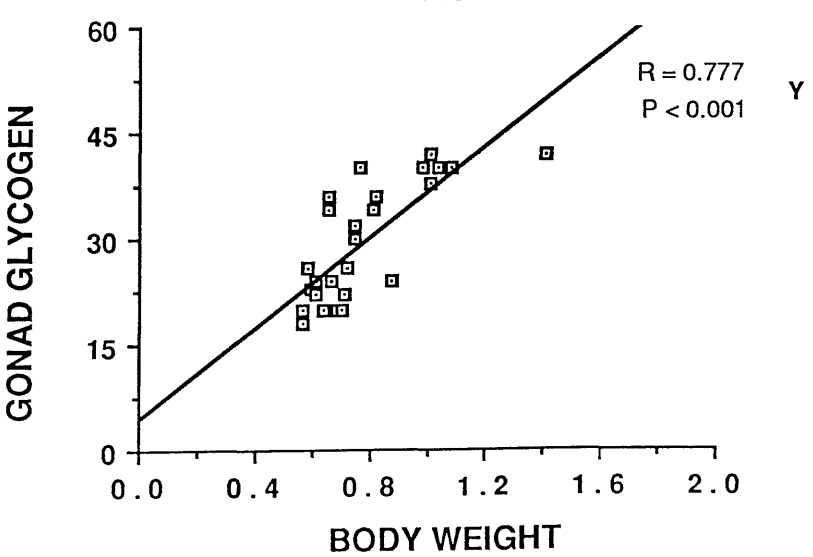

NOVEMBER
GONAD GLYCOGEN
60
45
30
15
0
R = 0.782
P < 0.01
Y
Y
R = -0.271
P = NS
A
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT

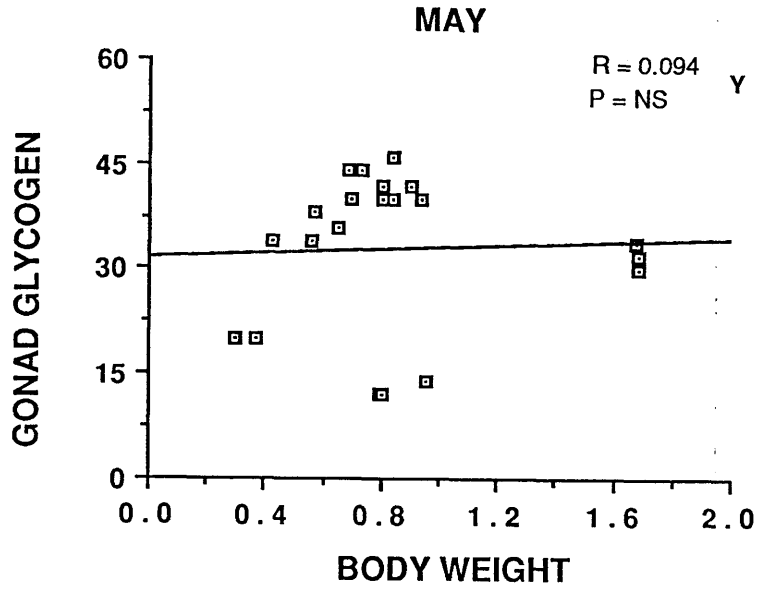

DECEMBER
GONAD GLYCOGEN
60
45
30
15
0
R = 0.604
P < 0.01
Y
R = -0.578
P = NS
A
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT


JANUARY
GONAD GLYCOGEN
55
40
25
10
R = 0.953
P < 0.001
Y
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT


FEBRUARY
GONAD GLYCOGEN
60
45
30
15
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT
R = 0.946
P < 0.001
Y


MARCH
GONAD GLYCOGEN
60
45
30
15
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT
R = 0.799
P < 0.001
Y


APRIL
GONAD GLYCOGEN
60
45
30
15
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT
R = 0.777
P < 0.001
Y


MAY
R = 0.094
P = NS
Y
GONAD GLYCOGEN
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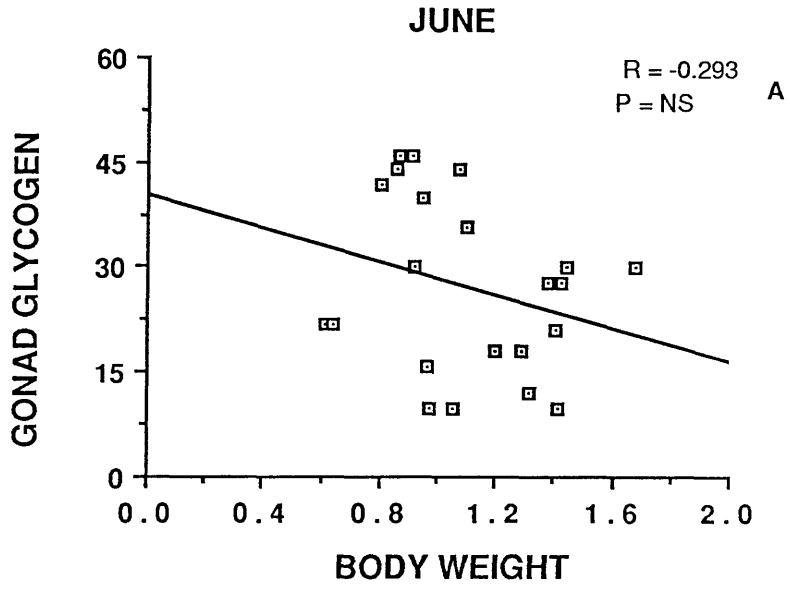
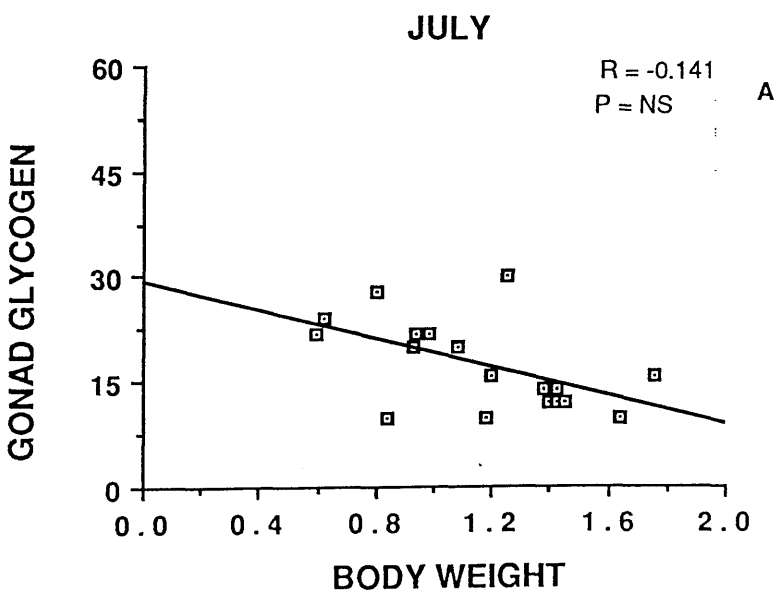
45
30
15
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT


JUNE
R = -0.293
P = NS
A
GONAD GLYCOGEN
60
45
30
15
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT


JULY
R = -0.141
P = NS
A
GONAD GLYCOGEN
60
45
30
15
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT

3.7.5.3 Body weight and carcass glycogen reserves

Figure 3.6 show the scatter plots of carcass glycogen against body weight for each month over a period of one year. From August through to April there was a strong and significant positive relationship between carcass glycogen and body weight. The relationship was weaker in May but still significant and positive. By June there was no significant relationship between body weight and carcass glycogen reserves. By July, when all the adult sexually mature male fish were breeding the relationship between body weight and carcass glycogen became negative.

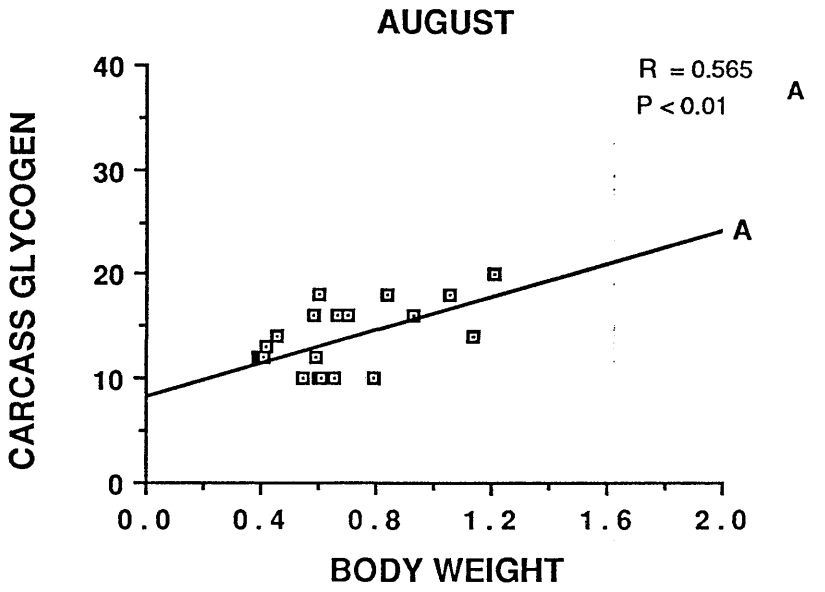
The adult fish caught in August had failed to breed and the relationship between their carcass glycogen and body weight was significant and positive. Again, this supports the suggestion that the reversal of this relationship discussed in the previous paragraph, was the result of breeding-induced depletion in large fish. This relationship remained the same during September and October but disappeared during November and December when carcass glycogen levels dropped to almost undetectable levels.

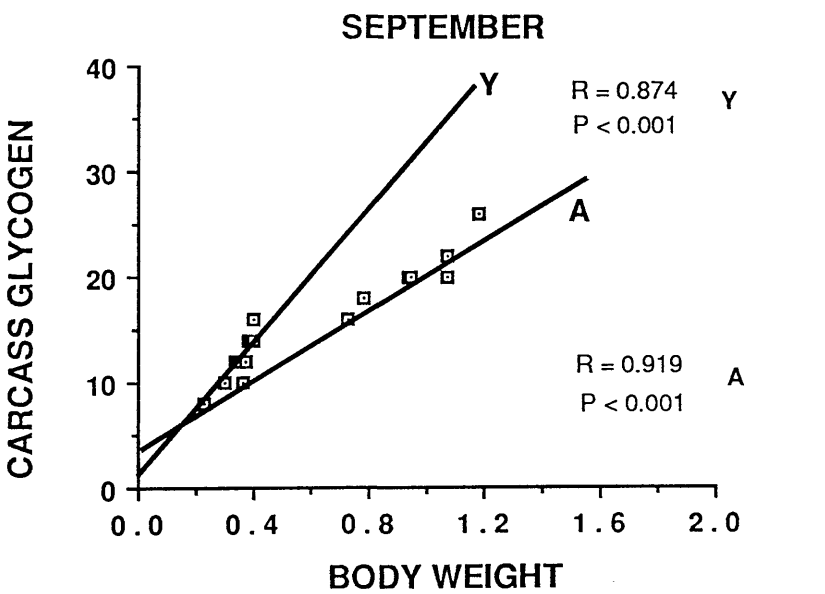
FIGURE 3.6 Scatter plots of carcass glycogen against body weight for each month over a period of one year. Glycogen values are given in μ mol g^{-1} d.w and body weight in gms.

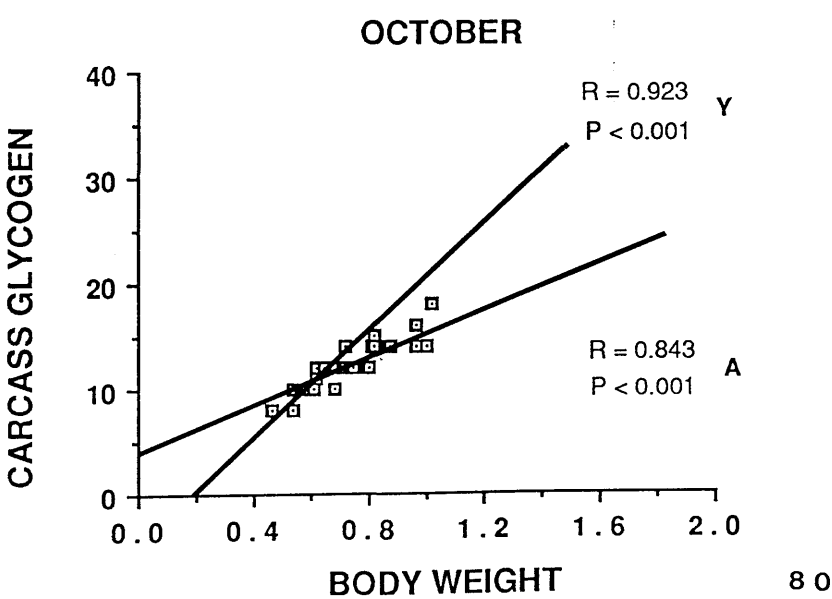
A = non-breeding adult males

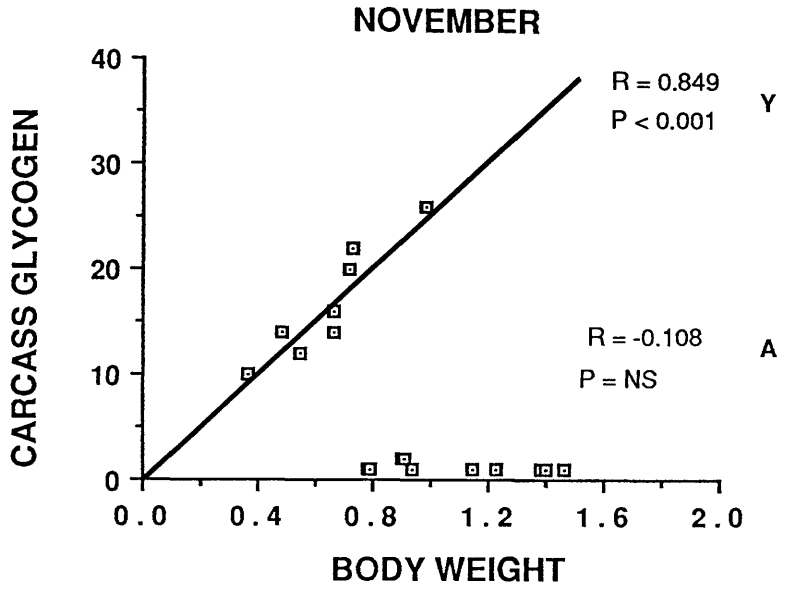
Y = young of the year

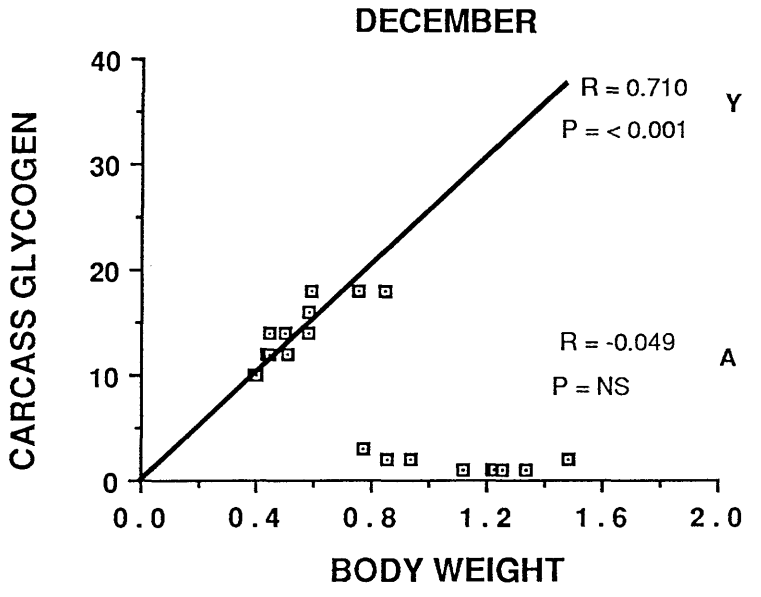
A′= breeding adult males

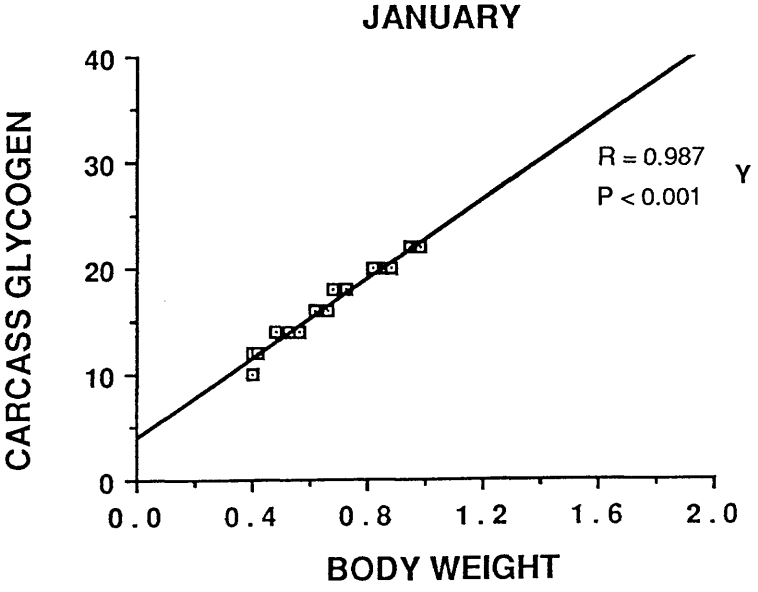

AUGUST
CARCASS GLYCOGEN
BODY WEIGHT
R = 0.565
P < 0.01
A
A
0
10
20
30
40
0.0
0.4
0.8
1.2
1.6
2.0

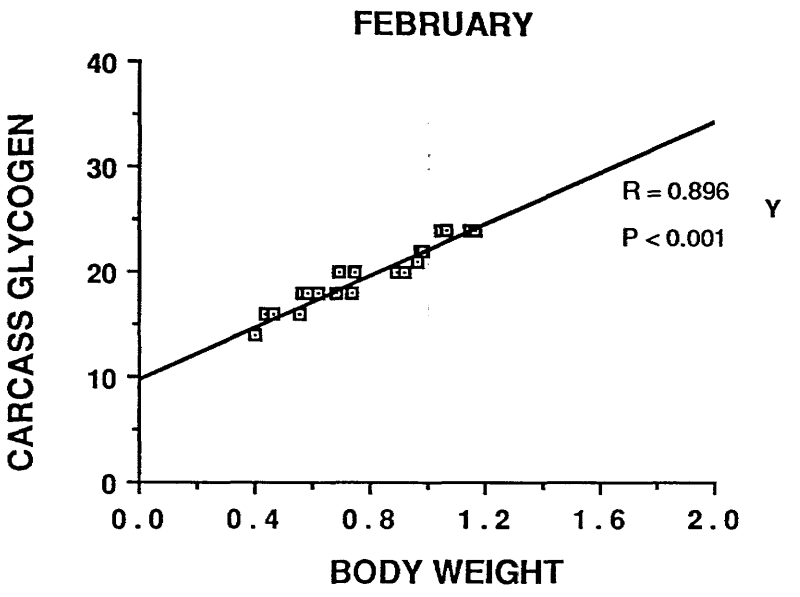

SEPTEMBER
CARCASS GLYCOGEN
BODY WEIGHT
Y
R = 0.874
P < 0.001
Y
A
R = 0.919
P < 0.001
A
0
10
20
30
40
0.0
0.4
0.8
1.2
1.6
2.0

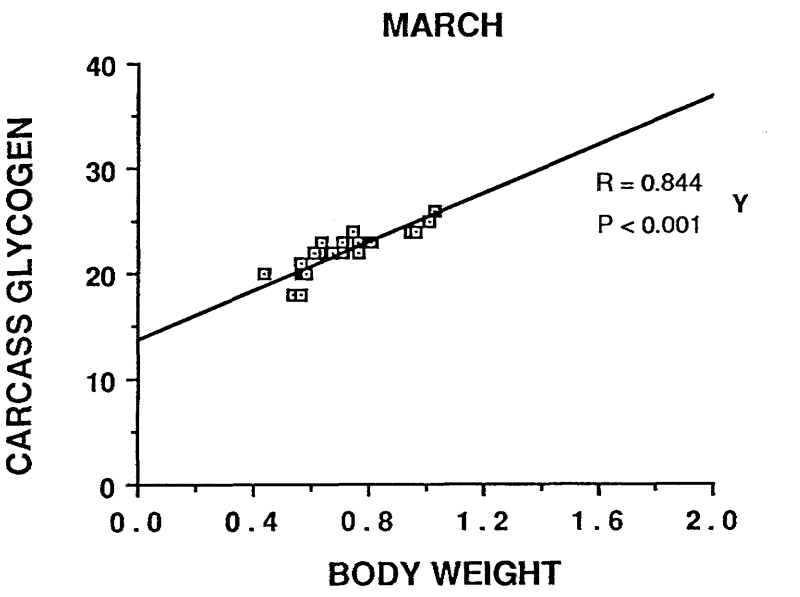

OCTOBER
CARCASS GLYCOGEN
BODY WEIGHT
R = 0.923
P < 0.001
Y
R = 0.843
P < 0.001
A
0
10
20
30
40
0.0
0.4
0.8
1.2
1.6
2.0

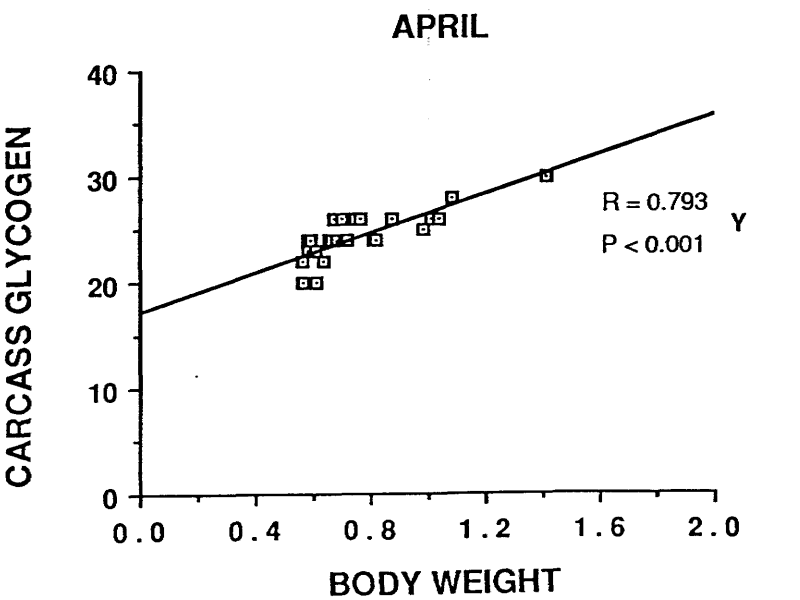

NOVEMBER
CARCASS GLYCOGEN
40
30
20
10
0
R = 0.849
P < 0.001
Y
R = -0.108
P = NS
A
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT

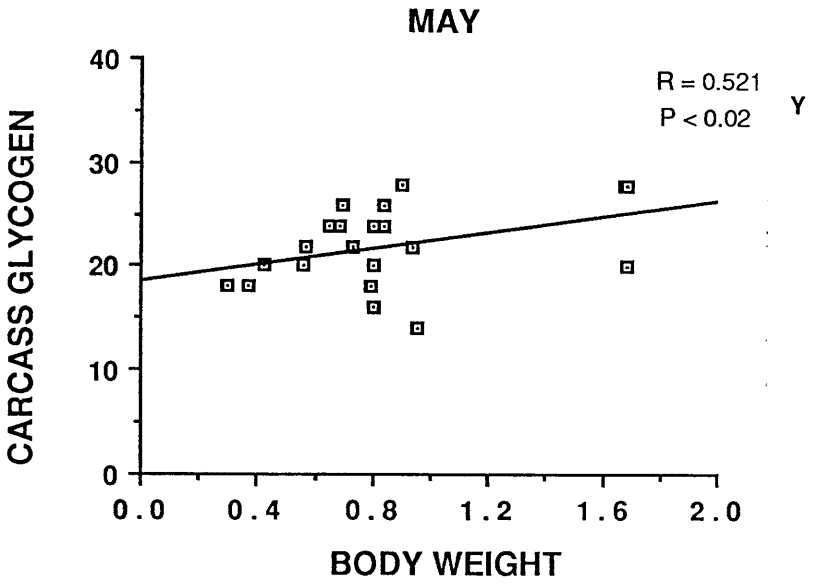

DECEMBER
CARCASS GLYCOGEN
40
30
20
10
0
R = 0.710
P = < 0.001
Y
R = -0.049
P = NS
A
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT

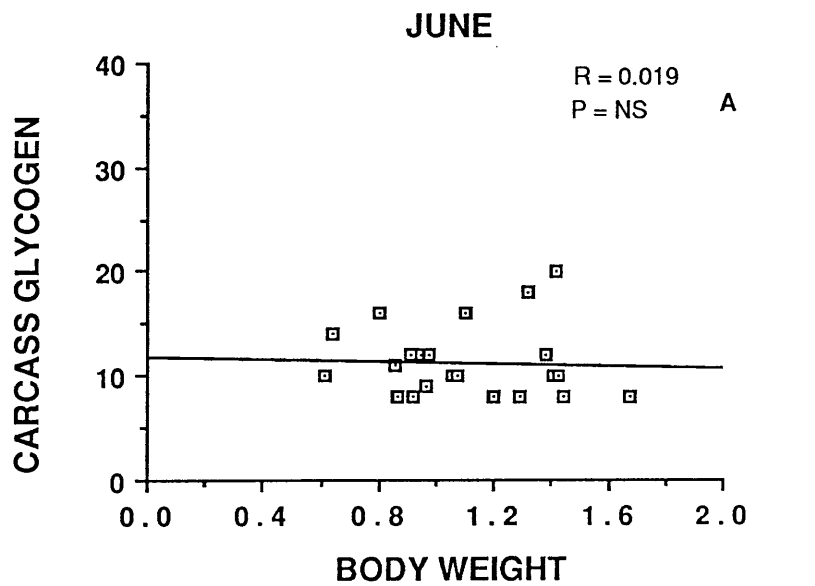

JANUARY
CARCASS GLYCOGEN
40
30
20
10
0
R = 0.987
P < 0.001
Y
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT

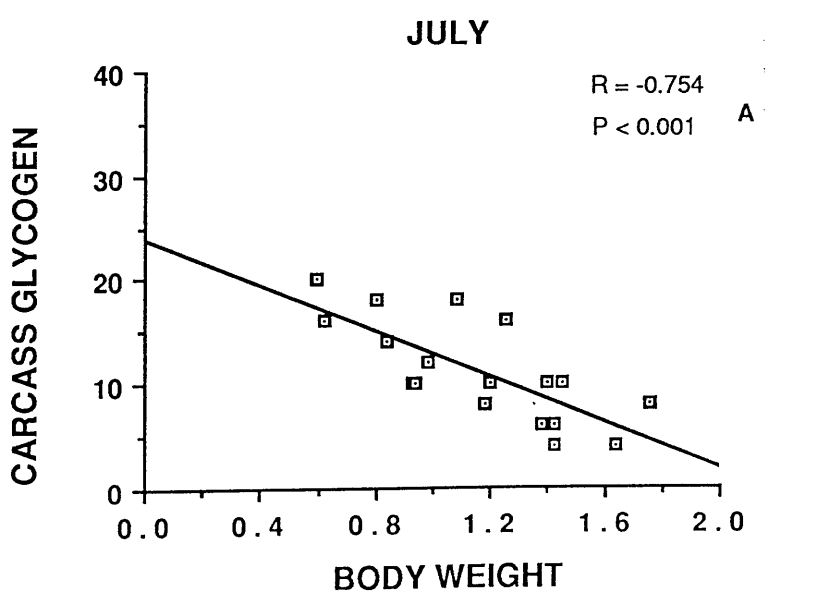

FEBRUARY
CARCASS GLYCOGEN
40
30
20
10
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT
R = 0.896
P < 0.001
Y


MARCH
CARCASS GLYCOGEN
40
30
20
10
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT
R = 0.844
P < 0.001
Y


APRIL
CARCASS GLYCOGEN
40
30
20
10
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT
R = 0.793
P < 0.001
Y


MAY
R = 0.521
P < 0.02
Y
CARCASS GLYCOGEN
40
30
20
10
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT


JUNE
R = 0.019
P = NS
A
CARCASS GLYCOGEN
40
30
20
10
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT


JULY
R = -0.754
P < 0.001
A
CARCASS GLYCOGEN
40
30
20
10
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT

3.8 DISCUSSION

The level of energy reserves in the individual fish can be regarded as an indication of its physiological condition and nutritional state (Love, 1970). The results indicate that the glycogen reserves in all the body compartments of the male stickleback change with the seasons. The general pattern of these changes is similar in the liver, gonad and carcass.

Young of the year start with low glycogen levels in August followed by a sharp increase in glycogen reserves by the end of autumn, which level off during the winter months of November to January. The glycogen stores of the young fish are fairly stable throughout their first winter though there is a slight decrease in November. In Autumn, the supply of food to the young of the year presumably exceeds the rate of utilization and the surplus energy is stored as glycogen reserves. This results in a sharp increase in glycogen stores of the young of the year by the end of Autumn. On the other hand, during winter months intake of food may become equal to or less than maintenance requirements of the young fish (for detail see chapter 1). Since the glycogen reserves of the young of the year are fairly stable throughout the winter, presumably food was available to meet maintenance requirements of their small bodies. During spring (February to April) just before the breeding season there is another sharp rise in the glycogen reserves. This may be due to the abundant food supply available in spring and/or the increase in appetite with increased temperature. This is followed by a steep fall from May and

by July the glycogen reserves are virtually depleted. Field observations reveal that many males die off at the end of the breeding season. Depletion of glycogen stores (following reproductive activity) in conjunction with increased body demands as a result of higher basal metabolism during the breeding season, may be the cause of their mortality.

The life span of the Scottish populations of male sticklebacks is just over a year (see chapter 1). Most of the male sticklebacks which spawn die off and the males which fail to breed during the summer survive only up to their second winter. The adults sampled from August onwards are non-breeders. They have relatively high glycogen reserves in August compared to the newly hatched fish. The glycogen stores of these adult fish show a slight increase in late summer and then drop to drastically low levels between September to December. These fish did not survive beyond their second winter. The relatively large bodies of the adult non-breeder males compared to the small bodies of the young of the year may be energetically more expensive to maintain. During the winter months, the mean level of food availability is presumably below maintenance requirements of the adult fish. As such, they mobilize the glycogen reserves of the body as fuel for survival. Severe depletion of energy stores coupled with insufficient food supply during the harsh winter months may be the reason for the mortality of adult non-breeder male sticklebacks.

Generally, until the start of the breeding season (viz. May,) the larger fish have higher glycogen reserves. This may be because

the larger fish are more efficient at competing for food and make wise foraging decisions or because larger fish are older (which were hatched earlier in the previous breeding season) and had the richest feeding period for their early growth (Ibrahim, 1988). From the begining of the breeding season this relationship gradually reverses, so that the larger fish have lower glycogen reserves. This may be because the larger fish are very active during the breeding season and their large bodies may be energetically more expensive to maintain hence the glycogen reserves are depleted.

The glycogen reserves accumulated during spring are mobilized during the summer, perhaps for reproductive activities. Liver glycogen decreases by 98 percent between April and July in the male stickleback. As the body musculature of the stickleback lacks red muscle fibre, and consists only white muscle (see chapter 1), glycogen is the chief fuel for muscle contraction (Love, 1970). The carcass glycogen stores are depleted by 67 percent from April to July, whereas the depletion of glycogen stores from the gonad is 40 percent for the same period. Thus a significant decrease in glycogen level occurs during the breeding season.

In sticklebacks from the River Kelvin, spermatogenesis begin in late summer and continues through the autumn. Spermatocytes are common in the period between July to December when the young of the year are recruited into the population but are at lower levels for the rest of the year. Spermatids are most abundant between January to June as the males reach maturity and came into breeding condition. In this period

a steep and a sustained increase in gonad glycogen reserve is registered. Spermatozoa were abundant between April and August (Ukegbu, 1986). Thus the period of rapid decrease in gonad glycogen level corresponds to the period when spermatozoa were most abundant.

The maximum glycogen stores in a Latvian population of male and female stickleback were found from August to December when the post-spawned fish were regaining their energy reserves and the minimum was found during the spawning period of May to July (Immers, 1953). It appears that the Latvian populations of stickleback have a longer life span as there is a period of regeneration after the breeding season. The total glycogen reserves of the Latvian populations of stickleback were estimated by using the liver, gonad and skin (which was removed from the body proper) but the carcass was not used for the glycogen assays (for details see chapter 1). Immers found that the principal glycogen stores were in the liver in the male stickleback (42.5 to 89% of the total glycogen). The glycogen stores of the testes never exceeded 35% of the total glycogen, whereas in the female stickleback the largest glycogen reserves were found in the ovaries representing about 20 to 73% of the total glycogen .

The results of the present study differ from those of Immers (1953) in the following respects:

(i) The maximum glycogen reserves were found prior to the breeding season (March to May) in the River Kelvin population of male sticklebacks but in the Latvian population of sticklebacks, the maximum glycogen stores were found in the regenerating post-spawned

adult fish (August to December). The present study population of three-spined sticklebacks from the River Kelvin, has a maximum life span of one year, whereas the Latvian three-spined sticklebacks were not annual.

(ii) Immers found that the major glycogen stores were in the liver in the male stickleback, which constituted 42 to 89% of the total glycogen but he had not used the carcass for the glycogen assays. In the River Kelvin population of sticklebacks (prior to the breeding season when glycogen reserves were at their peak) the liver constituted 48% of the total glycogen reserves, whereas the carcass had 50% of the total glycogen reserves.

(iii) Immers found that the glycogen stores of the testes sometimes reached 35% of the total glycogen, whereas in the present study the maximum glycogen store in the testes was 2 to 3% of the total glycogen reserves. The higher percentage of glycogen stores in the testes calculated by Immers reflects the exclusion of the carcass from glycogen assays. However, in both studies, the minimum glycogen stores were found during the breeding season.

The study of Wootton et al.,(1978) (see chapter 1) confirms that the breeding season was a period of severe depletion of glycogen reserves from the liver and carcass in the female stickleback whereas the ovaries were not affected. The present study shows that in the male stickleback minimum depletion of glycogen reserves occurred in the testes compared to the liver and carcass during the winter and the

breeding season. This compares fairly well with the above cited work.

3.9 CONCLUSIONS

The work described in this chapter gives information on the annual changes in glycogen reserves of the different body compartments in male three-spined sticklebacks. Glycogen reserves accumulated prior to the breeding season were mobilized during the breeding season. Male sticklebacks have drastically reduced energy reserves as a result of breeding activities. Mass mortality of spawned males occurred soon after the breeding season. Glycogen stores in the liver, gonad and carcass were positively correlated outside the breeding season but became either negatively correlated (in June) or weakly positively correlated (in July) due to depletion of glycogen reserves. Positive correlations between body weights and glycogen reserves during the non-breeding season were reversed to negative correlations with the onset of the breeding season. This change in the relationship between body weight and glycogen reserves was due to depletion of glycogen stores in the large fish, presumably as a result of breeding activities.

CHAPTER 4

SEASONAL VARIATION IN LIPID RESERVES

CHAPTER 4. SEASONAL VARIATION IN LIPID RESERVES

4.1 INTRODUCTION

In mammals, the two major energy reserves are glycogen and lipids. The relative importance of these two biochemical components as sources of energy at any instant depends upon the interaction of many complex nutritional, metabolic and hormonal factors. In terms of the total energy reserves of the body, lipid is of greater quantitative importance than glycogen. In mammals, on a weight for weight basis, triglycerides are able to supply by oxidation about two and a half times more ATP than carbohydrates (Bartley et al., 1968). For example, the average amount of energy made avilable by oxidation of carbohydrate is 17.57 KJ per gm and in the case of protein it is 23.43 KJ per gm, whereas lipids supply 38.91 KJ per gm.

Lipids can be either simple or complex. Chemically, the simple lipids are esters of fatty acids with alcohols; for example fats and oils are esters of fatty acids and glycerol whereas waxes are esters of fatty acids with long chain aliphatic alcohols or cyclic alcohols. The complex lipids include the phospholipids, glycolipids, sulpholipids and lipoproteins. The simple lipids, such as fats and oils represent the chief form in which excess nutrients are stored in the animal body. Of these the neutral fats viz. triglycerides, are important because as much as 90% of the lipid stores in the adipose tissue of the mammalian body is neutral fat and represents a concentrated form of energy stored until required for metabolic purposes. Since the caloric value of fat is more than twice that of an

equivalent weight of carbohydrate or protein (see above) fat is a more efficient energy reserve. In addition to its function as stored energy source, fat serves as an insulator and provides physical support to internal organs. The fact that the swimbladder volume varies inversely with the lipid stores as in the Atlantic herring (Clupea harengus) suggests that lipids also play a role in the buoyancy mechanism in fish (Brawn, 1969 a). Phospholipids, glycolipids, sulpholipids and lipoproteins are present in the body primarily as essential structural elements of cell walls and other structures, rather than as stored energy. The complex lipids are relatively unavailable as an energy source. For example, in mammals which had died of starvation there is little decrease in the lipid content of the structural elements of the cells (Oser, 1965).

Fish lipids are characteristically rich in polyunsaturated fatty acids (Sargent, 1976a). These polyunsaturated fatty acids with five or six double bonds originate from the planktonic organisms which form part of the food chain of fishes. For example, when the triglycerides and wax esters of the copepods are compared with the lipid composition of Capelin (Mallotus villosus), mackerel (Scomber scombrus) and Atlantic herring (Clupea harengus) they are found to be very similar (Ackman, 1980 as reviewed by Gunstone et al. 1986). The ability of fish mitochondria to oxidise both very long chain monoethylenic fatty acids and polyunsaturated fatty acids indicate that fish mitochondria are well adapted to utilise the spectrum of fatty acids present in natural fish food (Osmundsen and Bremer, 1978 as reviewed by Henderson and Sargent, 1985).

The concentration of lipids vary enormously in different parts of the body of fish (Love, 1970). In fatty fish, there is usually a high concentration immediately under the skin. In fast swimming fish like the tunnies (Thunnus sp) and marlins (Makaira sp) oily tissues are sandwiched between the surface layers of connective tissue. The belly wall of fatty fish like the Coho salmon (Oncorhynchus kisutch) shows the highest concentration of lipids. The rainbow trout (Salmo gairdneri) possesses definite perivisceral adipose tissue which is composed of fat cells or adipocytes. There is an inverse relationship between the lipid and water contents in the muscle tissue of fatty fish such as the Atlantic herring (Clupea harengus). In non-fatty fish such as Atlantic cod (Gadus morhua), where the muscle lipid concentration is only about 0.5%, the liver is used to store large quantities of lipid (15-65% liver weight) which is later utilized during gonad maturation and spawning (Jangaard et.al., 1967). In herrings (Clupea sp) and sturgeons (Acipenser sturio) the bulk of fat is concentrated in the muscle, while in perch (Perca flavescens) fat is concentrated in the body cavity.

The fat content in the body of the fish changes depending on the time of the year, environmental conditions, stage of maturity of the gonads, state of nutrition and age. The selectivity of the fish in utilizing its lipid stores is interesting, although information is scanty. Atlantic herring preferentially use the highly saturated lipids when stores are being utilized (Lovern, 1938). In the muscle of the Rainbow trout (Salmo gairdneri) the proportion of highly unsaturated fatty acids increases during starvation, presumably

indicating utilization of the saturated acids (Kaneko et al., 1967 as reviewed by Love, 1970).

Lipid is not the major energy store in all fish. For example in Northern pike (Esox lucius) endogenous lipid is not considered as an important source of energy because of its low concentration in the muscle, instead endogenous protein is considered important as an energy substrate in the metabolism. However, if percentage composition data on muscle of this species is considered, the trend is for lipid to be utilized preferentially during the spawning season. In males and females of Northern pike the decrease in muscle protein concentration was 5.7% compared with a decrease of 16.7% for muscle lipid (Medford and Mackay, 1978).

4.2 LIPID METABOLISM

Fats and oils arise from ingested lipid and from the metabolism of carbohydrates and proteins. The liver is the organ in which most lipid metabolism occurs.

The triglycerides of the adipose tissue are continuously hydrolysed by the adrenaline-sensitive lipase to yield fatty acids and glycerol. If there is an adequate supply of glucose, this hydrolysis can be matched by re-synthesis because glucose supplies the L-alpha - glycerophosphate needed for triglyceride formation.

In the liver long chain fatty acids can be metabolised via three main pathways :-

i) Oxidation to CO_2, H_2O and energy.

On hydrolysis, neutral fats yield glycerol and fatty acids. Glycerol can be metabolised via conversion to glycerol phosphate and dihydroxyacetone phosphate, which enters the glycolytic pathway and may either be converted to glycogen or oxidized to carbon dioxide water and energy (see chapter 3).

ii) Synthesis of triglyceride.

By reverse process, the glycerol portion of lipid molecules may be formed from carbohydrate.

iii) Conversion to Acetyl coenzyme (COA).

The major pathway whereby fatty acids are oxidized is by the process known as Beta- oxidation. By this process the fatty acid chain is oxidized at the carbon atom β to the carboxyl group and is subsequently split into acetyl coenzyme A which mixes with the acetyl coenzyme A pool derived from glycogen and aminoacid metabolism.

These pathways are depicted in Figure 4.1 which indicates the interrelationship between the metabolism of glycogen, lipids and proteins, and shows the important role of acetyl COA as a metabolic intermediate (Oser, 1965).

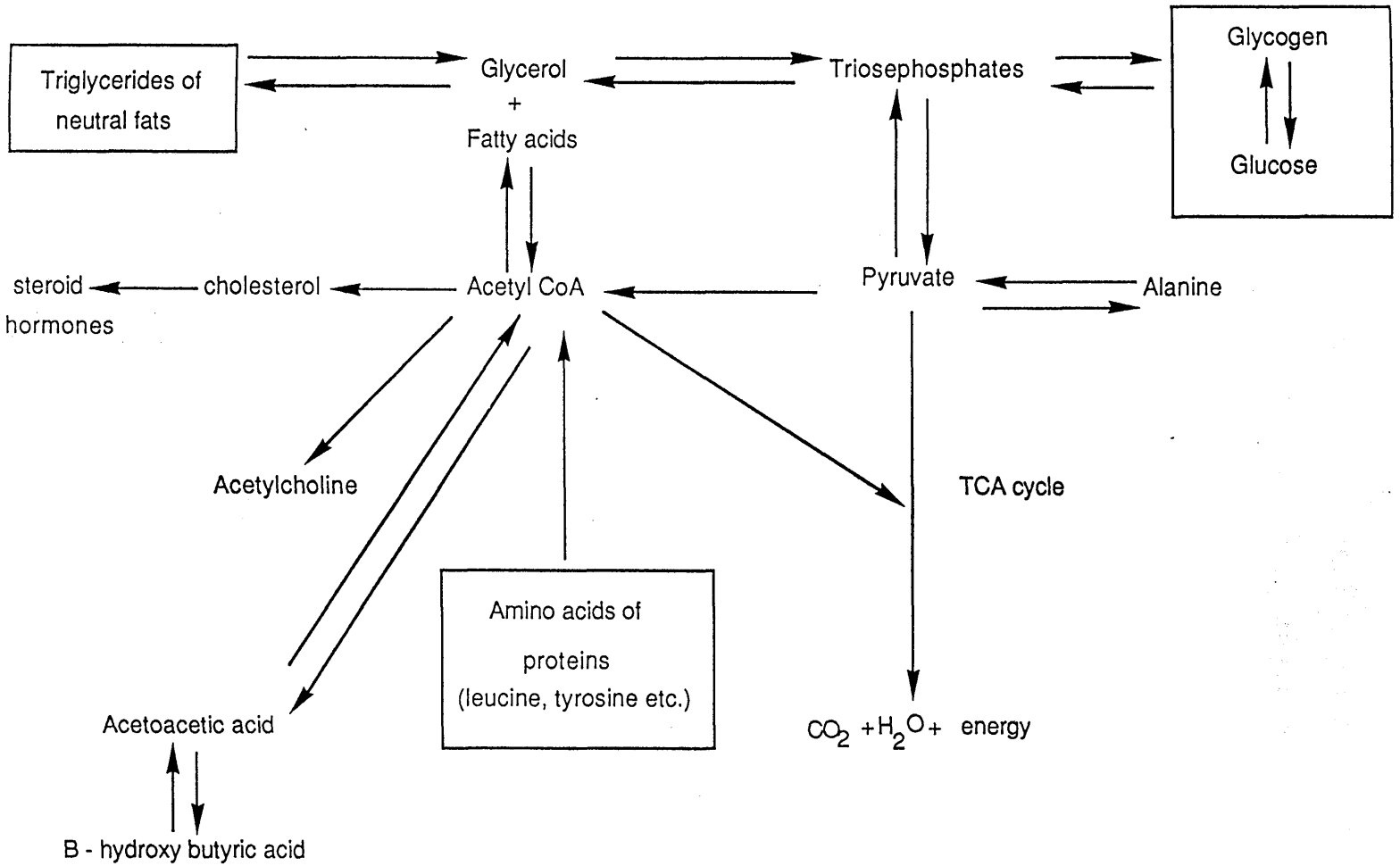


FIG. 4.1 THE INTERRELATIONSHIP BETWEEN METABOLISM OF GLYCOGEN, LIPID AND PROTEIN (AFTER OSER, 1965)

4.3 METHODS OF LIPID DETERMINATION

Lipids are characterized by insolubility in water and solubility in organic fat solvents such as acetone, chloroform, ether, benzene and boiling alcohol. Many investigators were discouraged from the study of lipids because of their physical properties (lack of water solubility and crystalization difficulties) are unfavourable for routine methods of analysis and purification. As a consequence, research in carbohydrate and protein chemistry advanced at a greater rate than studies with lipids. In recent years, however, there has been a reawakening of interest in lipid biochemistry.

4.3.1 Quantitative determination of fish lipids

Extraction techniques using organic solvents for a minimum period of 24 hours, can be used for quantitative determination of lipids. A simple method for the extraction of total lipids is by homogenizing the tissue with a 2:1 (by volume) chloroform - methanol mixture (Folch, Lees and Stanley, 1957). Lipid values have been obtained by many workers adapting this basic technique (Lambert and Dehnel, 1974; Medford and Mackay, 1978; Wootton et al., 1978; Gill and Weatherley, 1984). Lipids can also be determined by hexane extraction on whole fish (Gardiner and Geddes, 1980; Deegan, 1986).

4.3.2 Qualitative determination of lipids

Lipid composition may vary not only between species but also between male and female, juvenile and adult of the same species. The

polarity of lipid classes range from non-polar (eg. hydrocarbons) to highly polar (eg. fatty acids). Efficient separation can be achieved by matching solvent polarity to that of the lipid classes. A simple way to analyse lipids qualitatively is to use thin layer chromatography (TLC). In this technique (Stahl, 1965) a lipid solution is applied to a uniformly thick layer of adsorbent such as silica gel spread on a glass plate. An organic solvent system is then used to develop the TLC plate which separates the lipid into its classes of compounds. These classes form spots on the plate and can be located by iodine vapour, which turns the spots brown.

4.4. SEASONAL VARIATION IN LIPID RESERVES IN STICKLEBACK

As discussed in the introduction, the lipid reserves vary during the different periods of the life cycle of many animals. Quantitative lipid estimations had been carried out on female stickleback from two Welsh populations (Wootton et al., 1978). As mentioned in chapter 3, though the sample was collected on a monthly basis, the material was pooled to estimate the seasonal values for lipid reserves. It has been found that the carcass of the female stickleback had minimum lipid stores during the winter and the breeding season. The lipid store of the liver was also at a minimum in the breeding season, but that of the ovaries was relatively constant (for values see Table 1.2).

So far no systematic sampling had been carried out on male stickleback to investigate their lipid reserves. This is of considerable interest as territorial defense, courtship and parental care in male stickleback demand expenditure of energy; hence good body

condition of the male is important for successful breeding. One of the aims of this study is to examine the seasonal variation in lipid reserves in males of a Scottish population of three-spined stickleback using samples collected systematically on a monthly basis over one complete year.

4.5 MATERIAL AND METHODS

Male sticklebacks were collected and processed as described in chapter 2. Dr R.Y.Thompson of the Biochemistry Department of Glasgow University, gave valuable advice and participated in useful discussions throughout this project. Dr K.H.Lockey of the Department of Zoology, University of Glasgow, gave technical advice and useful suggestions for analysis of the lipid composition of the male stickleback.

4.5.1 Lipid extraction

The method used to extract the lipid from male stickleback was based on the chloroform-methanol mixture technique (Folch, Lees and Stanley, 1957). The procedure is depicted below in the form of a flow chart.

Fish killed in liquid nitrogen at -196 ^{o} C and transferred to a deep freezer of -70 ^{o} C

↓

Wet weight and standard length recorded and fish dissected on melting ice. Wet weights of body components recorded and kept at -30 ^{o} C for one hour. Fish components dehydrated by freeze-drying for 24 hours in Edwards EF03 freeze-drier.

↓

Dry weight of tissues recorded

↓

The dried tissue was ground into powder and dried again in a thermostat at 60 ^{o} C for 14 hours and the final dry weight recorded

↓

Known weight of powdered dry tissue was introduced into transparent seamless dialysis tubes and the margins sealed.

↓

The dialysis tubes were serially numbered and introduced into the extractor of the Soxhlet extraction apparatus. The conical flask of the Soxhlet apparatus was filled to two thirds with 2:1 chloroform-methanol mixture (v/v). The entire assembly was housed in a fume cupboard.

↓

The chloroform - methanol mixture was gently heated by the electric heater for 32 hours.

↓

The dialysis tubes with powdered tissue were dried at 60 ^{o} C for 4 hours in a thermostat and the final weights of the tissues were recorded.

4.5.2 Fractionation of lipids

In order to obtain a preliminary identification of the different classes of lipids in the male three-spined sticklebacks, the lipid extracted in chloroform and methanol (from 20 mature whole male sticklebacks prior to the breeding season) was fractionated as follows (Stahl, 1965):

100 ml of the lipid extract in chloroform and methanol was filtered using anhydrous sodium sulphate powder to remove traces of water.

↓

Lipid extract evaporated and concentrated in a rotary film evoporator for 1 hour.

↓

The concentrated lipid extract transferred to tubes of known weight, wrapped with an aluminium foil and left overnight

↓

Lipid extract subjected to steady stream of nitrogen gas and hot air for 20 minutes to evoporate the chloroform

↓

Lipid weighed and dissolved in a known volume of chloroform

↓

Lipid solution drawn-out using capillary tube applicators and lightly applied on the silica gel surface of the TLC plate

↓

Standard solution containing aliphatic hydrocarbon,ester fatty acid, alcohol and triglyceride was applied to the TLC plate to identify sample spots

↓

The TLC plate left upright in one inch of petroleum spirit solvent in a tank (first elution) for about 30 minutes

↓

The TLC plate removed from the solvent, hot air blown to evoporate petroleum spirit

↓

The TLC plate left upright in one inch of the second solvent mixture of petroleum spirit - diethyl ether - acetic acid 70:30:1 v/v/v (second elution) in a tank for about 20 minutes

↓

The TLC plate removed and dried with hot air blower

↓

The TLC plate exposed to iodine vapour to stain the spots brown

↓

Spot positions marked and lipid classes identified.

Complete identification would involve the analysis of each lipid class by combined gas chromatography and mass spectrometry. Gas chromatography could separate each lipid class into its constutuents while mass spectrometry could provide a mass spectrum of each constituent from which identity can be deduced. However, in the present context only the different classes of the lipids (extracted from mature whole male stickleback) were identified and a general picture of the lipid composition obtained.

4.6 DATA ANALYSIS

Mean monthly levels of lipid reserves were calculated for fish of each age class viz. the young of the year and the non-breeding adult fish. The fish were assigned to their respective age class using weight as a criteria for distinguishing young of the year from adult fish. The data were checked for normal distribution and transformed when necessary. Product-moment correlation coefficients were calculated between body weight and lipid reserves of the liver, gonad

and carcass. Oneway analysis of variance (ANOVA) was used to check for a seasonal variation in lipid reserves. The relationship between body weight and lipid reserves was investigated by regression analysis for each month, considering the young of the year and non-breeding adult fish separately.

4.7 RESULTS

Table 4.1 summarizes the results of one way analysis of variance (ANOVA) on lipid reserves for the entire year. Figure 4.2 A to C shows the annual changes in liver, gonad and carcass lipid reserves. The mean values and 95% confidence intervals of the mean are plotted against the months of the year. The months have been arranged on the X-axis to depict the complete life cycle of male sticklebacks from this population.

TABLE 4.1 ONE WAY ANALYSIS OF VARIANCE BY MONTH ON LIPID RESERVES

VARIABLE	SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROBABILITY
LIVER LIPID						
	Between groups	11	549594.35	49963.12	21.80	0.0000
	Within groups	271	621004.28	2291.52	--	--
GONAD LIPID						
	Between groups	11	13802.35	1254.76	51.26	0.0000
	Within groups	271	6633.30	24.47	--	--
CARCASS LIPID						
	Between groups	11	249093.01	22644.82	26.35	0.0000
	Within groups	271	232857.98	859.25	--	--

FIGURE 4.2 ANNUAL CHANGES IN LIPID RESERVES

A) Mean and 95% confidence intervals of liver lipid

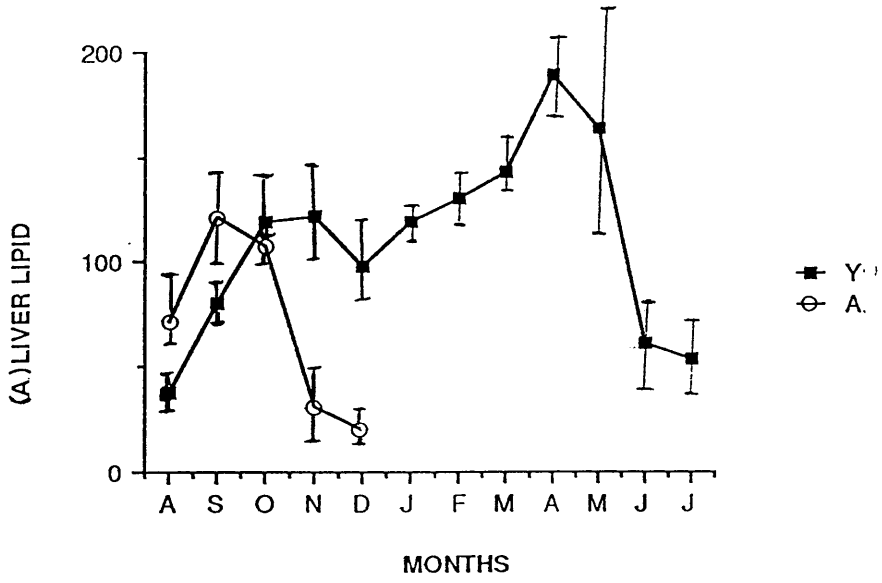
B) Mean and 95% confidence intervals of gonad lipid

C) Mean and 95% confidence intervals of carcass lipid

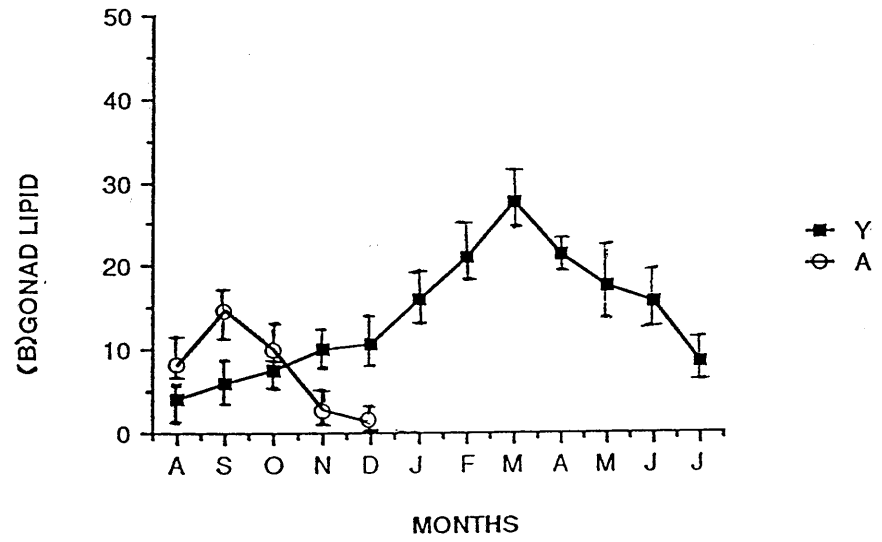
(The months are arranged on the X-axis to depict the complete life cycle of the male three-spined stickleback from the River Kelvin)

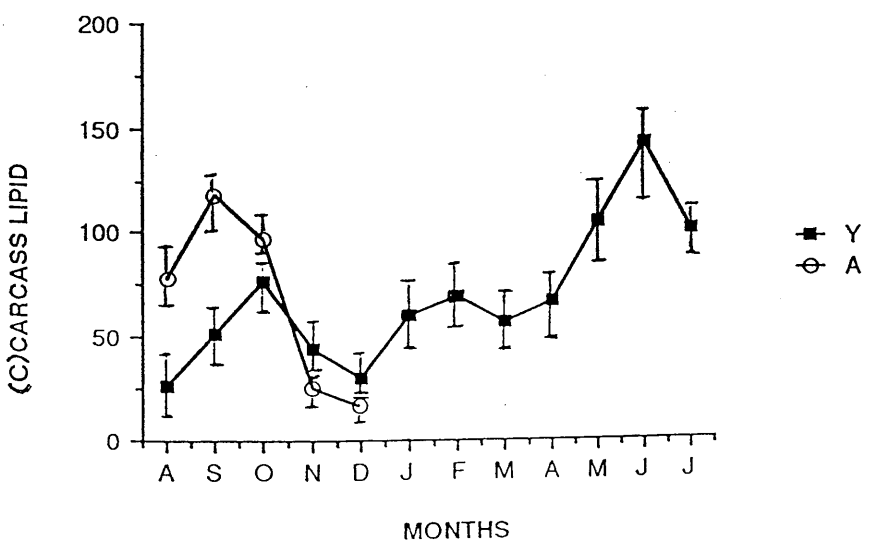
Y = Young of the year

A = Non-breeder adult



LIPID (mg g-1 d.w)





4.7.1 Annual variation in liver lipid reserves

Fig 4.2 A shows that from August to October there was a steady and marked increase in liver lipid reserves of the young of the year, but this levelled off during the winter months. The mean lipid level remained constant from October up until March, although there was a slow increase from December onwards. Between March to April of their first spring, the mean lipid levels in the young of the year increased markedly. This was followed by a dramatic fall during the breeding season. The great variability of the mean lipid level during the month of May probably reflected the lipid levels in the different categories of fish caught, as the sample consisted of fully mature males with and without breeding colouration and small immature males.

The adult male fish caught from August onwards were probably non-breeders (see chapter 2). Their mean lipid level showed an increase between August to September i.e. conditions were still favourable for accumulation of energy reserves in fish that had not expended energy on reproductive activities. From September to December there was a consistent drop in the lipid reserves of these fish and the mean lipid stores reached very low levels in December, after which no further fish of this age class were caught.

4.7.2 Annual variation in gonad lipid reserves

Fig 4.2 B shows that the mean gonad lipid level was generally low even at its peak. There was a gradual increase in the mean gonad

lipid levels from August to March in the young of the year, with a slight check in the winter. There was a gradual drop from March to July, indicating that the lipid reserves were being mobilized for gonad maturation. In the non-breeding adult males, there was a slight increase in the mean gonad lipid from August to September, followed by a decrease to very low levels in December.

4.7.3 Annual variation in carcass lipid reserves

Fig 4.2 C shows that mean carcass lipid levels were intermediate between the liver and gonad mean lipid levels. There was an increase in the mean carcass lipid level from August to October which was checked (and even fell slightly) during the winter. Then there was a more or less consistent increase up until June followed by a drop in July during the breeding season. In the non-breeding adult males caught from August onwards, there was an increase in the mean carcass lipid level up to September. From October to November there was a sharp drop in the carcass lipid level which reached low levels in December.

4.7.4 The relationship between lipid reserves in liver,gonad and carcass

Prior to the breeding season, when liver and gonad lipid reserves were at their peak they constituted 7% and 1% respectively whereas carcass lipid stores formed 92% of the total lipid reserves. Table 4.2 A to C gives product-moment correlation coefficients between lipid reserves in the different body compartments during the non-breeding season and in June and July together with levels of significance. There were highly significant positive relationships between lipid reserves in liver, gonad and carcass during the non-breeding season (viz. August to May). These relationships disappeared during the breeding season, due to depletion of lipid reserves (particularly liver and gonad lipid reserves).

TABLE 4.2 Correlation coefficients and levels of significance between lipid reserves in liver, gonad and carcass.

A - during the non-breeding season of August to May

B - during the breeding season - month of June

C - during the breeding season - month of July

Abbreviations:

R = Product-moment correlation coefficient

P = Levels of significance

*** = P <0.001

** = P <0.01

* = P <0.05

+ = P <0.1

NS = Non-significant

N = Number of samples

Table 4.2 A

LIPID

LIVER	GONAD	CARCASS	
	R = 0.593 P = ***	P = 0.628 P = ***	LIVER
N = 241		R = 0.344 P = ***	GONAD
			CARCASS

LIPID

Table 4.2 B

LIVER	GONAD	CARCASS	
	R = 0.075 P = NS	P = 0.399 P = +	LIVER
N = 22		R =-0.090 P = NS	GONAD
			CARCASS

LIPID

Table 4.2 C

LIVER	GONAD	CARCASS	
	R = 0.228 P = NS	P = 0.398 P = +	LIVER
N = 18		R = 0.407 P = +	GONAD
			CARCASS

4.7.5 The relationship between body weight and lipid reserves

4.7.5.1 Body weight and liver lipid reserves

Figure 4.3 shows the scatter plots of liver lipid against body weight for each month over a period of one year. In young of the year, there was a weak relationship between body weight and liver lipid reserves in August. In September and October there was a strong positive relationship which continued up until April. In June this relationship changed from positive to a negative one as lipid levels decreased in bigger fish as a result of breeding activities. In July, the relationship between body weight and liver lipid reserves disappeared, as the lipid levels had become very low in fish of all sizes.

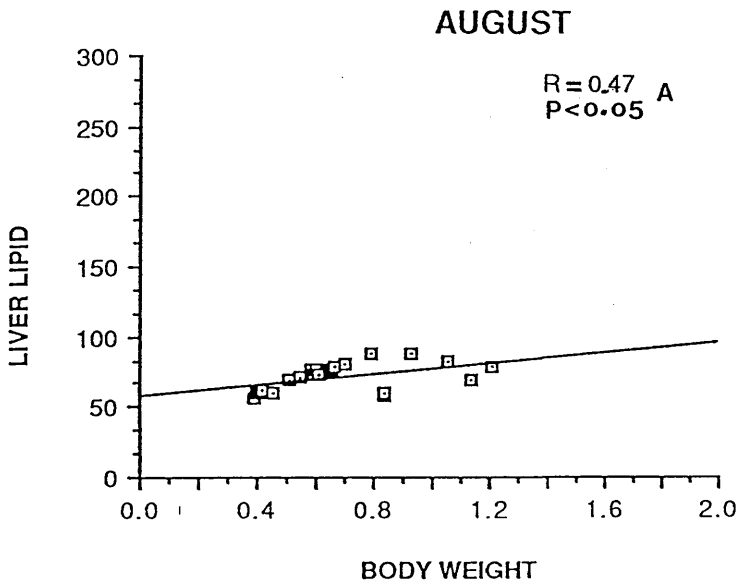
In the non-breeding adult fish caught from August onwards, the relationship between body weight and liver lipid remained positive from August to October. (This supports the above suggestion that the reversal of the relationship between body weight and energy reserves in the sexually mature males in June is the result of breeding). However, by November and December there was no relationship. This is because all adult fish regardless of size had very low liver lipid reserves in their second winter.

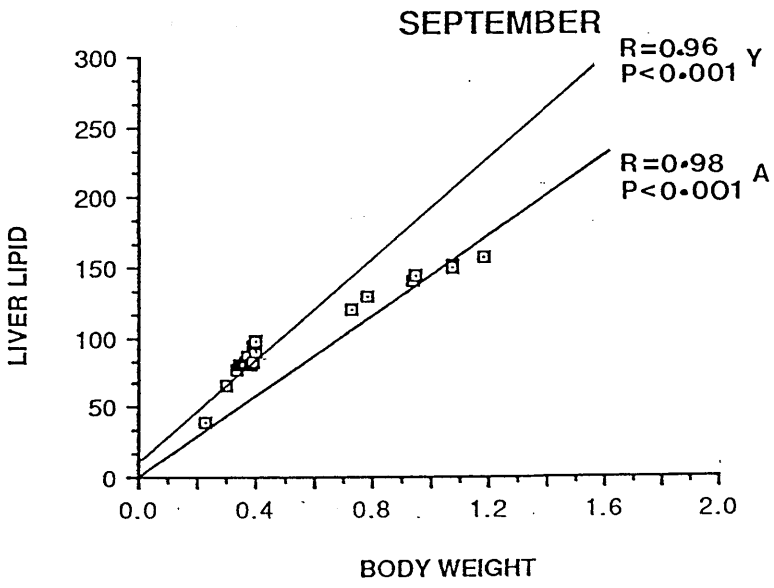
FIGURE 4.3 Scatter plots of Liver lipid against body weight for each month over a period of one year. Lipid values are given in mg g^{-1} d.w and body weight in gms.

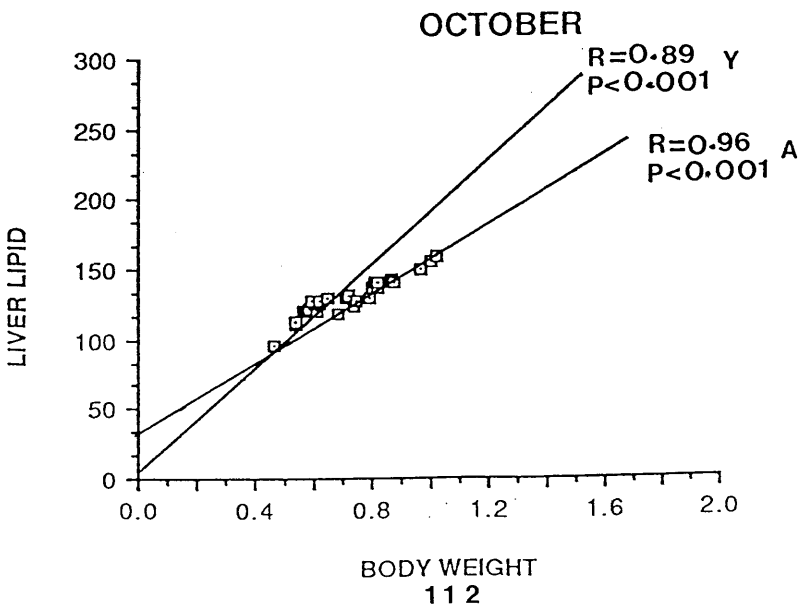
A = non-breeding adult males

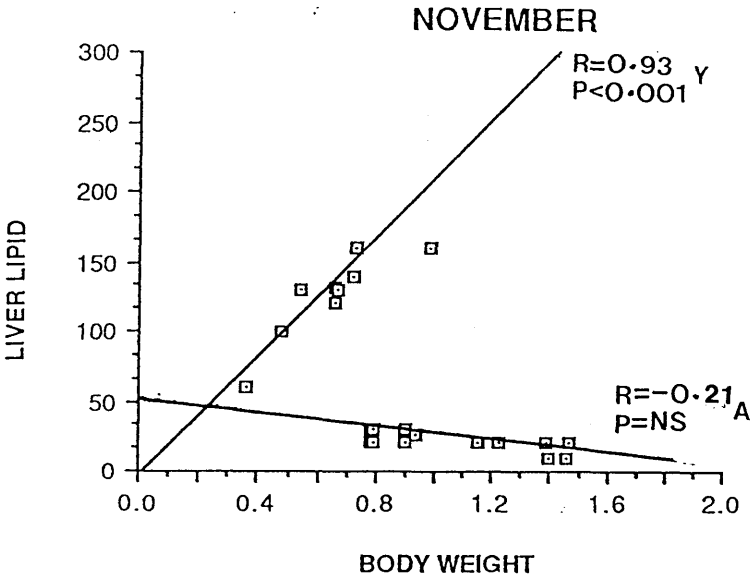
Y = young of the year

A′= breeding adult males


AUGUST
R=0.47 A
P<0.05
LIVER LIPID
300
250
200
150
100
50
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT


SEPTEMBER
R=0.96 Y
P<0.001
R=0.98 A
P<0.001
LIVER LIPID
300
250
200
150
100
50
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT


OCTOBER
R=0.89 Y
P<0.001
R=0.96 A
P<0.001
LIVER LIPID
300
250
200
150
100
50
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT


NOVEMBER
R=0·93 Y
P<0·001
R=−0·21 A
P=NS
LIVER LIPID
BODY WEIGHT
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250
200
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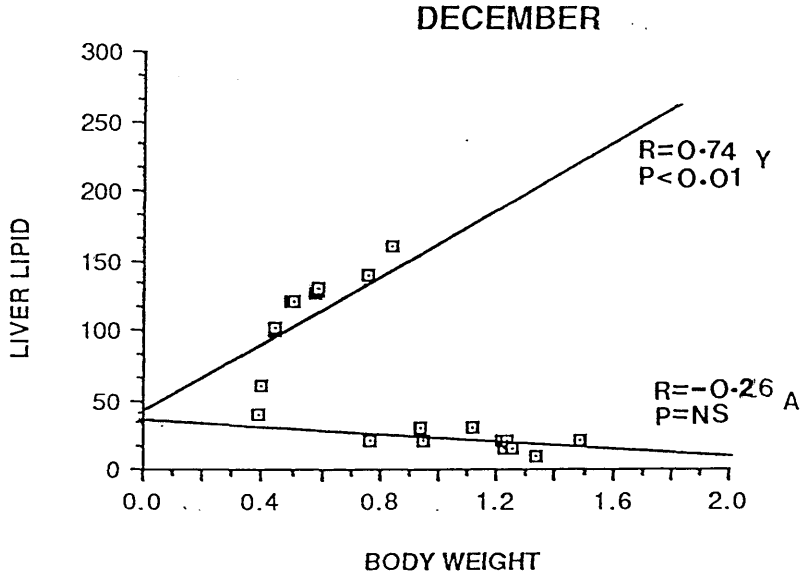
100
50
0
0.0
0.4
0.8
1.2
1.6
2.0


DECEMBER
R=0·74 Y
P<0·01
R=−0·26 A
P=NS
LIVER LIPID
BODY WEIGHT
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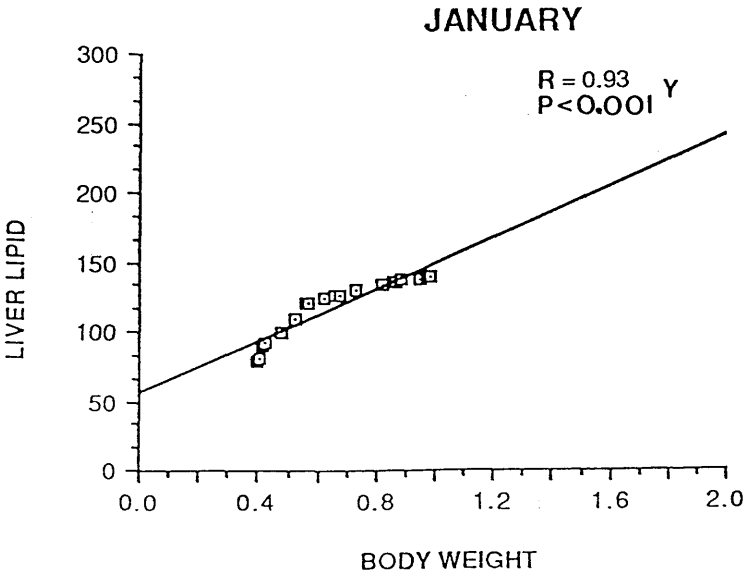
250
200
150
100
50
0
0.0
0.4
0.8
1.2
1.6
2.0


JANUARY
R = 0.93 Y
P<0.001
LIVER LIPID
BODY WEIGHT
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100
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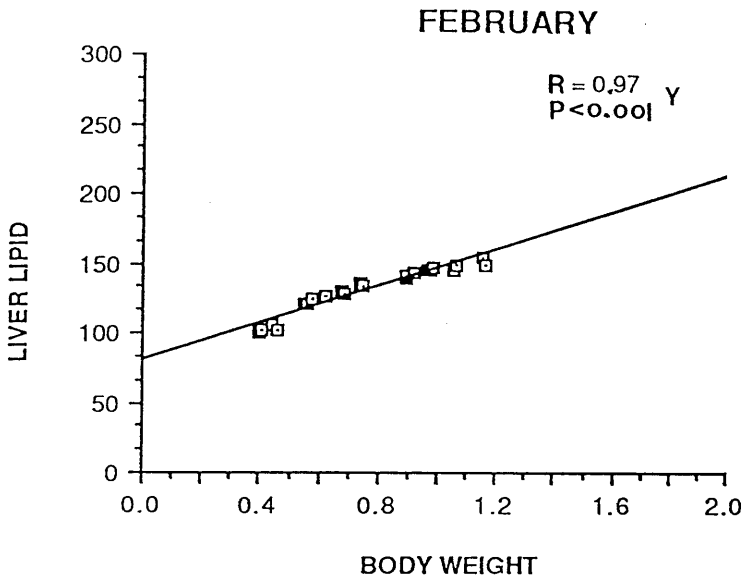
0.4
0.8
1.2
1.6
2.0


FEBRUARY
R = 0.97
P<0.001
Y
LIVER LIPID
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200
150
100
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0
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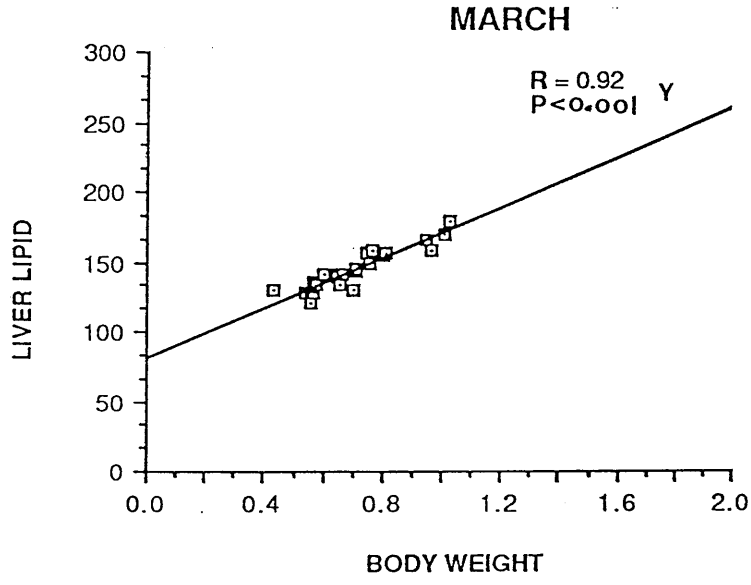
0.8
1.2
1.6
2.0
BODY WEIGHT


MARCH
R = 0.92
P<0.001
Y
LIVER LIPID
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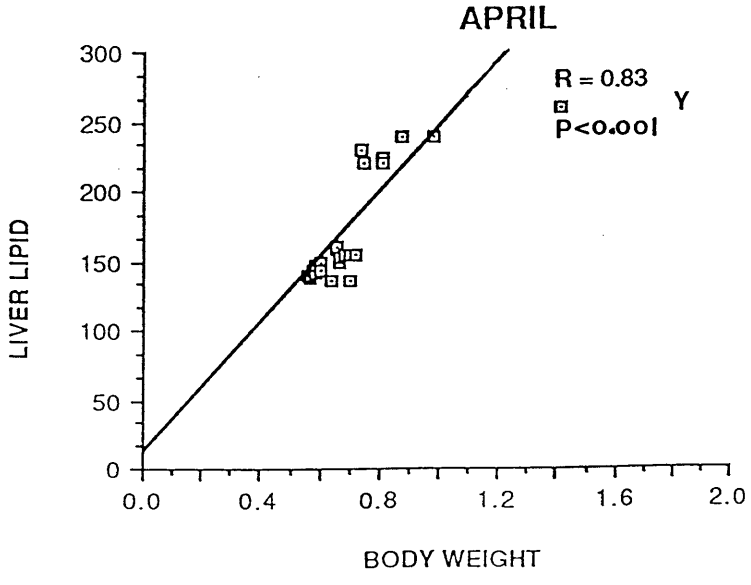
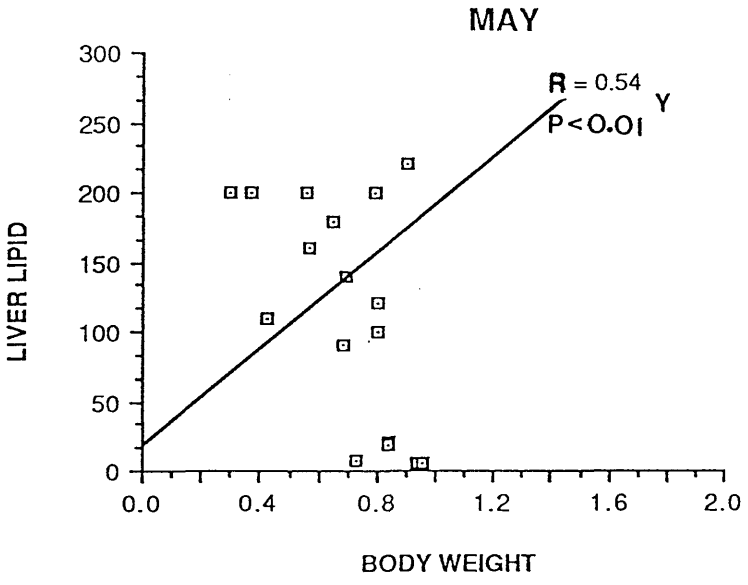
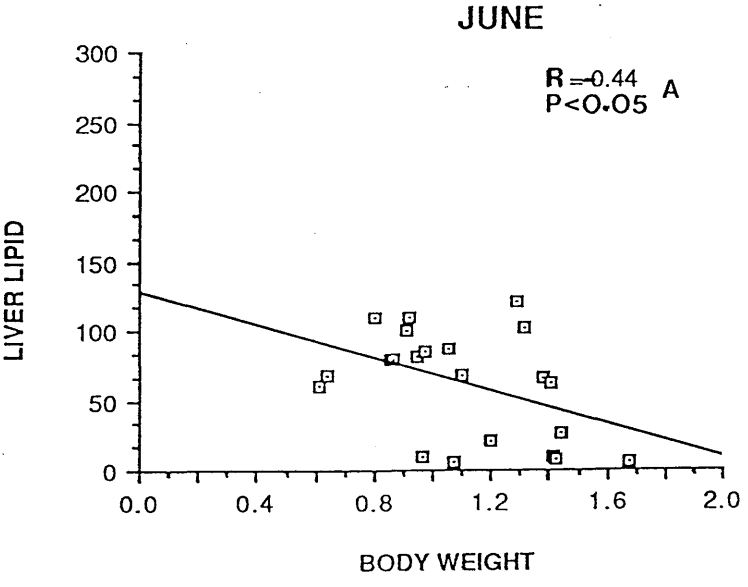
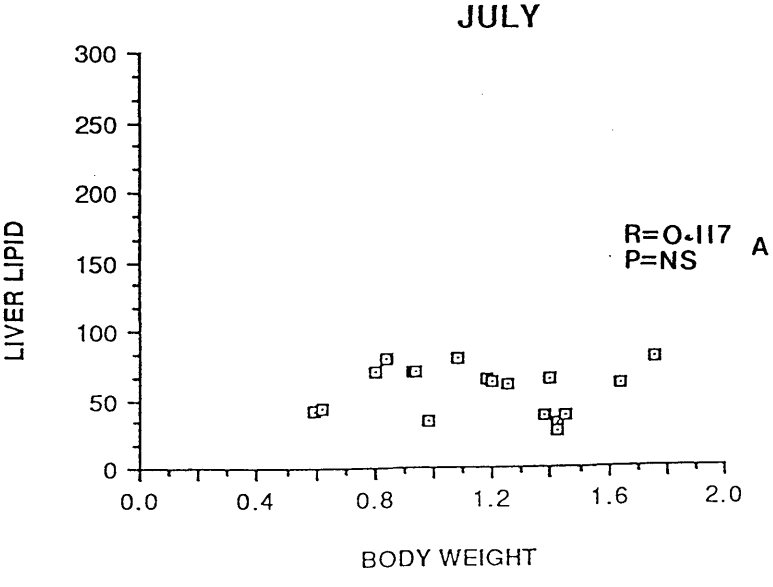
250
200
150
100
50
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT


APRIL
R = 0.83
P<0.001
Y
LIVER LIPID
300
250
200
150
100
50
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT


MAY
R = 0.54
P<0.01
Y
LIVER LIPID
300
250
200
150
100
50
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT


JUNE
R=-0.44
P<0.05
A
LIVER LIPID
300
250
200
150
100
50
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT


JULY
R=0.117
P=NS
A
LIVER LIPID
300
250
200
150
100
50
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT

4.7.5.2 Body weight and gonad lipid reserves

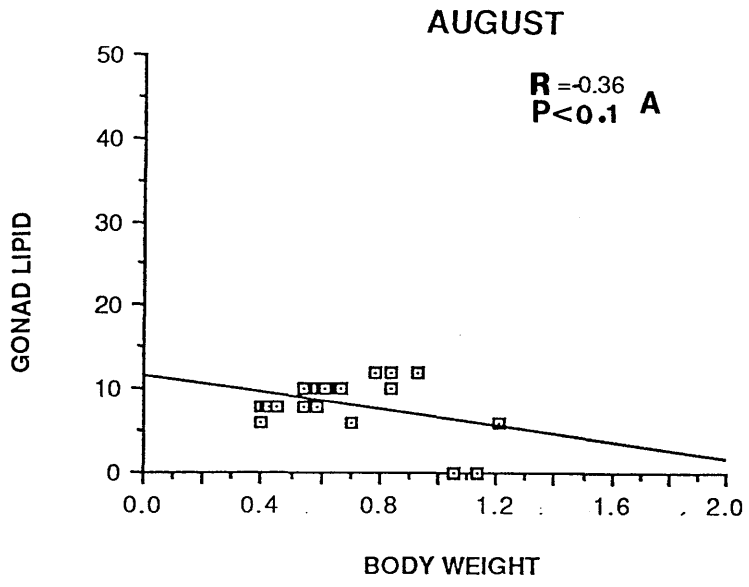
Figure 4.4 shows the scatter plots of gonad lipid against body weight for each month over a period of one year. In the young of the year there was a strong positive relationship between body weight and gonad lipid reserves from September up until March. In April the positive relationship was a weak one which became weaker still in May. In June there was a negative relationship between body weight and gonad lipid reserves as lipid levels dropped most markedly in larger fish. Probably because lipid reserves were used up for gonad maturation. This disappeared altogether in July as the lipid reserve levels became very low in all fish. In the non-breeding adult fish caught from August onwards there was no relationship between body weight and gonad lipid levels up until December, after which no further fish of this age class were encountered in the sample.

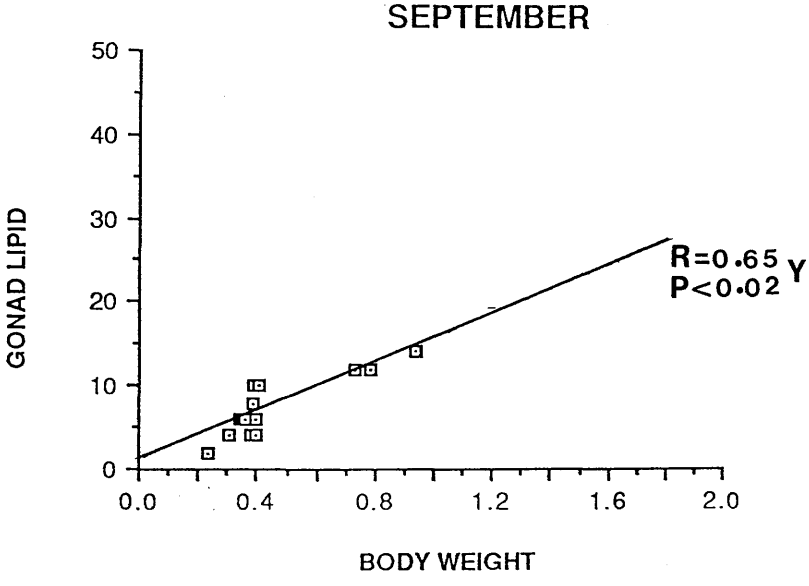
FIGURE 4.4 Scatter plots of Gonad lipid against body weight for each month over a period of one year. Lipid values are given in mg g^{-1} d.w and body weight in gms.

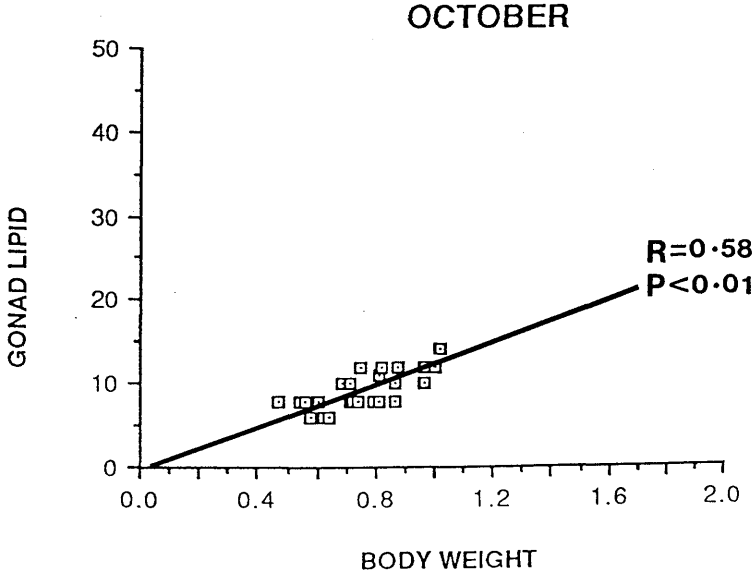
A = non-breeding adult males

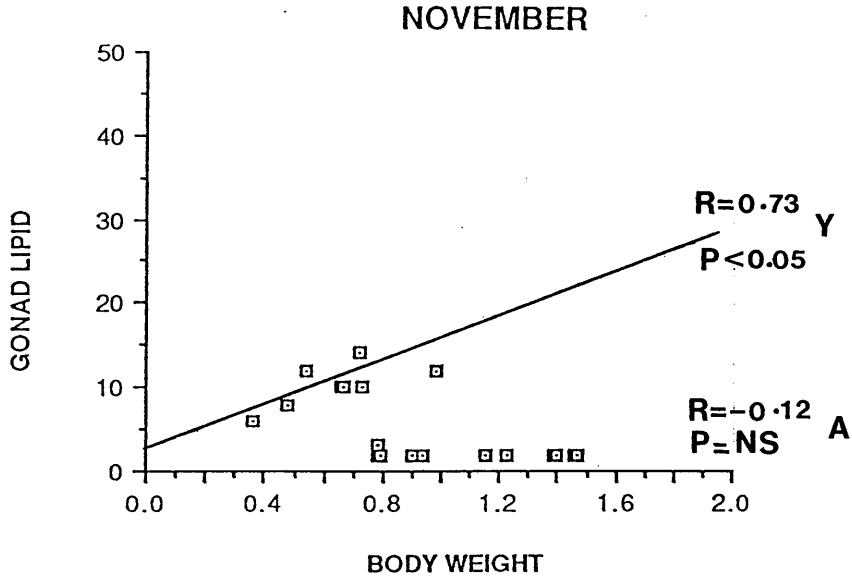
Y = young of the year

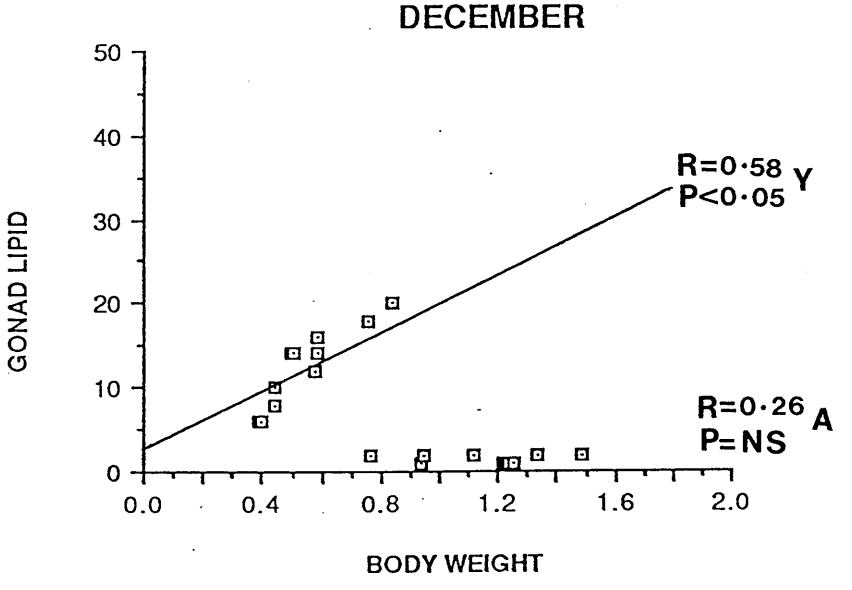
A′= breeding adult males

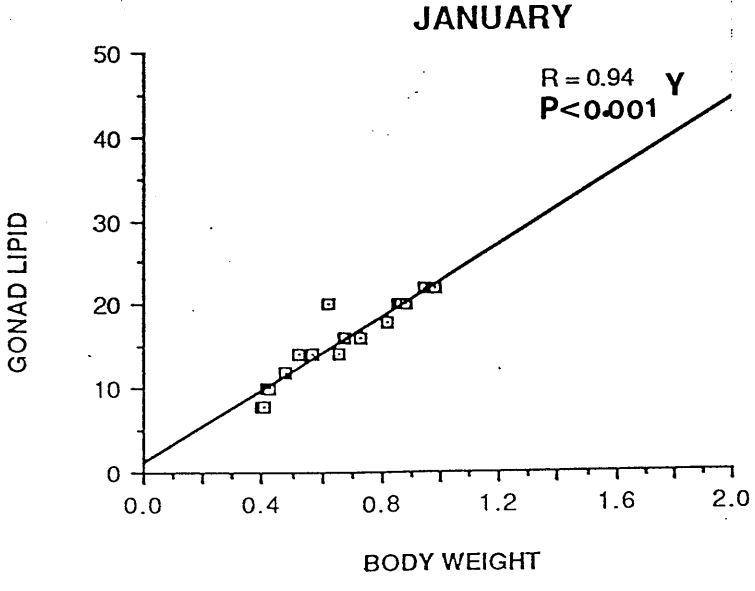

AUGUST
R =-0.36
P<0.1 A
GONAD LIPID
50
40
30
20
10
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT

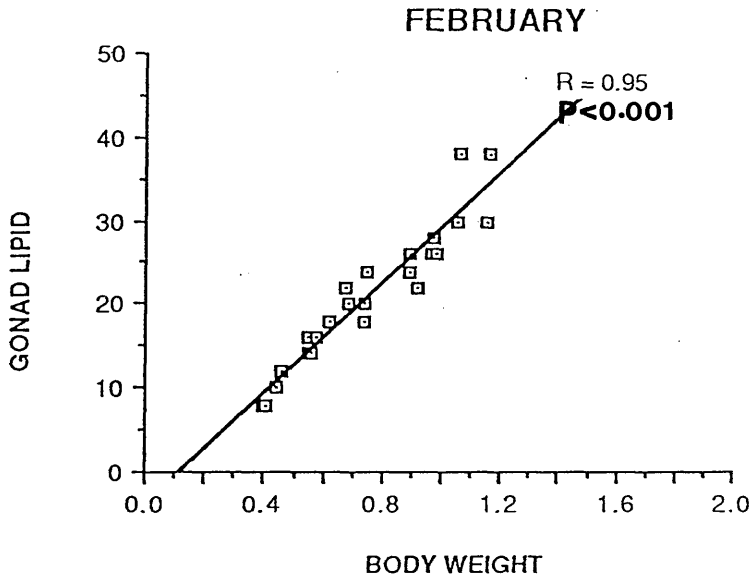

SEPTEMBER
R=0.65 Y
P<0.02
GONAD LIPID
50
40
30
20
10
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT

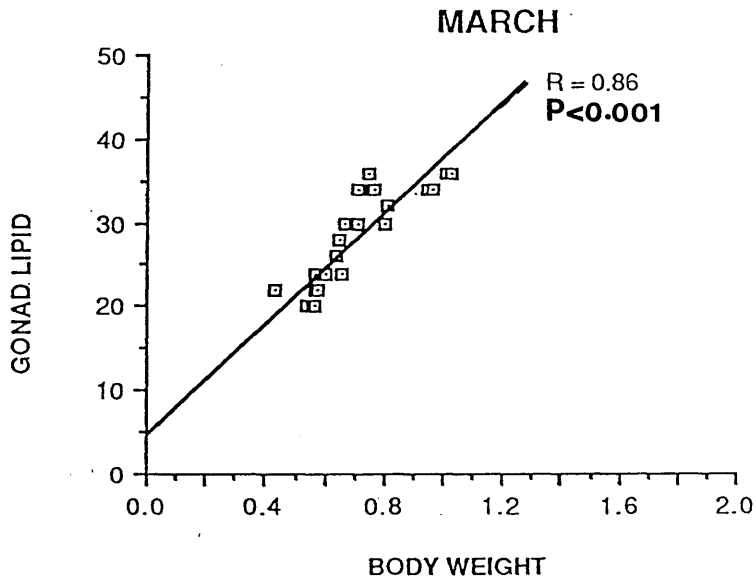

OCTOBER
R=0·58
P<0·01
GONAD LIPID
50
40
30
20
10
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT

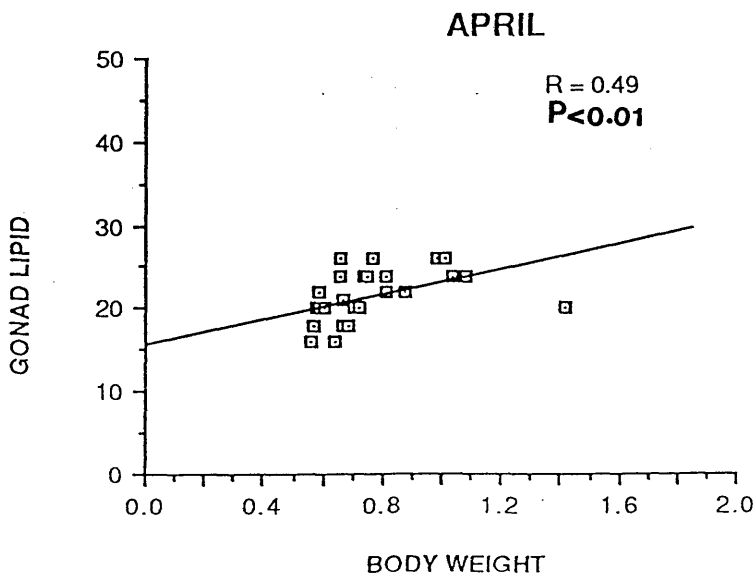

NOVEMBER
GONAD LIPID
50
40
30
20
10
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT
R=0·73 Y
P<0.05
R=-0·12 A
P=NS

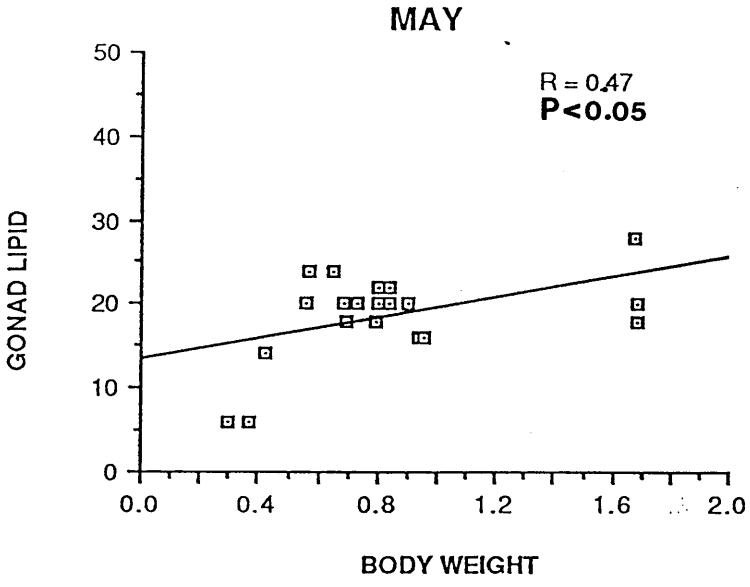

DECEMBER
GONAD LIPID
50
40
30
20
10
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT
R=0·58 Y
P<0·05
R=0·26 A
P=NS

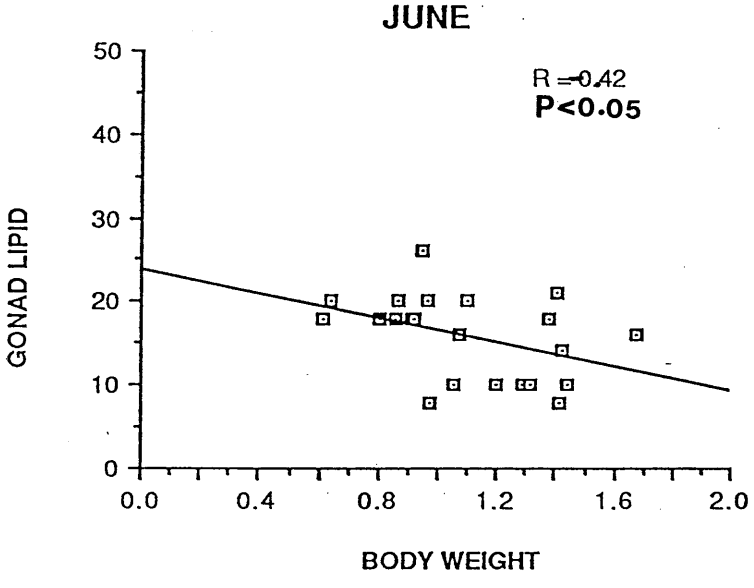

JANUARY
GONAD LIPID
50
40
30
20
10
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT
R = 0.94 Y
P<0.001

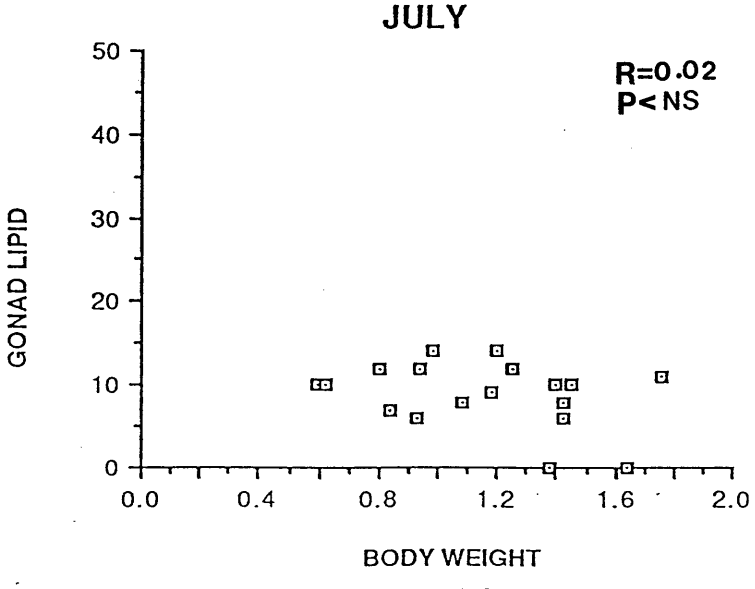

FEBRUARY
R = 0.95
P<0.001
GONAD LIPID
BODY WEIGHT
0
10
20
30
40
50
0.0
0.4
0.8
1.2
1.6
2.0


MARCH
R = 0.86
P<0.001
GONAD LIPID
BODY WEIGHT
0
10
20
30
40
50
0.0
0.4
0.8
1.2
1.6
2.0


APRIL
R = 0.49
P<0.01
GONAD LIPID
BODY WEIGHT
0
10
20
30
40
50
0.0
0.4
0.8
1.2
1.6
2.0


MAY
R = 0.47
P<0.05
GONAD LIPID
BODY WEIGHT
0
10
20
30
40
50
0.0
0.4
0.8
1.2
1.6
2.0


JUNE
R =-0.42
P<0.05
GONAD LIPID
BODY WEIGHT
0
10
20
30
40
50
0.0
0.4
0.8
1.2
1.6
2.0


JULY
R=0.02
P< NS
GONAD LIPID
BODY WEIGHT
0
10
20
30
40
50
0.0
0.4
0.8
1.2
1.6
2.0

4.7.5.3 Body weight and carcass lipid reserves

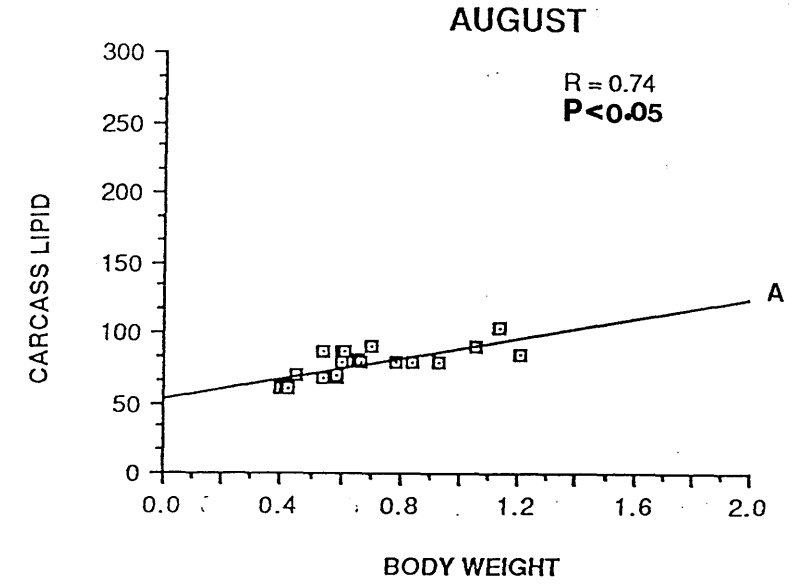
Figure 4.5 show the scatter plots of carcass lipid against body weight for each month over a period of one year. In the young of year, there was a strong positive relationship between body weight and carcass lipid reserves from August to October, and from January to April, but not during November and December when the carcass lipid levels were low. The relationship was weaker in May. In June there was a non-significant negative relationship which disappeared altogether in July, because regardless of body weight the carcass lipid reserve levels were low in July.

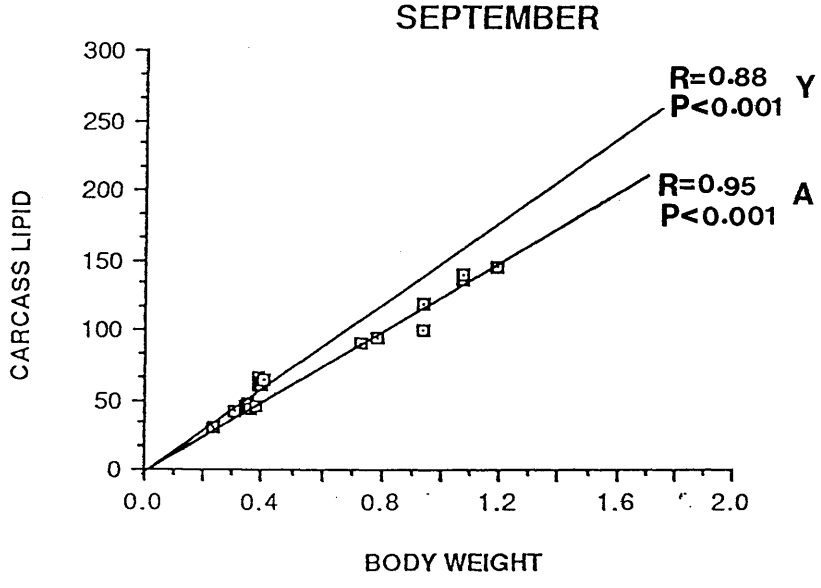
FIGURE 4.5 Scatter plots of Carcass lipid against body weight for each month over a period of one year. Lipid values are given in mg g^{-1} d.w and body weight in gms.

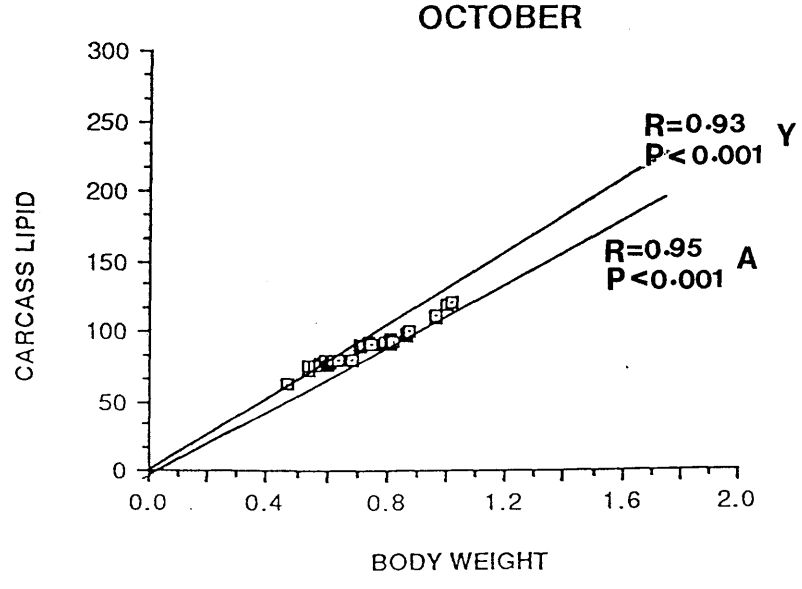
A = non-breeding adult males

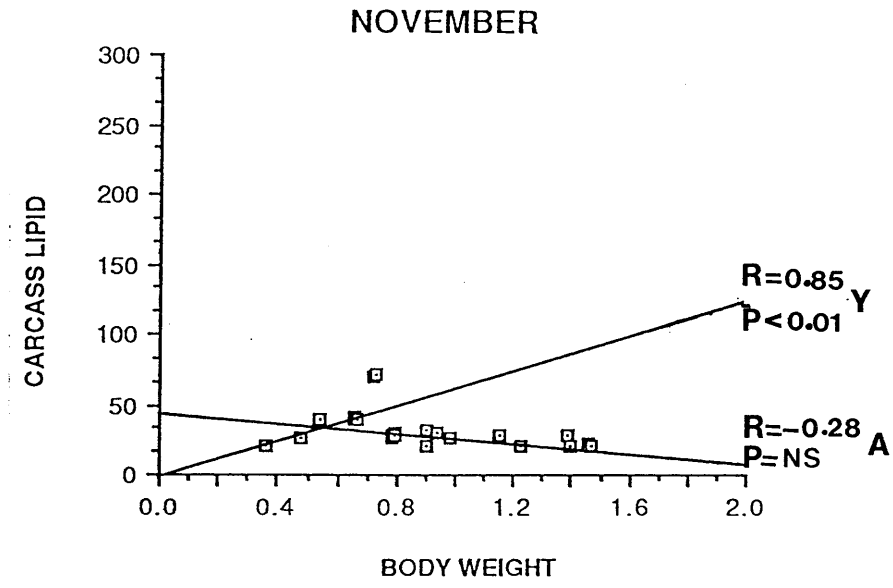
Y = young of the year

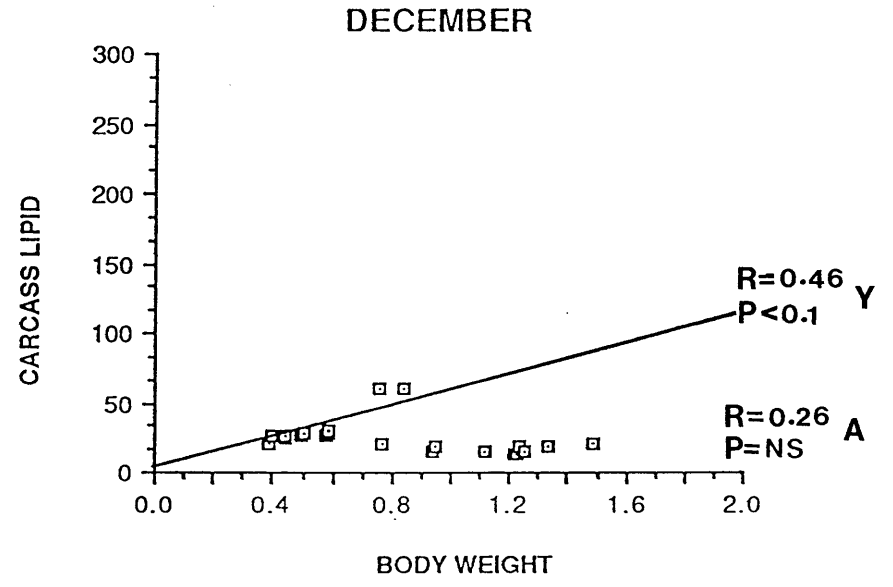
A′= breeding adult males

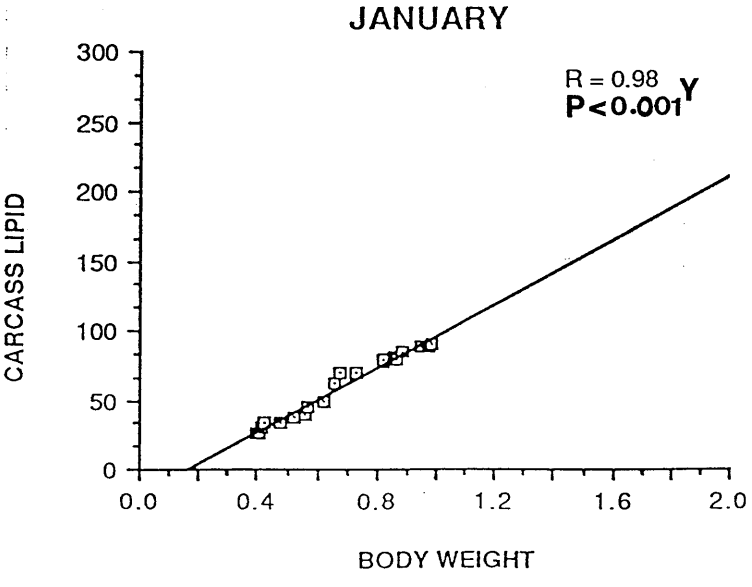

AUGUST
R = 0.74
P<0.05
A
CARCASS LIPID
BODY WEIGHT
300
250
200
150
100
50
0
0.0
0.4
0.8
1.2
1.6
2.0

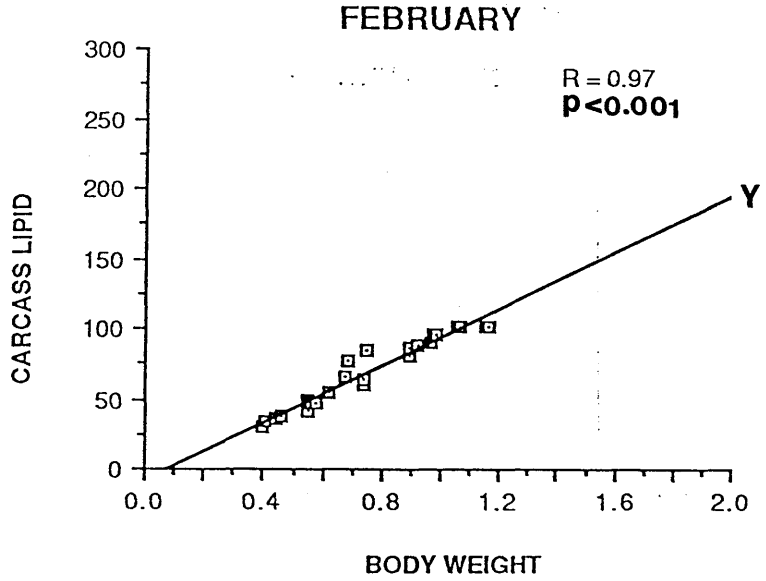

SEPTEMBER
R=0.88
P<0.001
Y
R=0.95
P<0.001
A
CARCASS LIPID
BODY WEIGHT
300
250
200
150
100
50
0
0.0
0.4
0.8
1.2
1.6
2.0

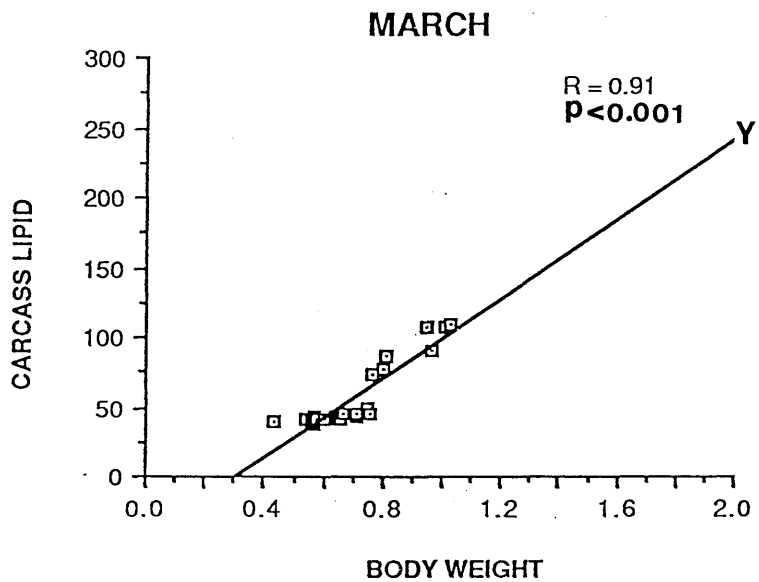

OCTOBER
R=0.93
P<0.001
Y
R=0.95
P<0.001
A
CARCASS LIPID
BODY WEIGHT
300
250
200
150
100
50
0
0.0
0.4
0.8
1.2
1.6
2.0

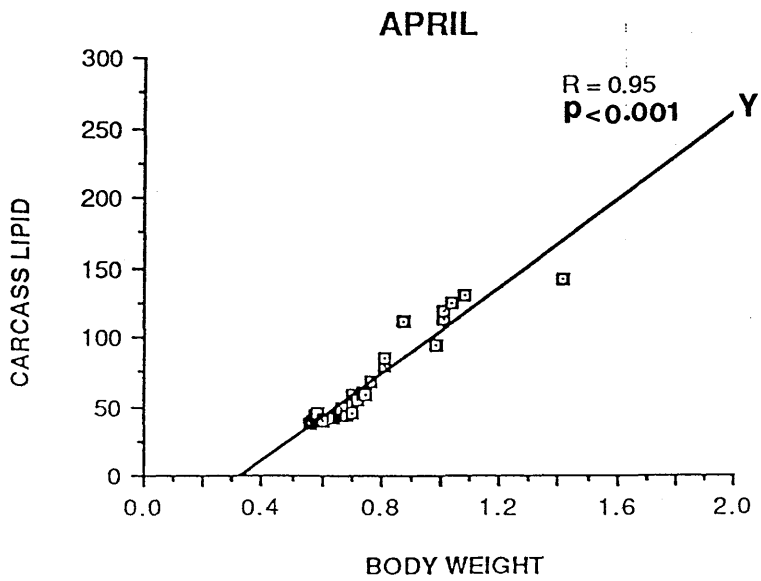

NOVEMBER
CARCASS LIPID
300
250
200
150
100
50
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT
R=0.85 Y
P<0.01
R=-0.28 A
P=NS

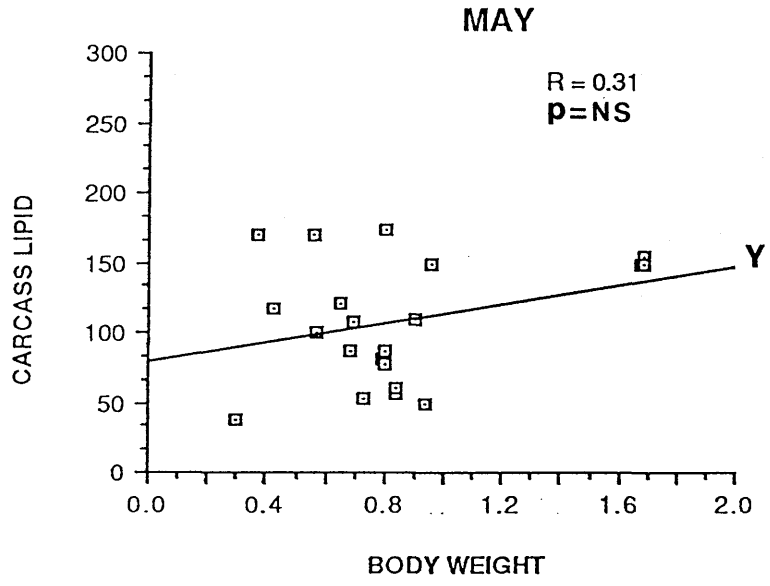

DECEMBER
CARCASS LIPID
300
250
200
150
100
50
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT
R=0.46 Y
P<0.1
R=0.26 A
P=NS

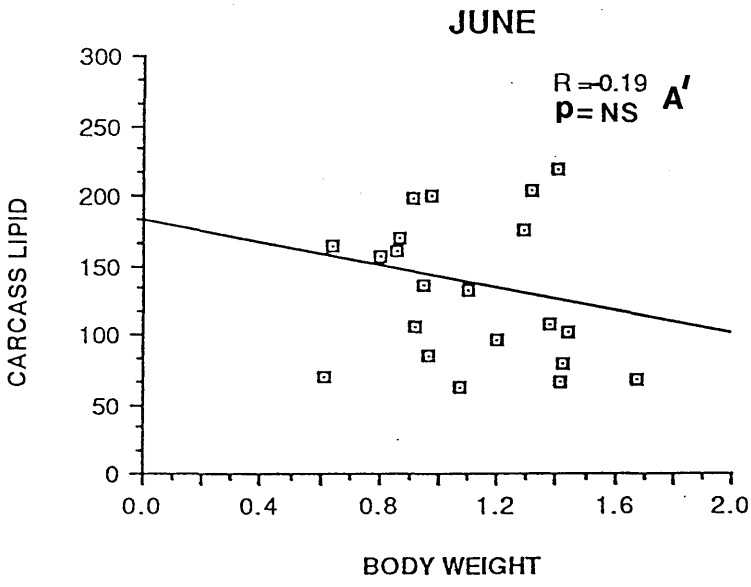

JANUARY
CARCASS LIPID
300
250
200
150
100
50
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT
R = 0.98 Y
P<0.001

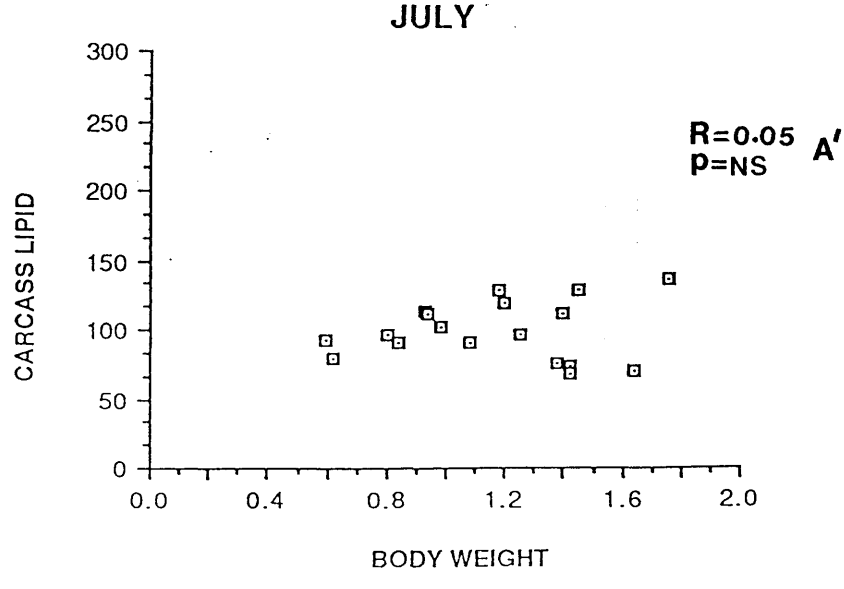

FEBRUARY
R = 0.97
p<0.001
Y
CARCASS LIPID
300
250
200
150
100
50
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT


MARCH
R = 0.91
p<0.001
Y
CARCASS LIPID
300
250
200
150
100
50
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT


APRIL
R = 0.95
p<0.001
Y
CARCASS LIPID
300
250
200
150
100
50
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT


MAY
R = 0.31
p=NS
Y
CARCASS LIPID
300
250
200
150
100
50
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT

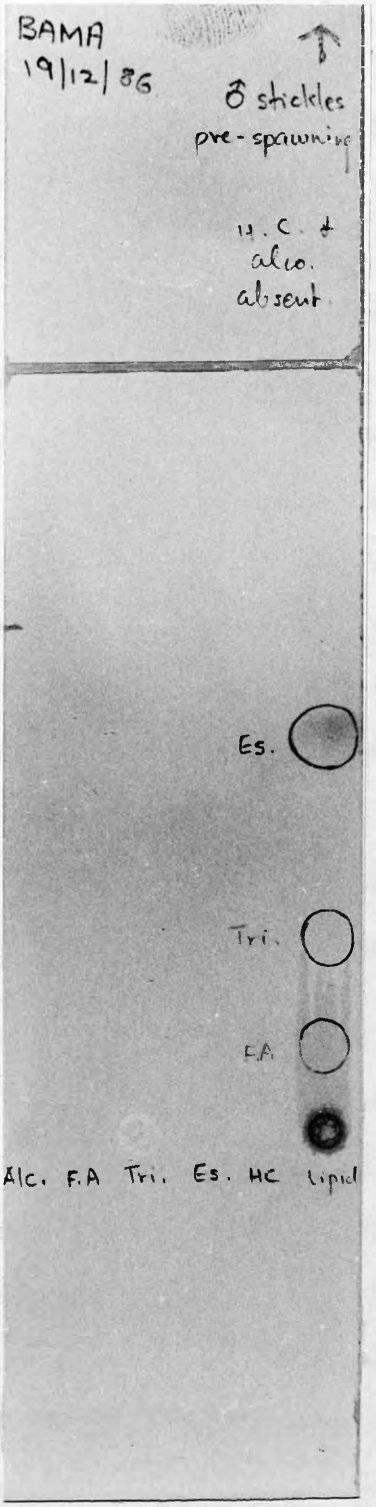

JUNE
R =-0.19
p= NS
A'
CARCASS LIPID
300
250
200
150
100
50
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT


JULY
R=0.05
p=NS
A'
CARCASS LIPID
300
250
200
150
100
50
0
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT

4.8 FRACTIONATION OF LIPIDS

Thin layer chromatography technique was used to analyse the lipid classes found in the lipids extracted from mature stickleback before the breeding season. The different classes of the lipids identified were triglycerides, fatty acids and esters (see Plate 4.1). Alcohols and hydrocarbons were absent.

PLATE 4.1 Thin layer chromatography plate showing lipid fractions


BAMA
19/12/86
♂ stickles
pre-spawning
N.C. ♯
alco.
absent
Es.
Tri.
F.A
Alc. F.A Tri. Es. HC Lipid

4.9 DISCUSSION

The results of the present study indicate that the patterns of accumulation and depletion of lipid and glycogen reserves in the liver, gonad and carcass in male sticklebacks are similar. In the young of the year, there is an increase in the liver lipid reserves from August to October which levels off in the winter. Presumably the abundant food supply available in the late summer allows energy intake to exceed the rate of utilization of the young fish so surplus energy is stored as lipid reserves. In winter, either lack of food or low rates of food consumption cause the lipid reserves to level off in the young of the year. Thereafter the liver lipid levels are more or less constant up until March . In March and April (during their first spring) the liver lipid levels of male fish increase markedly as food availability and/or appetite rise. This is followed by a steep fall during the breeding season as lipid reserves accumulated in spring are mobilized in June and July to provide energy for breeding.

On the other hand, there was a gradual drop in the gonad lipid reserves earlier in the year from March to July; presumably they were mobilized for gonad maturation, as this corresponds to the period when spermatozoa are high in levels (Ukegbu, 1986). The drop in liver and gonad lipid reserves during the breeding season indicate that lipid reserves are mobilized to meet the energetic cost of reproduction. The general pattern of the seasonal changes in the lipid reserves of liver, gonad and carcass are more or less similar apart from the fact that the lipid reserves of the carcass are not totally depleted by the

end of the breeding season. This is probably due to preferential depletion of lipid reserves in the liver and gonads. After breeding, the male sticklebacks have severely depleted energy reserves and often die. In contrast, those males that do not breed continue to accumulate energy reserves over the summer.

Lipid reserves in many teleost species show marked seasonal changes and seasonal fattening cycles are most pronounced in fish inhabiting temperate regions (Vlaming et al., 1978). Probably these seasonal fattening cycles are correlated with food availability. The present study showsthat male sticklebacks from the River Kelvin have a similar seasonal lipid cycle. According to Shulman (1974) lipids are the major source of energy for gamete production in fish. In the present study 95% of liver lipid and 30% of carcass lipid were depleted between May and July in breeding male sticklebacks. This supports the suggestion that lipids (particularly liver lipid reserves) are the major source of energy during the breeding season. Similarly the liver lipid stores in female sticklebacks (from Welsh populations) are at a minimum in the breeding season (Wootton et al., 1978).

Throughout the non-breeding season lipid reserves are strongly positively correlated with body weights, indicating that growth is not occurring at the expense of energy reserves. Similarly the fact that liver glycogen increases during the period of maximum spermatozoa production indicates that gonadal maturation does not deplete liver energy reserves. On the other hand, the fact that larger fish (which

breed earlier in the season) show more marked depletion in liver glycogen stores than smaller ones reinforces the conclusion that the behavioural aspect of breeding in male sticklebacks represent a major drain on energy reserves.

Lipid extracts from mature male sticklebacks yield triglycerides, fatty acids and esters on fractionation. Though this is a preliminary result, it conformswith lipid classes of freshwater fish (Gunstone et al., 1986). Further studies on analysis of each lipid class in the different body compartments (by combined gas chromatography and mass spectrometry) at different stages of life cycle of the male sticklebacks, may explain why liver lipids are selectively depleted over carcass lipid during the breeding season.

4.10 CONCLUSIONS

This chapter describes the annual changes in lipid reserves of the different body compartments in male three-spined stickleback from the River Kelvin. There is a striking parallelism in the accumulation and depletion of lipid and glycogen reserves in male sticklebacks. Lipid reserves in the different body compartments are positively correlated during the non-breeding season but become weakly positively correlated due to depletion during the breeding season (June and July). The picture that emerges is of accumulating energy reserves during growth and gonad maturation, but of drastic depletion (particularly liver and gonad lipids) during development of secondary sexual characteristics and breeding activities.

CHAPTER 5

SEASONAL VARIATION IN PROTEIN CONCENTRATION

CHAPTER 5. SEASONAL VARIATION IN PROTEIN CONCENTRATION

5.1 INTRODUCTION

The main sources of energy in fish appear to be protein and lipid; this is in contrast to mammals in which carbohydrate and lipid are more important. This is perhaps due to the following reasons : a) the diet of fish generally consists of high protein and their metabolism is very well adapted to deal with such a diet; b) unlike mammals, fish have the ability to eliminate nitrogenous waste rapidly and continuously; over 90 percent of the nitrogen excretion is in the form of ammonia via the gills; c) specific activities of lysosomal enzymes (viz. lysosomal proteinases) which are involved in protein breakdown, are found to be higher in fish than those in mammals (Fauconneau, 1985; Walton, 1985).

From measurements of ammonia excretion and oxygen uptake it is possible to estimate the contribution of amino acid catabolism to energy production under aerobic conditions. Such studies have shown that standard metabolism in fish is almost completely based on amino acid catabolism (Brett and Zala, 1975; Cho and Kaushik, 1985) but as the metabolic rate increases the lipid catabolism becomes increasingly important. The relative contributions of lipids and amino acids to energy production are dependent on a number of factors such as the species involved, nutritional state and environmental temperature. In salmonids, during routine activity more than 40% of energy production

is due to amino acid catabolism. The maintenance energy needs of fish are considerably lower than those of homeothermic animals. For example, the maintenance energy expenditure in Rainbow trout (Salmo gairdneri) with body weight between 50-150 gms, at 20 ^{o} C is 33 KJ/Kg $^{0.75}$ day whereas that of a rat (Rattus sp) weighing 130 gms at 22 ^{o} C is 552 KJ/Kg $^{0.75}$ day (as reviewed by Cho & Kaushik, 1985).

Fish can withstand periods of starvation, during which they catabolise their body proteins. Generally protein concentration in fish varies from 13% to 19% of the body wet weight. During depletion, fish break down contractile protein more readily than connective tissue protein. For example, collagen (which is the principal connective tissue protein) is not mobilized during starvation. Levels of amino acids such as glycine and histidine of the muscle drop to about one sixth of their normal values during starvation in the common carp (reviewed by Love, 1970). The protein concentration of the muscle of most severely starved fish reach low levels; for example in black back (Hippoglossoides platessoides) the protein level reached a very low figure of 2.83% of body wet weight (Templeman and Andrews, 1956).

As the protein gets depleted, the water content increases due to shrinkage of the cells. In case of severe protein depletion, the water content may rise to as high as 88%, with a corresponding fall in protein level. Non-fatty fish for example the Atlantic cod have very low lipid reserves in the muscle, and an inverse relationship between protein and water. On the other hand, there is an inverse relationship between lipid and water in the muscle of fatty fish, for example the Atlantic herring (Love, 1970).

Mobilisation of protein in fast swimming fish such as the Atlantic mackeral is rapid. This is probably because the activity of lysosomal enzymes and the autolysis of the muscle are both high in this fish (reviewed by Love, 1970). Proteins can serve as a source for carbohydrates since many of the amino acids can be converted into glucose, adding to the carbohydrate pool of the body. This process is called gluconeogenesis. Lipid can also be derived from amino acids and stored as fat, and this process is called lipogenesis. The energy expended by fish may come from the oxidation of lipids or glucose derived from amino acids. This process is accelerated by adrenocortical steroids in fish (viz. cortisol and cortisone; Butler, 1968). The protein catabolic property of the corticosteroid hormones mobilizes protein from the body muscle and provides energy. For example, in Oncorhynchus sp a six fold increase in the blood plasma corticoids during spawning migration brings about catabolism of about 60 percent of the body proteins (Matty and Khalid, 1985).

5.2 PROTEIN METABOLISM

Amino acids are precursors for many biological compounds especially proteins and are primarily required for the synthesis of new body protein and other compounds such as hormones, purines and neurotransmitters. The body pool of amino acids arises from two main sources, namely, from the diet and from the catabolism of body protein. In fish, amino acids from the gut enter the portal blood system and pass through the liver, which has an important role in the metabolism of amino acids. Several fish species have been shown to

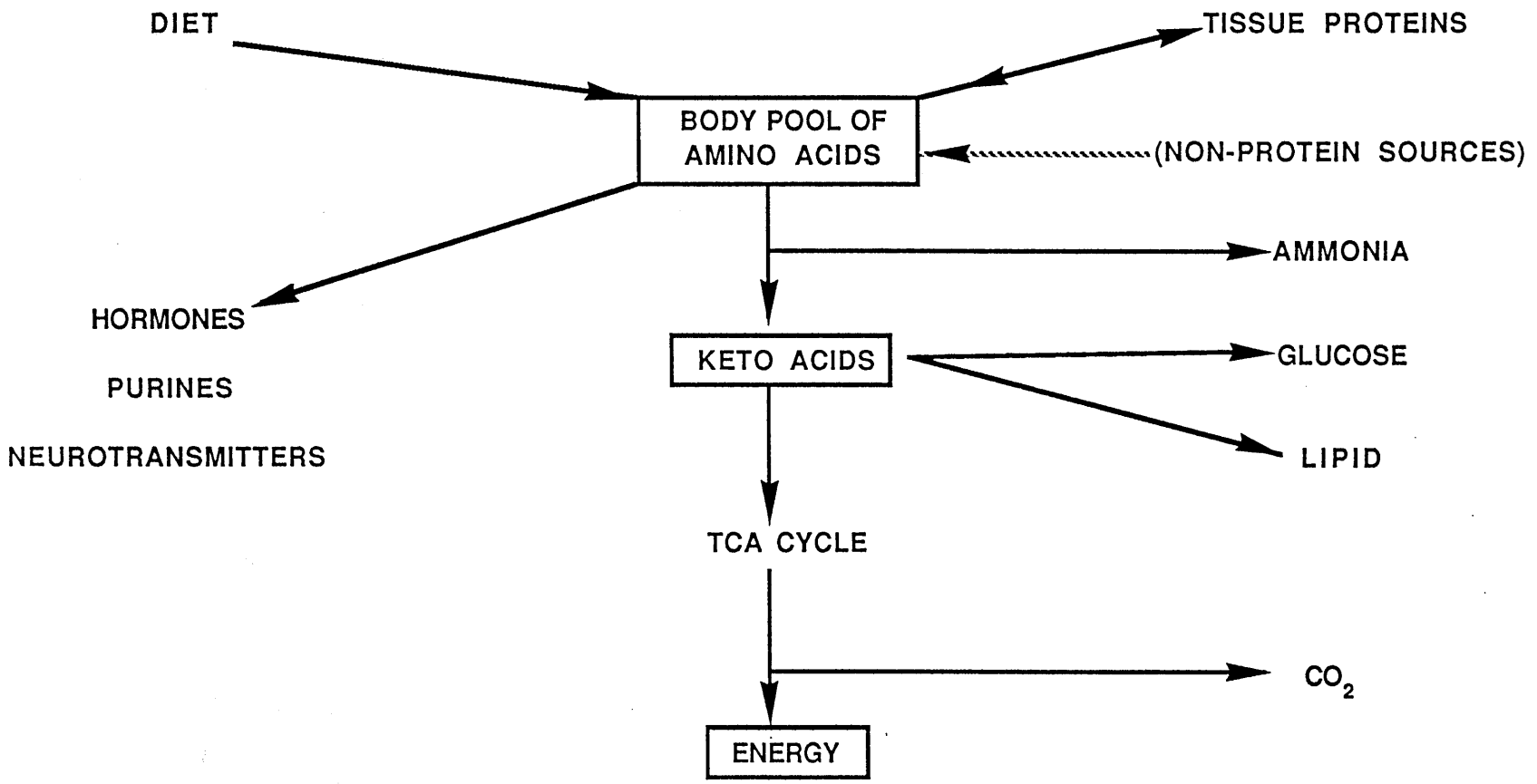


FIG. 5.1 OVERVIEW OF AMINO ACID METABOLISM IN FISH (AFTER WALTON, 1985)

require ten essential amino acids namely, arginine, histidine, isoleucine, leucine, methionine, phenylalanine, threonine, tryptophane and valine (Shanks et al., 1962). There are no known body stores of amino acids, as excess amino acids are rapidly deaminated. During this process the amino group is degraded and ultimately excreted as ammonia and the carbon skeleton gets oxidized via the tricarboxylic acid cycle (TCA) to yield energy or gets converted either to glucose or lipid. These pathways are depicted in Fig 5.1

5.3 SEASONAL VARIATION IN PROTEIN CONCENTRATION OF FISH

In healthy fish protein (when considered as percentage body weight) tends to be relatively constant for a given species. For example, the protein concentration does not change significantly during development from elver to adult stage in silver eel (Anguilla anguilla) (Boetius and Boetius, 1985). In general, there is no significant seasonal variation in protein concentration of fish, but during the periods of starvation and gonad development, non-fatty fish draw on their carcass protein. For example, in female plaice (Pleuronectus platessa) 40% of the protein in the body is utilized during December to March, when they do not feed but grow large ovaries. It is noteworthy that during this period there is body protein break down and concentrationsof plasma cortisol are elevated (Dawson and Grimm, 1980).

So far no systematic annual sampling has been done on male stickleback to estimate the protein concentration. This is of

considerable interest as activities such as territorial defense, courtship and parental care in male stickleback demand expenditure of energy. Good body condition is essential for successful breeding of the male stickleback. One of the aims of this study is therefore to estimate the protein concentration in the male stickleback at different stages in their annual life cycle.

5.4 METHODS OF PROTEIN DETERMINATION

Some workers have estimated the total nitrogen in the dry tissue and use this value to calculate protein concentration. Total nitrogen can be determined either on a Technicon autoanalyser employing the micro Kjeldahl method (Deegan, 1986) or using a Nitrogen gas analyser (Medford and Mackay, 1978). Protein is calculated as total nitrogen X 6.025. A nitrogen - protein conversion factor of 6.025 is considered appropriate for fish muscle.

The Lowry procedure has been found to be the most reliable and satisfactory method for quantitative analysis of soluble protein (Lowry et al., 1951). For many proteins, the Lowry procedure can be run directly on the protein solution by treating with alkaline copper reagent to precipitate the protein, which is then reduced by the Folin reagent. The insoluble proteins are initially treated with trichloroacetic acid or perchloric acid to precipitate the protein. The precipitate is dissolved in 1N sodium hydroxide, then treated with a carbonate copper solution and then reduced by Folin reagent. In both

cases, the optical density is read in a spectrophotometer. However, in the direct Lowry procedure interference is caused by commonly used chemicals, such as sucrose, citrate, ammonium sulphate, peptide buffers and phenols.

The Peterson's modification of micro-Lowry method utilizes sodium dodecylsulphate to facilitate the dissolution of relatively insoluble lipoproteins (Peterson, 1977). The principle involved in this method is based on the following reactions: An alkaline cupric tartrate reagent complexes with the peptide bonds and forms a purple-blue colour when a phenol reagent is added. Absorbance is read at a suitable wavelength between 500 nm and 800 nm. The protein concentration is determined by comparing with a calibration curve of the protein standard prepared from bovine serum albumin (BSA).

5.5 MATERIAL AND METHODS

The procedure used for the determination of protein in the male stickleback was based on the Peterson's modification of micro-Lowry method (Peterson, 1977). The procedure is depicted below in the form of a flow chart.

Fish killed in liquid nitrogen at -196 ^{o} C and transferred to a deep freezer of -70 ^{o} C

Wet weight and standard length recorded and fish dissected on melting ice. Wet weights of body compartments recorded and kept at -30 ^{o} C for one hour. Fish tissue dehydrated by freeze-drying for 24 hours in Edwards EF03 freeze-drier

↓

Dry weight of freeze-dried tisuues recorded

↓

Dried tissue ground into powder and used for lipid extraction (see chapter 4). Lipid-free tissue was used for protein determination

↓

Known weight of the dry lipid-free tissue introduced into labelled Eppendorf micro-centrifuge tube and 1 ml of water added

↓

0.1 ml of sodium deoxycholate solution (DOC) added and mixed well using a vortex-mixer. Kept at room temperature for 10 minutes

↓

0.1 ml of 72% trichloroacetic acid w/v (TCA) added and mixed well using a vortex-mixer

↓

Centrifuged for 10 minutes (at 10.000 g) to pellet the precipitate

↓

Supernatant decanted and dissolved the pellet in 1 ml modified Lowry reagent solution (Sigma chemical company)

↓

Solution transferred to labelled test tube, rinsed Eppendorf centrifuge tube with 1 ml water and rinsings added to test tube Contents in test tube mixed well using a vortex-mixer and allowed to stand at room temperature for 20 minutes

↓

0.5 ml Folin and Ciocalteu's phenol reagent added with rapid mixing

↓

The solution allowed to stand at room temperature for 30 minutes for the purple-blue colour to develop

↓

The test solution transferred to the spectrophotometer cuvette, optical density read at 750 nm and matched with a standard protein calibration curve

Freshly prepared protein standards were used for every batch of test solutions from bovine serum albumin (BSA). The absorbance values of the protein standards were plotted against their concentrations to obtain the protein calibration curve. The optical density readings of the test solutions were matched with the standard protein calibration curve and the protein concentrations obtained.

Protein values expressed as mg g^{-1} dry weight refer to relative levels of protein concentration. Analysis based on absolute levels leads to equivalent conclusions throughout this thesis.

5.6 DATA ANALYSIS

Mean monthly protein concentrations were calculated for the young of the year and for adult non-breeding fish. The fish were assigned to their respective age classes using body weight as the criteria for distinguishing young of the year from adult fish. Sample sizes, mean length and weights of the fish collected from the River Kelvin are given in Table 2.1 (see chapter 2).

The data were checked for normal distribution and transformed when necessary. Product-moment correlation coefficients were calculated between body weights and protein concentrations in the liver, gonad and carcass. Oneway analysis of variance (ANOVA) was used to check for seasonal variation in protein concentration. The relationship between body weight and protein concentration was investigated by regression analysis for each month over a period of one year, considering the young of the year and the adult non-breeding fish separately.

5.7 RESULTS

Table 5.1 summarizes the results of oneway analysis of variance (ANOVA) on protein concentrations for the entire year. Table 5.2 gives the correlations between protein concentrations in the liver, gonad and carcass. Figure 5.2 A to C shows the annual changes in protein concentrations in the liver, gonad and carcass respectively. The mean values and 95% confidence intervals of the means have been plotted against the months of the year. The months have been arranged on the X-axis to depict the complete life cycle of the male sticklebacks from this population.

TABLE 5.1 ONE WAY ANALYSIS OF VARIANCE BY MONTH ON PROTEIN CONCENTRATION

VARIABLE	SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROBABILITY
LIVER PROTEIN						
	Between groups	11	367876.19	33443.29	8.22	0.0000
	Within groups	271	1102723.71	4069.09	--	--
GONAD PROTEIN						
	Between groups	11	419802.76	38163.88	8.88	0.0000
	Within groups	271	1164124.06	4295.66	--	--
CARCASS PROTEIN						
	Between groups	11	926887.35	84262.48	21.97	0.0000
	Within groups	271	1039423.66	3835.51	--	--

FIGURE 5.2 ANNUAL CHANGES IN PROTEIN CONCENTRATION

A) Mean and 95% confidence intervals of liver protein

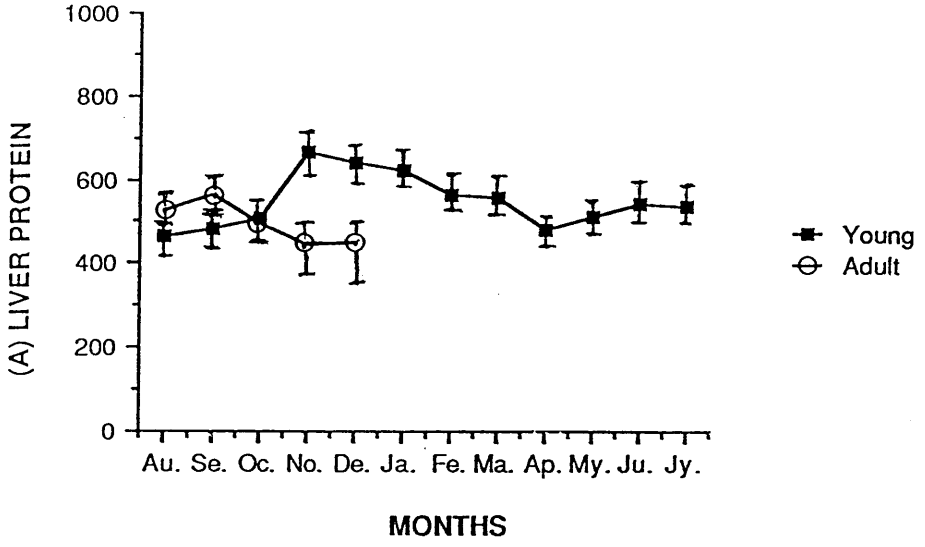
B) Mean and 95% confidence intervals of gonad protein

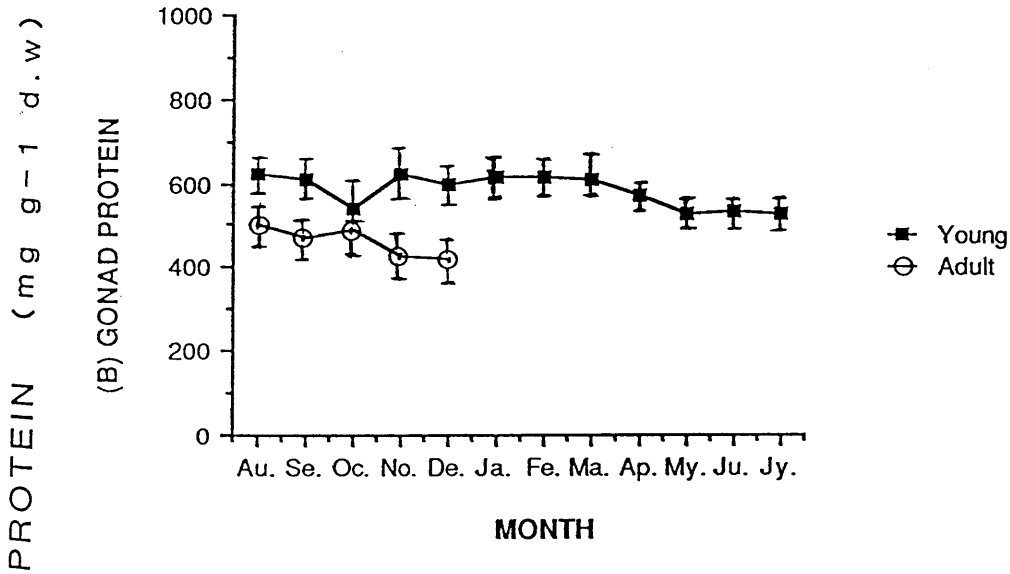
C) Mean and 95% confidence intervals of carcass protein

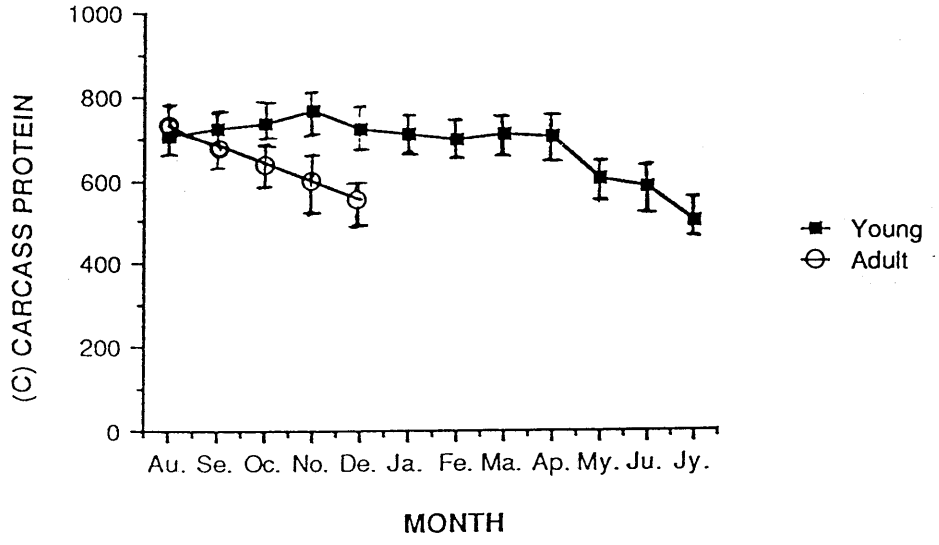
(The months are arranged on the X-axis to depict the complete life cycle of the male three-spined stickleback from the River Kelvin)

Y = Young of the year

A = Non-breeder adult


(A) LIVER PROTEIN
1000
800
600
400
200
0
Au. Se. Oc. No. De. Ja. Fe. Ma. Ap. My. Ju. Jy.
MONTHS
Young
Adult


PROTEIN (mg g-1 d.w)
(B) GONAD PROTEIN
1000
800
600
400
200
0
Au. Se. Oc. No. De. Ja. Fe. Ma. Ap. My. Ju. Jy.
MONTH
Young
Adult


(C) CARCASS PROTEIN
1000
800
600
400
200
0
Au. Se. Oc. No. De. Ja. Fe. Ma. Ap. My. Ju. Jy.
MONTH
Young
Adult

5.7.1 Annual variation in liver protein concentration

In general, the annual changes in protein concentrations were much less marked than for glycogen and lipids. In the young of the year, there was an increase in liver protein concentrations from August to November, followed by a gradual but small decline through the spring months and a small (non-significant) increase in early summer.

In August, the non-breeding adult fish had slightly higher protein concentrations than the young of the year (but not significantly so). Protein concentrations of the non-breeding fish fell from September and December to concentrations significantly below those of young of the year. This group of fish was not found beyond winter.

5.7.2 Annual variation in gonad protein concentration

In the young of the year gonad protein concentration did not vary across the year. Non-breeding adults had lower gonad protein concentrations than young of the year and these declined slightly up to December.

5.7.3 Annual variation in carcass protein concentration

In the young of the year carcass protein concentrations remained broadly constant from August to April. There was a decline in the carcass protein concentration from the begining of the breeding season until July. In the non-breeding male fish carcass protein concentration declined (from September onwards) to levels significantly lower than those of young of the year.

TABLE 5.2 Correlation coefficients and levels of significance between protein concentrations in the liver gonad and carcass.

A - during the non-breeding season of August to May

B - during the breeding season - month of June

C - during the breeding season - month of July

Abbreviations:

R = Product-moment correlation coefficient

P = Levels of significance

*** = $P < 0.001$

** = $P < 0.01$

* = $P < 0.05$

+ = $P < 0.1$

NS = Non-significant

N = Number of samples

Table 5.2 A

	PROTEIN: LIVER	PROTEIN: GONAD	PROTEIN: CARCASS	
		R = 0.572 P = ***	R = 0.542 P = ***	LIVER
N = 241			R = 0.649 P = ***	GONAD
				CARCASS

Table 5.2 B

	PROTEIN: LIVER	PROTEIN: GONAD	PROTEIN: CARCASS	
		R =-0.061 P = NS	R =-0.101 P = NS	LIVER
N = 22			R =-0.221 P = NS	GONAD
				CARCASS

Table 5.2 C

	PROTEIN: LIVER	PROTEIN: GONAD	PROTEIN: CARCASS	
		R =-0.249 P = NS	R =-0.364 P = NS	LIVER
N = 18			R =-0.529 P = *	GONAD
				CARCASS

5.7.4 The relationship between protein concentration in the liver, gonad and carcass

Prior to the breeding season, the liver, gonad and carcass constituted 2.5%, 1.5% and 96% of protein respectively (of the total body proteins). Table 5.2 A to C show the product-moment correlation coefficients between protein concentrations in the different body compartments. There were positive (highly significant) relationships between protein concentration in the liver, gonad and carcass during the non-breeding season (viz. August to May). In June the relationships were non-significant (but weakly negative) and by July these had larger negative values (significant only in the case of gonad and carcass). The change from a positive to a negative relationship over this period is probably due to differential depletion of gonad protein in fish with high liver and carcass protein concentration (presumably those that had depleted their reserves as a result of breeding).

5.7.5 The relationship between body weight and protein concentration

5.7.5.1 Body weight and liver protein concentration

Figure 5.3 show the scatter plots of liver protein concentration against body weight for each month over a period of one year. In young of the year the relationship between body weight and liver protein concentration was positive in September but this reversed to a negative correlation in October. In November there was no significant relationship. From December to May the relationship was negative and significant but there was no significant correlation in June. In July there was a weakly positive relationship. These changes in the relationship between body weight and protein concentration probably came about as follows: the liver protein concentration increased in young of the year from September resulting in positive correlation; from December to May the bigger fish accumulated lipid and glycogen reserves in their livers and had a lower relative protein concentration than the smaller fish; this results in a negative correlation. In June the bigger fish bred actively using up their energy reserves hence there was no significant relationship between body weight and liver protein concentration.

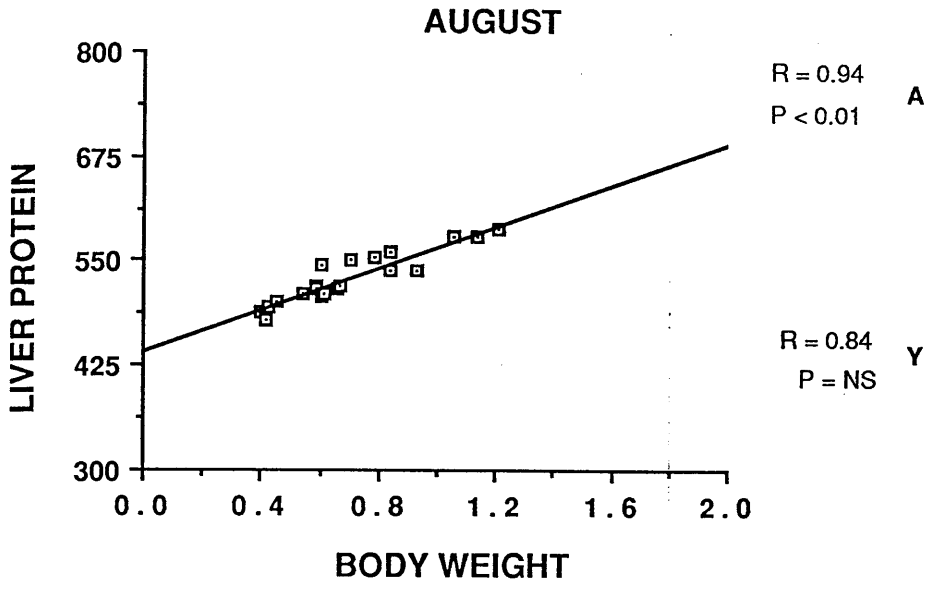
In the adult non-breeding fish the relationship between body weight and liver protein concentration was positive in August but there was no significant relationship from September to December.

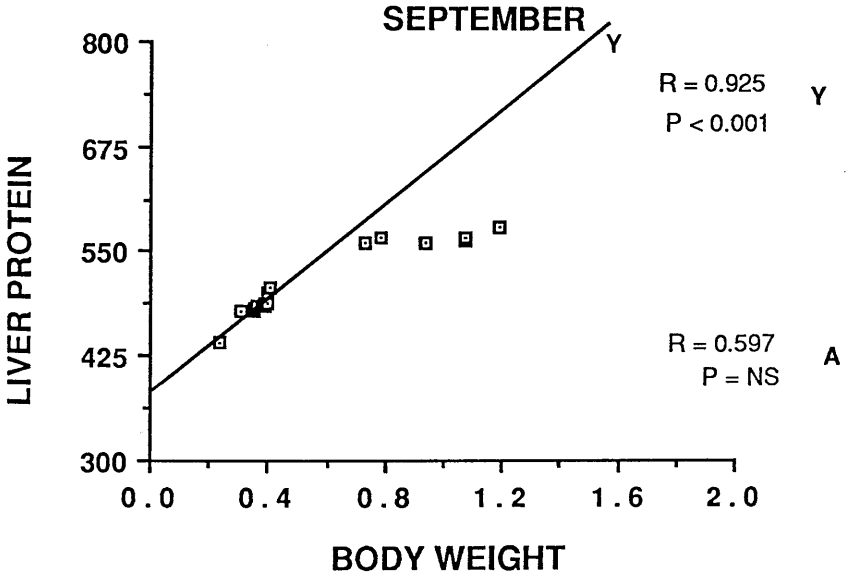
FIGURE 5.3 Scatter plots of Liver protein against body weight for each month over a period of one year. Protein values are given in mg g^{-1} d.w and body weight in gms.

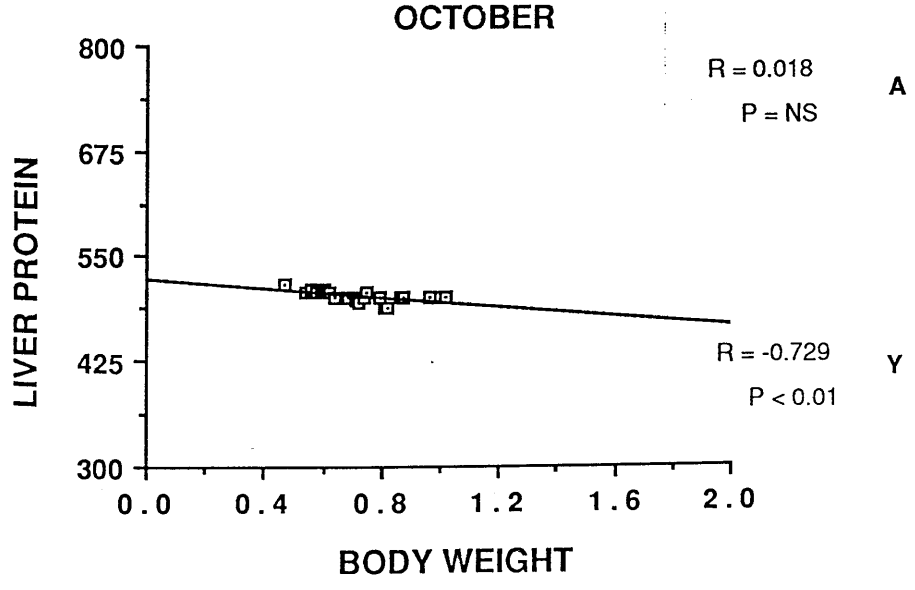
A = non-breeding adult males

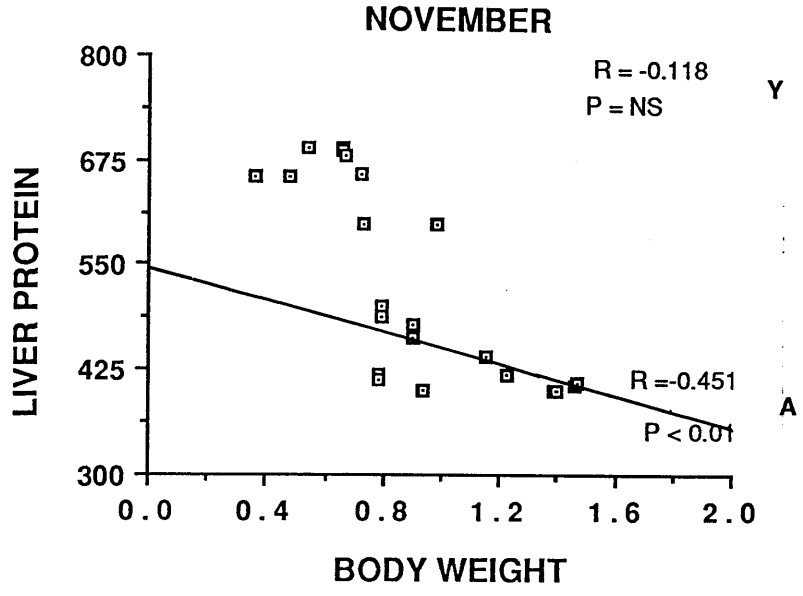
Y = young of the year

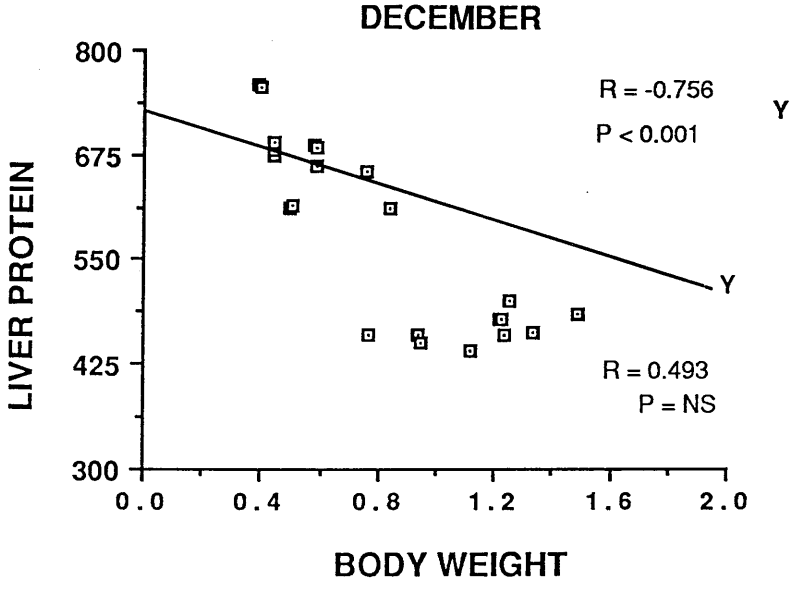
A'= breeding adult males


AUGUST
800
675
550
425
300
LIVER PROTEIN
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT
R = 0.94
P < 0.01
A
R = 0.84
P = NS
Y


SEPTEMBER
800
675
550
425
300
LIVER PROTEIN
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT
Y
R = 0.925
P < 0.001
Y
R = 0.597
P = NS
A


OCTOBER
800
675
550
425
300
LIVER PROTEIN
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT
R = 0.018
P = NS
A
R = -0.729
P < 0.01
Y


NOVEMBER
R = -0.118
P = NS
Y
R =-0.451
P < 0.01
A
LIVER PROTEIN
800
675
550
425
300
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT


DECEMBER
R = -0.756
P < 0.001
Y
Y
R = 0.493
P = NS
LIVER PROTEIN
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300
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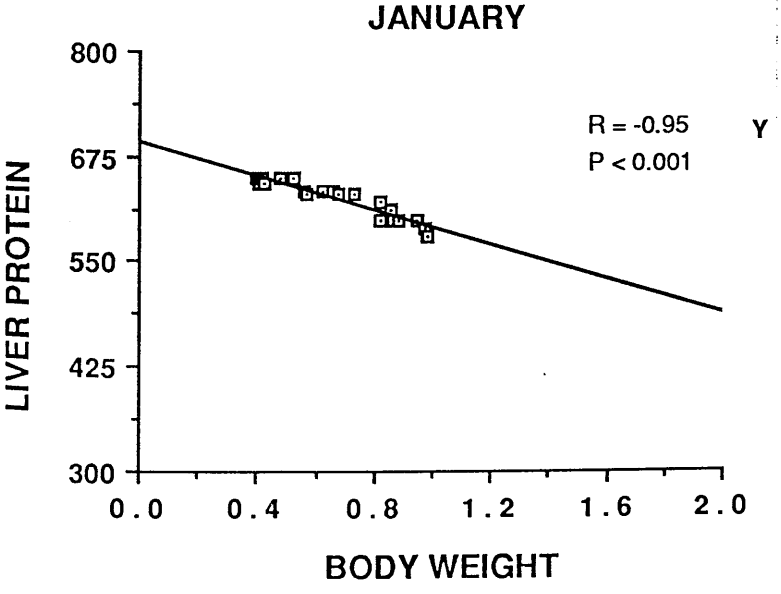
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT


JANUARY
R = -0.95
P < 0.001
Y
LIVER PROTEIN
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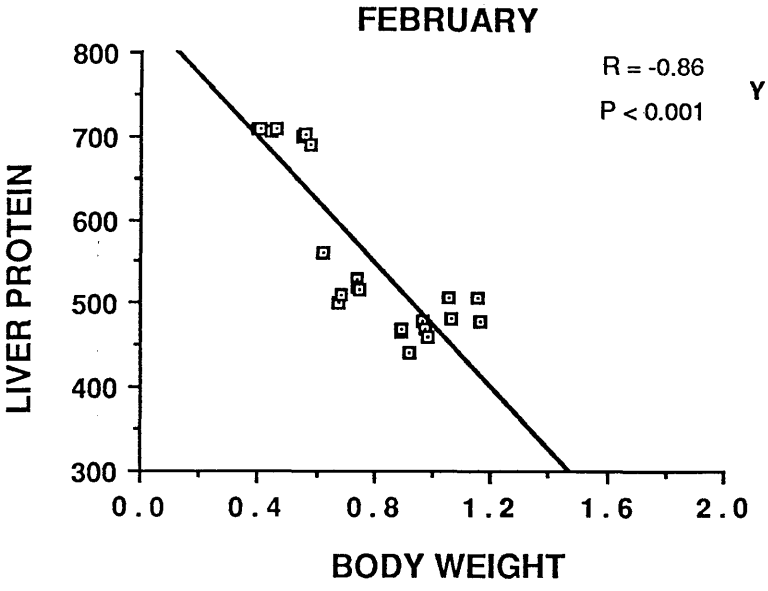
1.2
1.6
2.0
BODY WEIGHT


FEBRUARY
R = -0.86
P < 0.001
Y
LIVER PROTEIN
800
700
600
500
400
300
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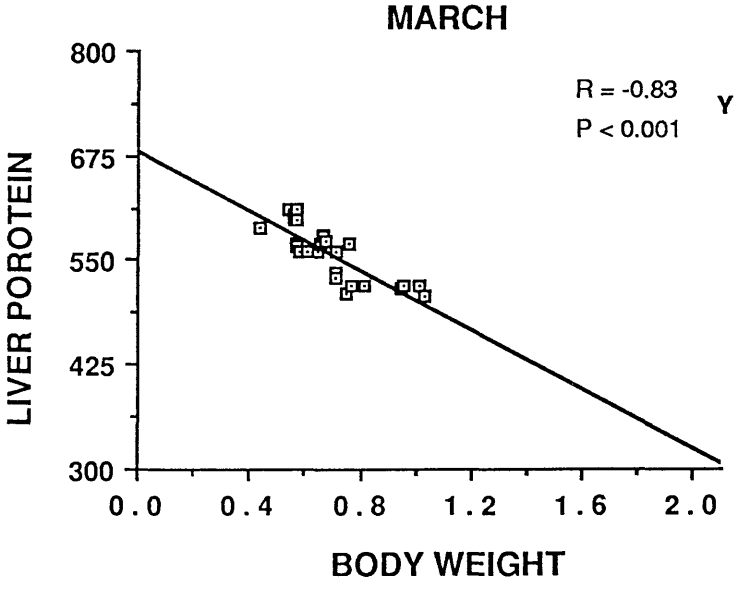
0.8
1.2
1.6
2.0
BODY WEIGHT


MARCH
R = -0.83
P < 0.001
Y
LIVER POROTEIN
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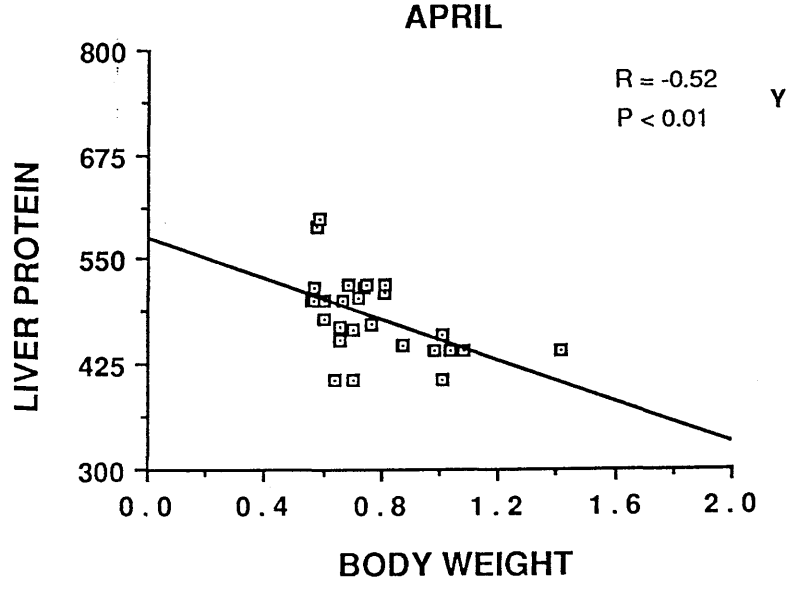
550
425
300
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT


APRIL
R = -0.52
P < 0.01
Y
LIVER PROTEIN
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675
550
425
300
0.0
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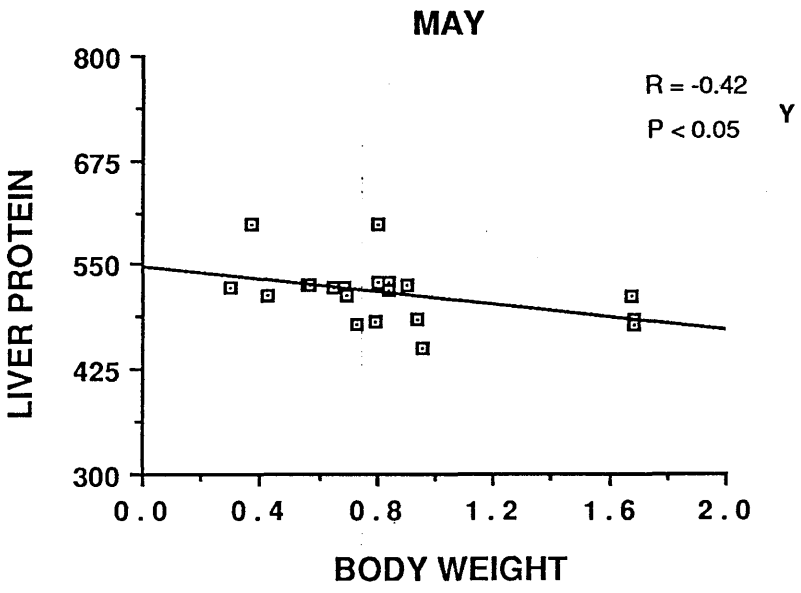
0.8
1.2
1.6
2.0
BODY WEIGHT


MAY
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P < 0.05
Y
LIVER PROTEIN
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550
425
300
0.0
0.4
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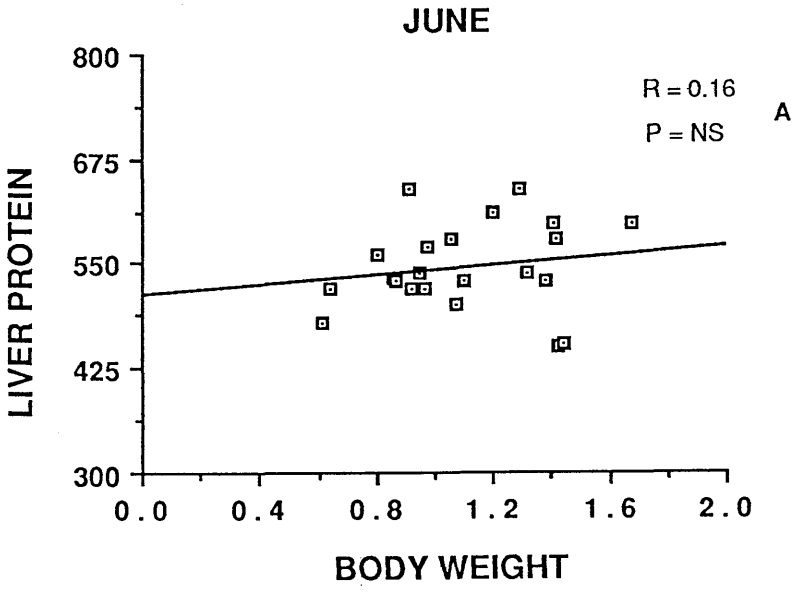
1.2
1.6
2.0
BODY WEIGHT


JUNE
R = 0.16
P = NS
A
LIVER PROTEIN
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675
550
425
300
0.0
0.4
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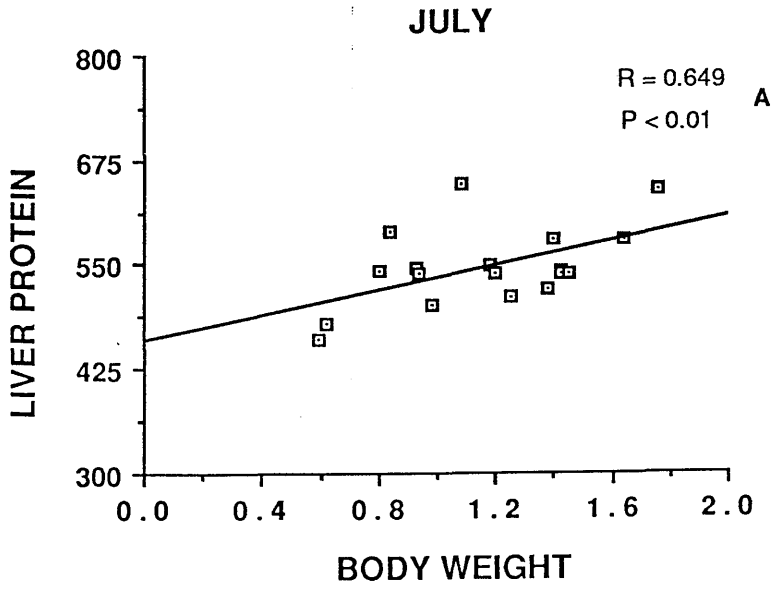
1.2
1.6
2.0
BODY WEIGHT


JULY
R = 0.649
P < 0.01
A
LIVER PROTEIN
800
675
550
425
300
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT

5.7.5.2 Body weight and gonad protein concentration

Figure 5.4 shows the scatter plots of gonad protein concentrations against body weights for each month over a period of one year. In young of the year the relationship between body weight and gonad protein concentration was negative and significant for almost the whole year, apart from October to November and May to June when the relationship was non significant.

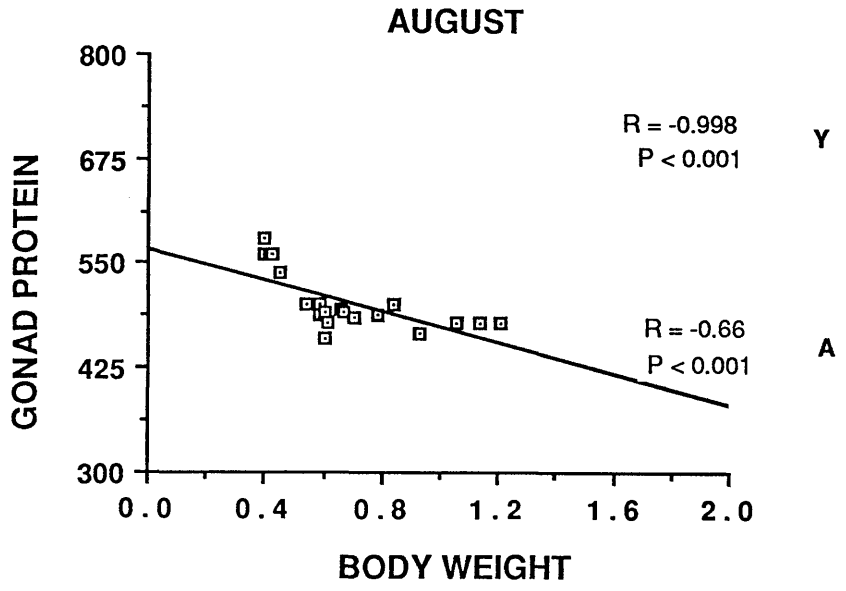
In the non breeding adult fish the relationship was negative and significant from August until October but was non-significant in November and December as gonad protein concentration dropped to very low levels.

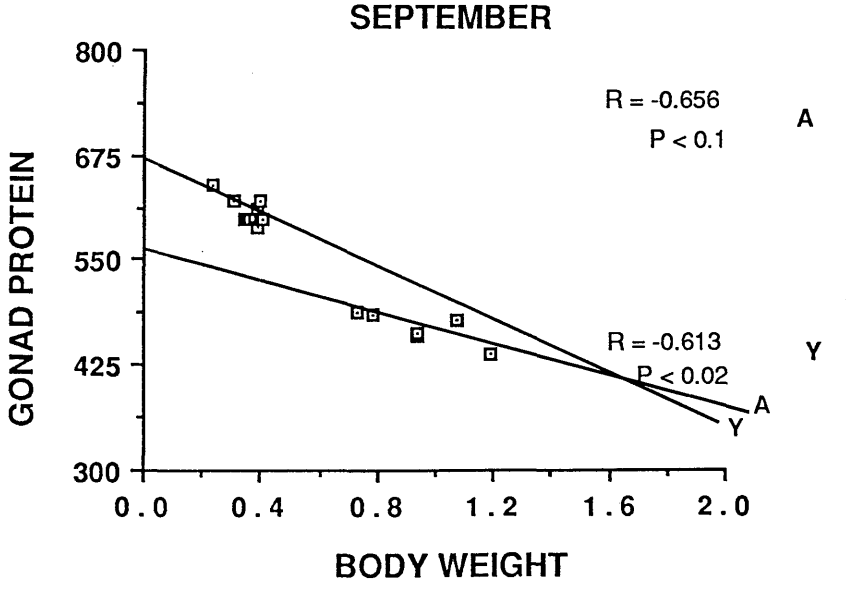
FIGURE 5.4 Scatter plots of Gonad protein against body weight for each month over a period of one year. Protein values are given in mg g^{-1} d.w and body weight in gms.

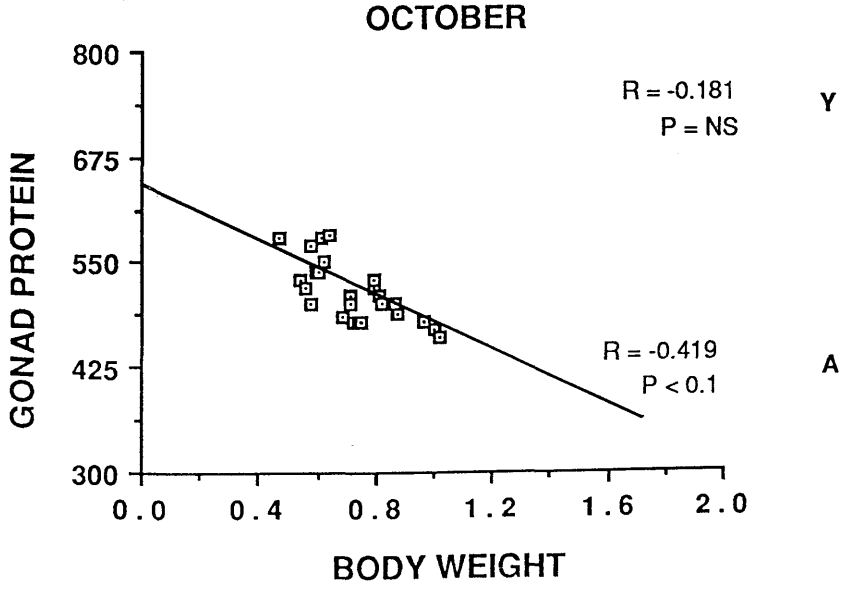
A = non-breeding adult males

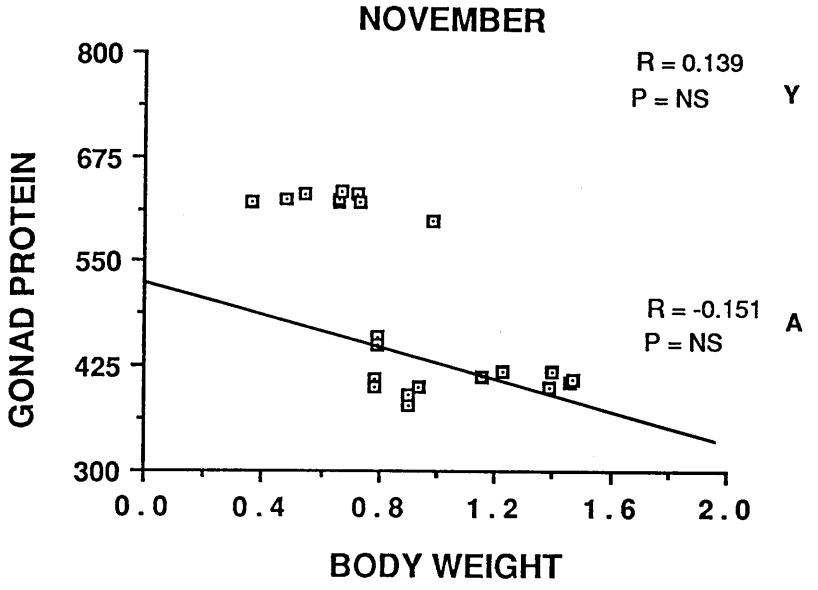
Y = young of the year

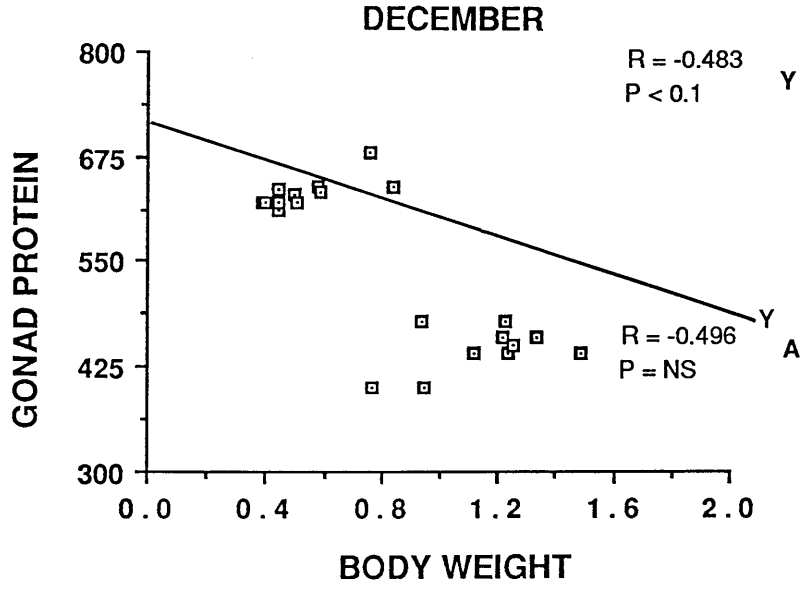
A′= breeding adult males

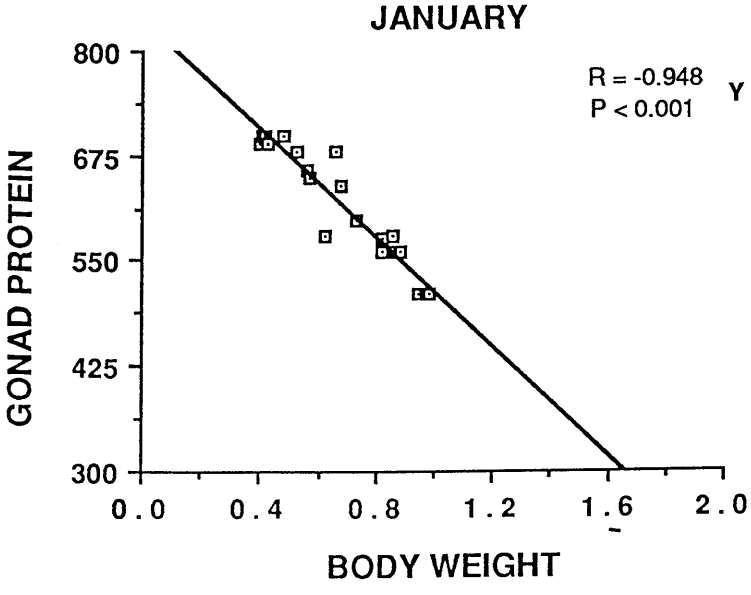

AUGUST
GONAD PROTEIN
800
675
550
425
300
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT
R = -0.998
P < 0.001
Y
R = -0.66
P < 0.001
A

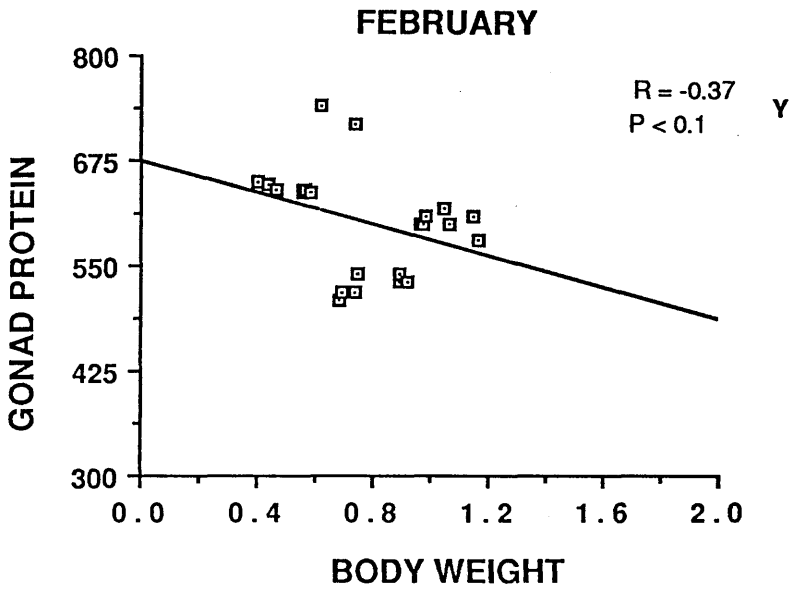

SEPTEMBER
GONAD PROTEIN
800
675
550
425
300
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT
R = -0.656
P < 0.1
A
R = -0.613
P < 0.02
Y
A
Y

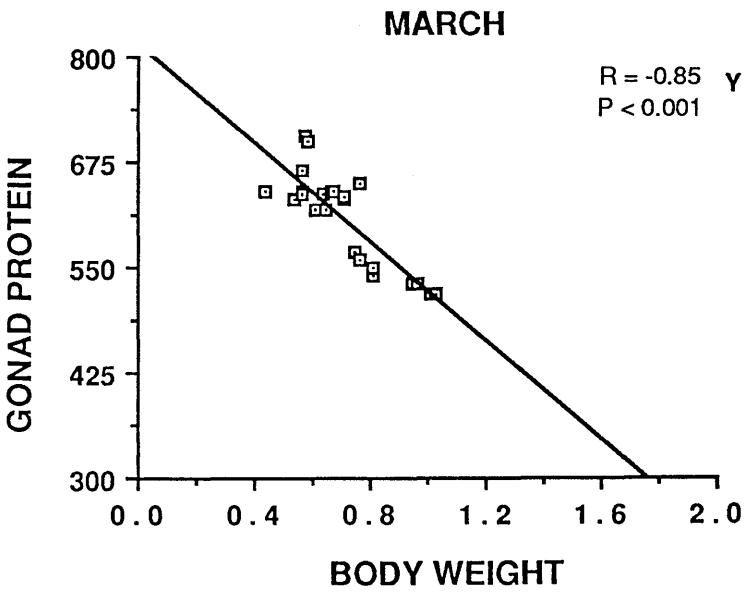

OCTOBER
GONAD PROTEIN
800
675
550
425
300
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT
R = -0.181
P = NS
Y
R = -0.419
P < 0.1
A

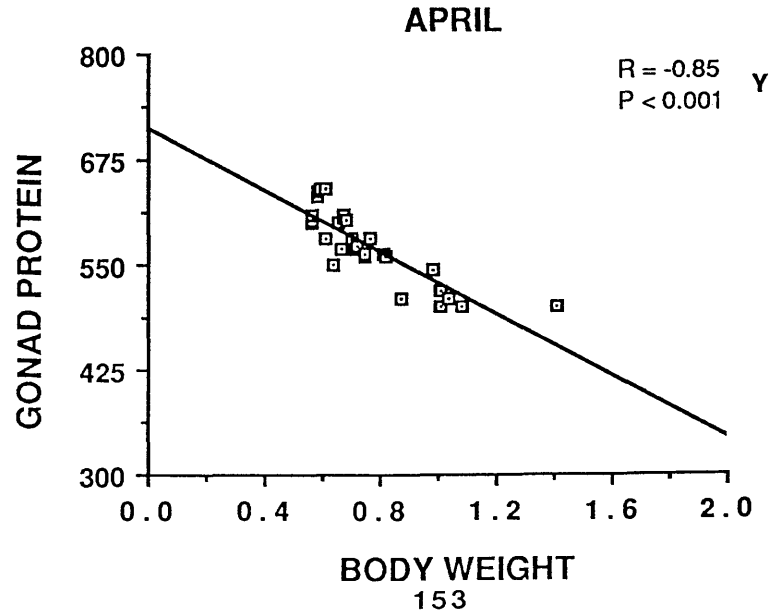

NOVEMBER
R = 0.139
P = NS
Y
R = -0.151
P = NS
A
GONAD PROTEIN
800
675
550
425
300
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT

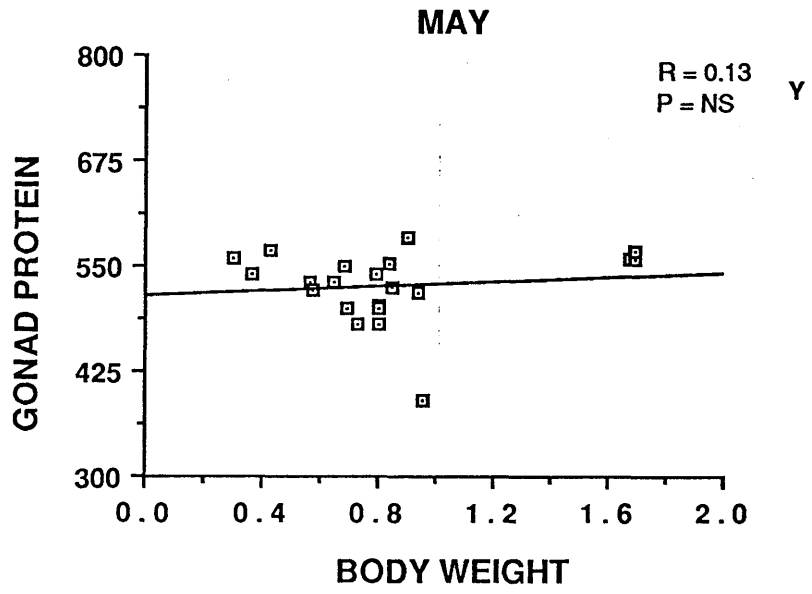

DECEMBER
R = -0.483
P < 0.1
Y
Y
R = -0.496
P = NS
A
GONAD PROTEIN
800
675
550
425
300
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT

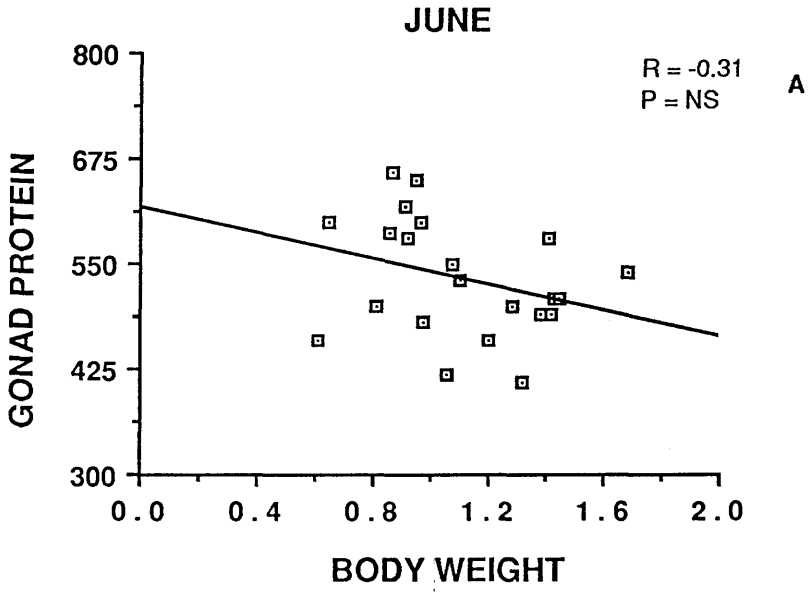

JANUARY
R = -0.948
P < 0.001
Y
GONAD PROTEIN
800
675
550
425
300
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT

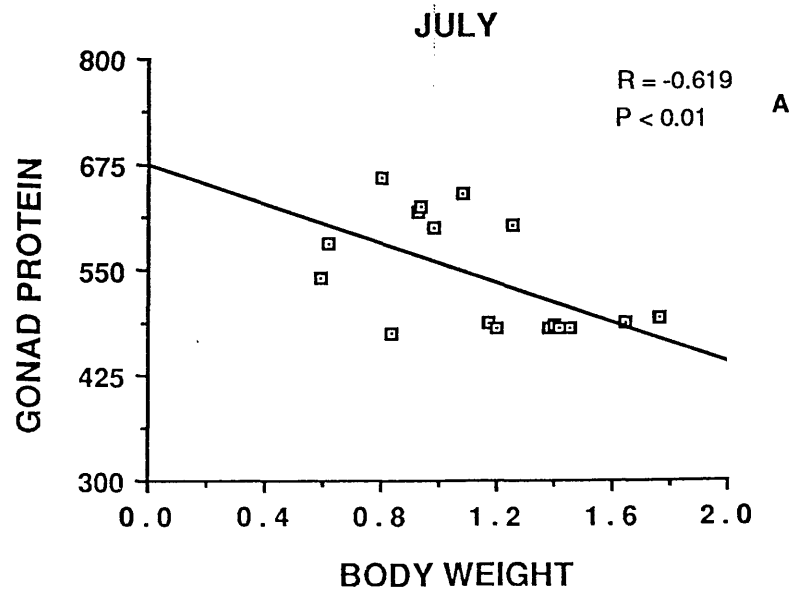

FEBRUARY
R = -0.37
P < 0.1
Y
GONAD PROTEIN
800
675
550
425
300
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT


MARCH
R = -0.85
P < 0.001
Y
GONAD PROTEIN
800
675
550
425
300
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT


APRIL
R = -0.85
P < 0.001
Y
GONAD PROTEIN
800
675
550
425
300
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT


MAY
R = 0.13
P = NS
Y
GONAD PROTEIN
800
675
550
425
300
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT


JUNE
R = -0.31
P = NS
A
GONAD PROTEIN
800
675
550
425
300
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT


JULY
R = -0.619
P < 0.01
A
GONAD PROTEIN
800
675
550
425
300
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT

5.7.5.3 Body weight and carcass protein concentration

Figure 5.5 shows the scatter plots of carcass protein concentration against body weight for each month over a period of one year. In young of the year the relationship between body weight and carcass protein concentration was negative and significant from September to May apart from November and May when it was negative but non-significant. The negative relationship arose because the larger fish had comparatively lower concentration of carcass protein than the smaller fish due to accumulation of lipid reserves. In June and July the relationship changed to a positive one as the carcass protein in the smaller fish was depleted more than in the bigger fish during the breeding season. This was probably because the smaller fish had lower energy reserves and had to draw on their carcass protein to meet the energy demands.

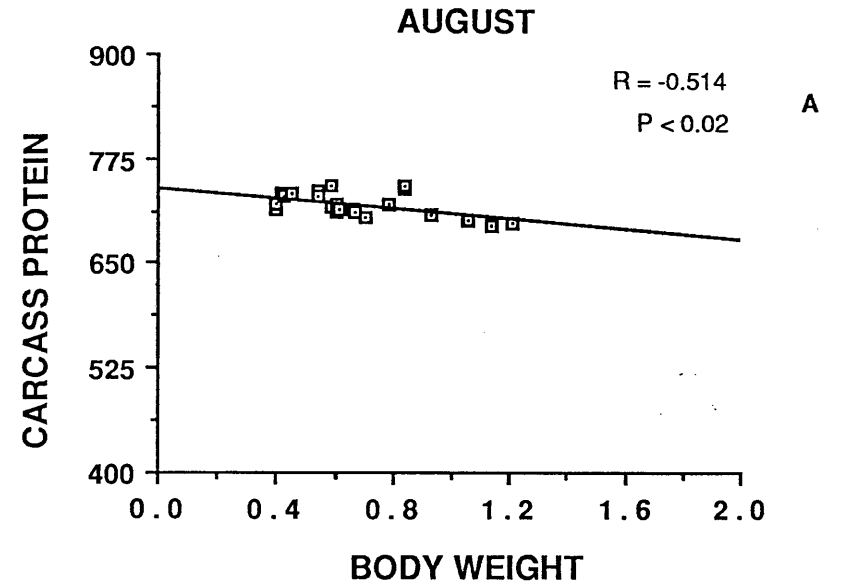
In the non-breeding adults the relationship between body weight and carcass protein concentration was negative and significant from August to December, after which this age group of fish was not encountered in the sample.

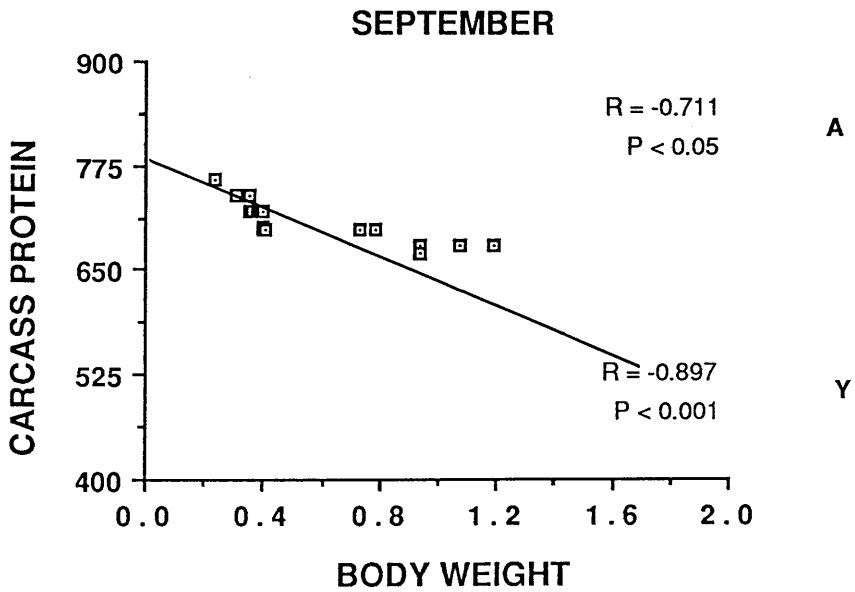
FIGURE 5.5 Scatter plots of Carcass protein against body weight for each month over a period of one year. Protein values are given in mg g^{-1} d.w and body weight in gms.

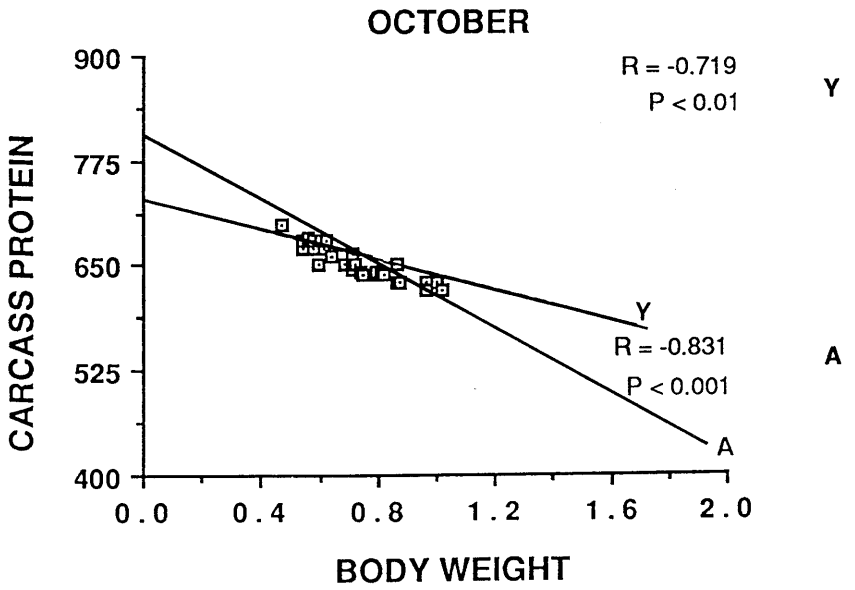
A = non-breeding adult males

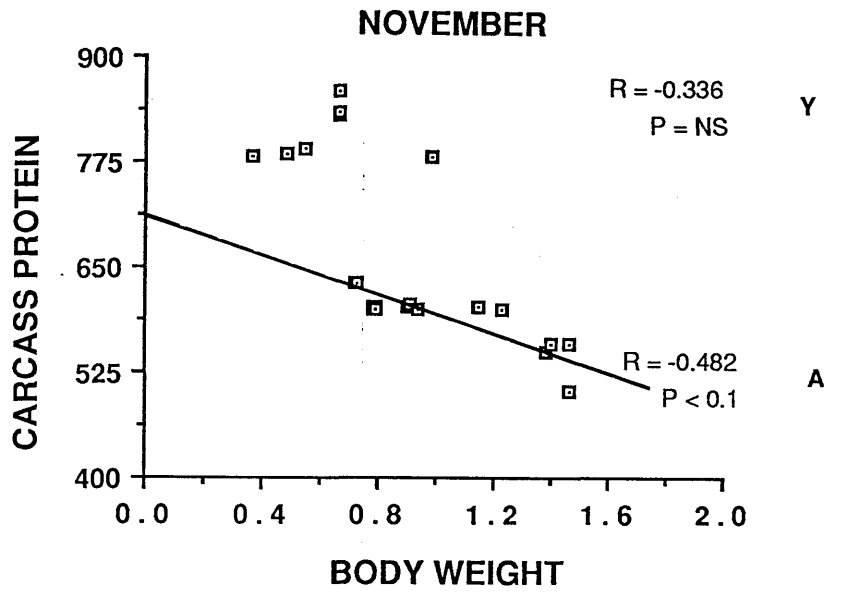
Y = young of the year

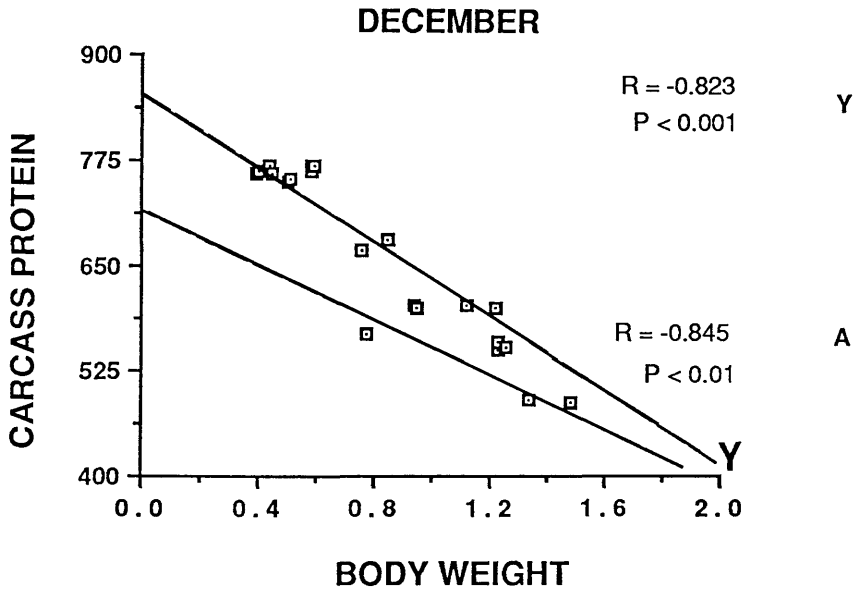
A′= breeding adult males

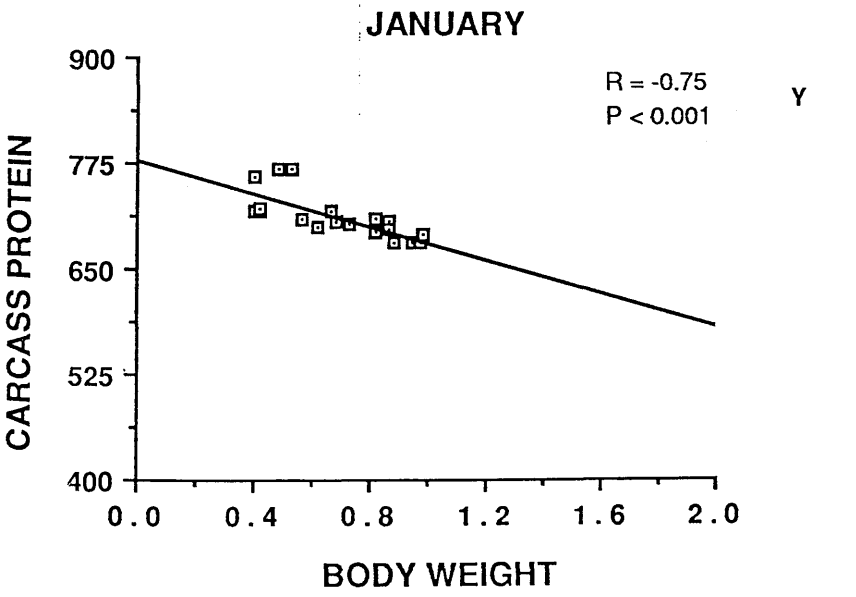

AUGUST
900
775
650
525
400
CARCASS PROTEIN
R = -0.514
P < 0.02
A
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT

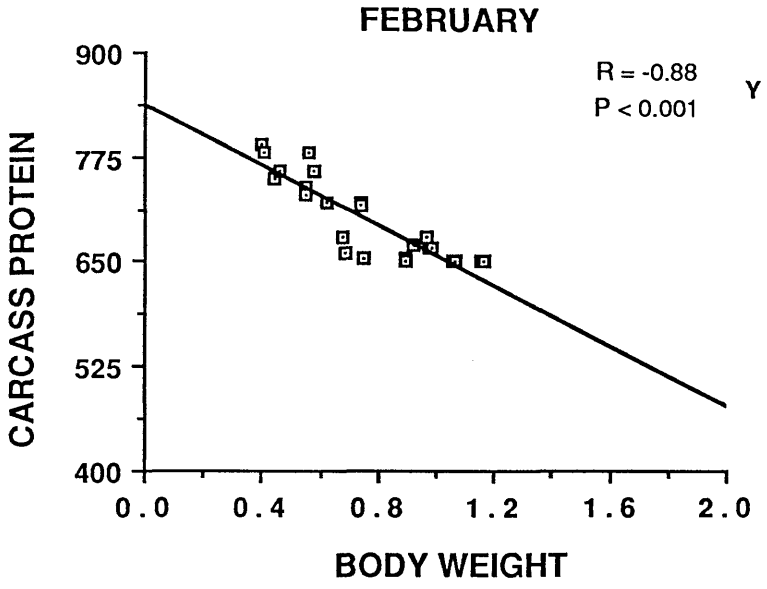

SEPTEMBER
900
775
650
525
400
CARCASS PROTEIN
R = -0.711
P < 0.05
A
R = -0.897
P < 0.001
Y
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT

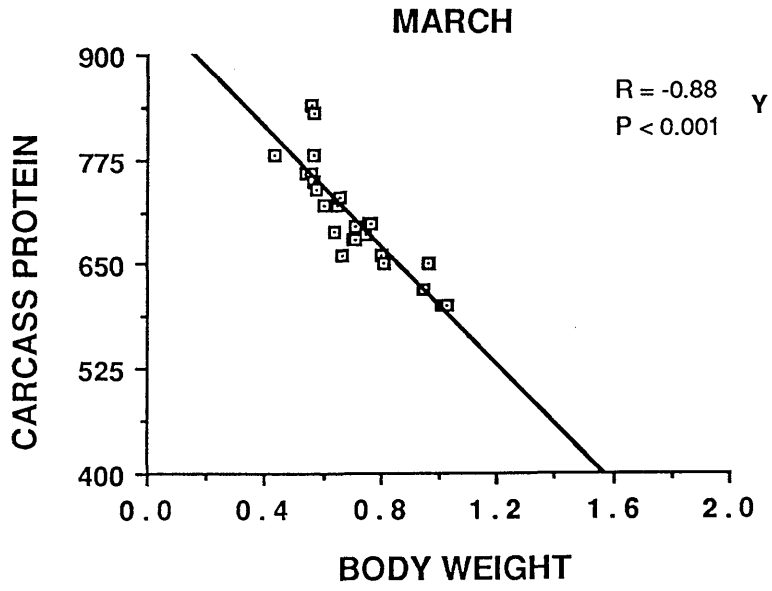

OCTOBER
900
775
650
525
400
CARCASS PROTEIN
R = -0.719
P < 0.01
Y
Y
R = -0.831
P < 0.001
A
A
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT

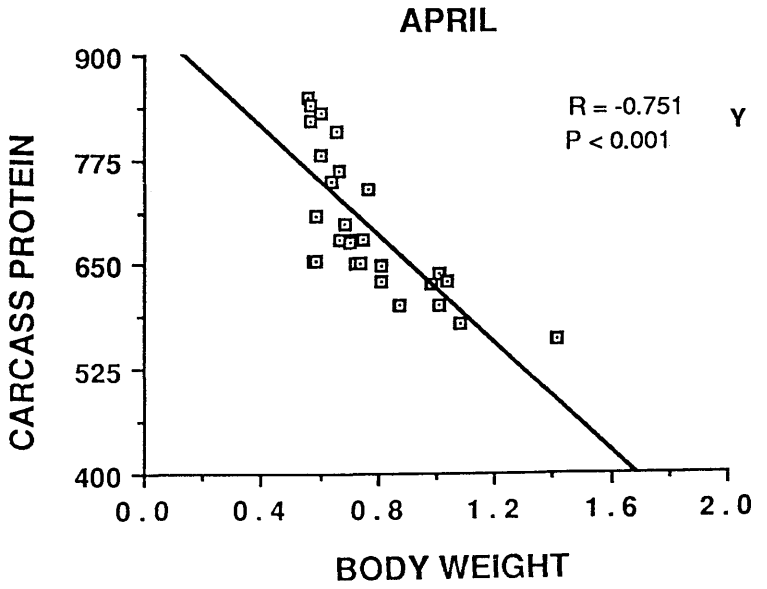

NOVEMBER
900
775
650
525
400
CARCASS PROTEIN
R = -0.336
P = NS
Y
R = -0.482
P < 0.1
A
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT


DECEMBER
900
775
650
525
400
CARCASS PROTEIN
R = -0.823
P < 0.001
Y
R = -0.845
P < 0.01
A
Y
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT


JANUARY
900
775
650
525
400
CARCASS PROTEIN
R = -0.75
P < 0.001
Y
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT


FEBRUARY
R = -0.88
P < 0.001
Y
CARCASS PROTEIN
900
775
650
525
400
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT


MARCH
R = -0.88
P < 0.001
Y
CARCASS PROTEIN
900
775
650
525
400
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT


APRIL
R = -0.751
P < 0.001
Y
CARCASS PROTEIN
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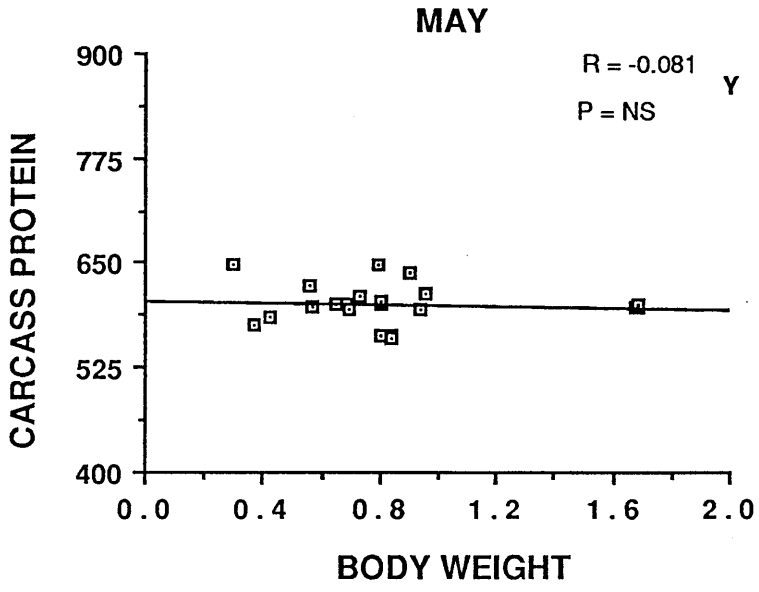
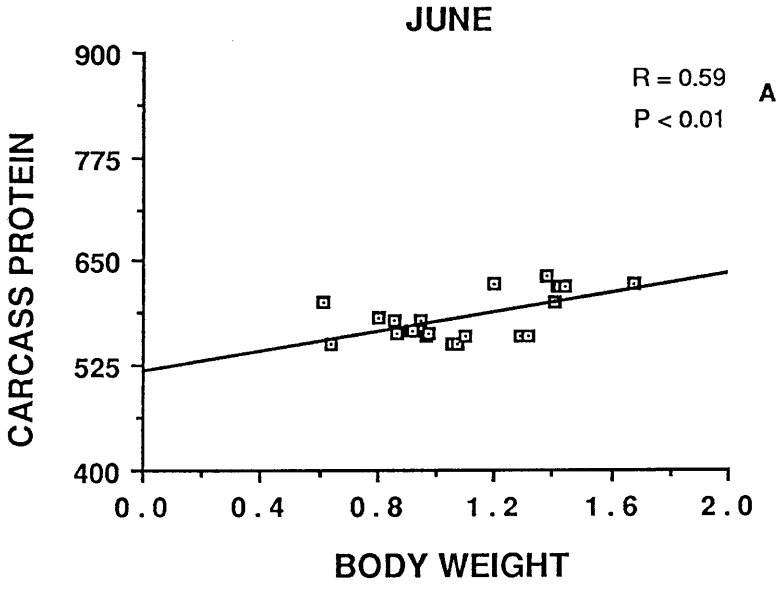
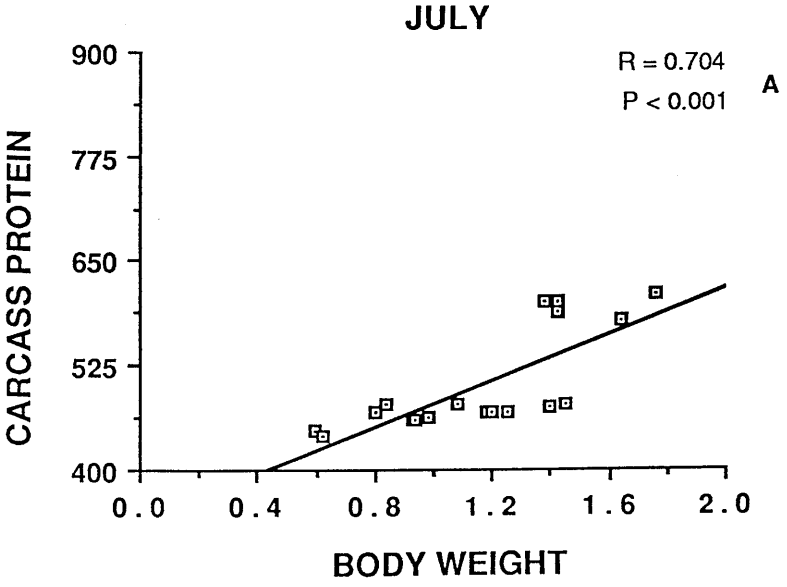
775
650
525
400
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT


MAY
R = -0.081
P = NS
Y
900
775
650
525
400
CARCASS PROTEIN
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT


JUNE
R = 0.59
P < 0.01
A
900
775
650
525
400
CARCASS PROTEIN
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT


JULY
R = 0.704
P < 0.001
A
900
775
650
525
400
CARCASS PROTEIN
0.0
0.4
0.8
1.2
1.6
2.0
BODY WEIGHT

5.8 DISCUSSION

Long range migratory animals such as birds and insects, normally acquire energy for their activities by an almost exclusive combustion of protein-free energy reserves (viz. glycogen and lipids). However, studies on fish indicate that there is no exclusive mobilisation of lipid reserves in fish during migration and breeding. Instead proteins are drawn upon in addition to lipid and glycogen reserves (as reviewed by Boetius and Boetius, 1985). Similar results have been obtained for the male three-spined stickleback in this project.

In the young of the year, protein concentrations remain more or less constant during the early non-breeding season. As the fish grow, there is a shift from initial protein growth to lipid accumulation. Thus there is a very gradual decrease in protein concentration as the fish tends to accumulate lipids. The liver and gonad protein concentrations do not change significantly during the breeding season, but the carcass protein concentration falls sharply during the months of June and July. Thus, the positive relationship between protein concentration in the liver, gonad and carcass (which exists during the non-breeding season) disappears during the breeding season due to depletion of protein.

Percentages calculated from monthly relative protein concentration (on dry weight basis) show that carcass protein constitutes about 71% of dry weight during the non-breeding season; this decreases to 58% in June and to 50% in July. These results indicate that glycogen and lipid reserves in the liver of the male

three-spined stickleback are probably insufficient to cover the energy expenditure involved in breeding especially during the month of July. Consequently protein from the carcass is drawn upon to meet the energy demand. There is preferential utilization of protein over lipid reserves of the carcass, which indicates that in the male sticklebacks there is no exclusive mobilisation of carcass lipids during the breeding season.

5.9 CONCLUSIONS

This chapter describes the annual variation in protein concentrations of the liver, gonad and carcass in the male sticklebacks. Protein concentration is less variable than glycogen and lipid reserves, although liver protein increases in the late autumn and carcass protein decreases very gradually from January onwards. This decrease in carcass protein is inversely related to lipid accumulation.

The relationship between body weight and protein concentration is predominantly negative during the non-breeding season, which reflects the relatively higher lipid reserves, particularly in the carcass of larger fish. In June and July, the negative relationship between the body weight and carcass protein concentration changes to a positive one. This is because carcass protein in the smaller fish is depleted more than in the bigger fish during the breeding season, probably due to their low levels of liver lipid and glycogen reserves.

These results indicate that protein as well as lipid and glycogen are mobilized to meet the energetic requirements of breeding male sticklebacks.

CHAPTER 6

THE RELATIONSHIP BETWEEN GLYCOGEN, LIPID AND PROTEIN CONCENTRATION

CHAPTER 6. THE RELATIONSHIP BETWEEN GLYCOGEN, LIPID AND PROTEIN CONCENTRATION

6.1 INTRODUCTION

Animal tissue consists of two major components: namely water and dry matter. The latter is divisible into subcomponents of protein, lipid, glycogen, minerals and vitamins (Love, 1970). The relative proportion of these components varies as different energy reserves are stored and depleted in different parts of the body. The relationship between the main constituents therefore varies depending on the species, season of the year, environmental parameters, stage of gonad maturity, state of nutrition and age. Studies on chemical changes in the body during periods of growth and reproduction can provide information about changes in metabolic activity.

The relationship of the main biochemical constituents in fish muscle tissue varies between species according to where the reserve lipid is stored. There is an inverse relationship between the lipid and water in fatty fish muscle such as the Atlantic herring (Clupea harengus), but in non fatty fish such as Atlantic cod and brook char (Salvelinus fontinalis) water content is inversely related to the protein level (Phillips and Livingston, 1960; Love, 1970).

In many fish from both freshwater and marine environments, marked changes occur during the breeding season in the levels of the biochemical components of the tissues. Gonad maturation is one of the important variables influencing the levels of the various biochemical

components of fish. For example, in the cat fish (Heteropneustes fossilis), the period of peak ripeness of the gonads is accompanied by a rapid depletion of the hepatic glycogen and lipid reserves. This is because the energy required for the gonad development is made available by mobilizing energy reserves from the liver. The pre-spawning period is generally characterized by a steep increase in the energy reserves. On the other hand, the breeding season registers the lowest values of energy reserves (Shreni, 1980).

In the Centracantid fish (Spicara chryselis) there is an increase in blood glucose levels during the pre-spawning period. The blood glucose comes from lipids or proteins which are all capable of being converted into glucose. In addition there is an increase in the concentration of free fatty acids (FFA) in the plasma during the pre-spawning period indicating mobilization of lipid reserves (Fernandez and Planas, 1980). All these changes result in differential depletion of biochemical components in the body compartments. For example, in the female plaice (Pleuronectes platessa) (which does not feed from December until March but grows large ovaries during this time) the body depletes 40% of its protein (out of which 7% is metabolised to provide energy and 33% is used for egg production) and 64% of its lipid (to supply energy for metabolic purposes). It is also found that there are elevated levels of plasma cortisol when nitrogen balance is negative and low levels of plasma cortisol when nitrogen balance is positive (Dawson and Grimm, 1980).

A study of changes in the relative proportions of protein, lipid and water of bluntnose minnow (Pimephales notatus) growing at different temperatures (viz. 15 ^{o}, 25 ^{o} and 30 ^{o} C) showed that temperature modifies body composition. For example, protein (as a percentage of body dry weight) decreased in fish at 25 ^{o} C and 30 ^{o} C but decreasing temperature led to significantly enhanced protein content during growth. The correlations between body constituents and body weight are high, so that estimates of body composition can be obtained from body weight for all temperature groups (Gill and Weatherley, 1984).

Biochemical analyses of the silver eel (Anguilla anguilla) show that there is no preferential mobilization of lipid reserves during a period of starvation. This is because the depletion of lipid reserves take place at a lower rate than that of protein (Boetius and Boetius, 1985). In the gulf menhaden (Brevoortia patronus) body composition changes from larvae to subadult stage. The changes include an increases in lipid reserves and a corresponding decrease in nitrogen content, showing a shift from protein growth to lipid storage which is associated with attainment of larger size (Deegan, 1986).

Results discussed in the previous chapters indicate that there are annual variations in the biochemical components in male three-spined sticklebacks. Data analyses and results described in this chapter are intended to supplement the information given in the previous chapters (Chapter 3,4 and 5).

In particular the aims are as follows:

1) To look at the interrelationship between biochemical components both outside and during the breeding season in the male three-spined stickleback.

2) To examine the annual variation in percentage composition of body of the male three-spined stickleback.

6.2 DATA ANALYSES

In order to examine the relationship between biochemical components, product-moment correlation coefficients were calculated for all possible combination of biochemical components (viz. glycogen, lipid and protein) in the different body compartments (viz. liver, gonad and carcass). Correlation coefficients were calculated separately for non-breeding season (August to May) and for the breeding season(June and July). Percentage values were calculated (from the mean absolute values of biochemical components) to obtain the annual variation in percentage composition of body.

6.3 RESULTS

Table 6.1 A to C shows correlations between the biochemical components in the liver, gonad and carcass during the non-breeding season (viz. August to May) and in June and July, the two months of the breeding season. Figures 6.1 A and 6.2 A are scattergrams showing the strong positive correlation between glycogen and lipid reserves in the liver and carcass respectively during the non-breeding season (viz. August to May). Figure 6.1 B is a scattergram showing equal degrees of depletion of glycogen and lipid reserves in the liver during the breeding season. Figure 6.2 B is a scattergram showing differential depletion of glycogen and lipid reserves in the carcass during the breeding season. Table 6.2 A-B shows the relative degrees of depletion of various energy sources during the breeding season. Figures 6.3 and 6.4 show the variation in percentage composition of the body (based on wet and dry weights respectively) of young of the year over a period of twelve months.

6.3.1 Interrelationship between biochemical components.

6.3.1.1 During the non-breeding season (August to May)

During the non-breeding season the relationship between lipid, glycogen and protein levels in the body components were as follows (See Table 6.1 A).

In the liver : Liver lipid and glycogen were positively correlated (highly significantly so) but there was no relationship between liver protein and lipid. There was a weak but significant negative relationship between liver protein and glycogen.

TABLE 6.1 A Correlations between biochemical components in in the liver, gonad and carcass during the non-breeding season (viz. August to May)

Abbreviations:

R = Product-moment correlation coefficient

P = Levels of significance

*** = P <0.001

** = P <0.01

* = P <0.05

+ = P <0.1

NS = Non-significant

N = 241 (Number of samples)

	LIPID			PROTEIN				
	LIVER	GONAD	CARCASS	LIVER	GONAD	CARCASS		
LIVER	R=0.602 P=***	R=0.842 P=***	R=0.416 P=***	R=-0.125 P=*	R=0.155 P=**	R=-0.061 P=+	LIVER	GLYCOGEN
GONAD	R=0.589 P=***	R=0.855 P=***	R=0.409 P=***	R=0.047 P= NS	R=0.289 P=***	R=-0.019 P=NS	GONAD	
CARCASS	R=0.714 P=***	R=0.826 P=***	R=0.484 P=***	R=0.141 P=**	R=0.365 P=***	R=0.159 P=**	CARCASS	
LIVER				R=0.030 P=NS	R=0.227 P=***	R=0.007 P=NS	LIVER	LIPID
GONAD				R=0.047 P=NS	R=0.306 P=***	R=0..47 P=NS	GONAD	
CARCASS				R=-0.188 P=**	R=-0.273 P=***	R=-0.322 P=***	CARCASS	

In the gonad : Gonad lipid and glycogen, gonad protein and glycogen, gonad protein and lipid were all positively correlated (highly significantly so), the relationship between glycogen and lipid being particularly strong.

In the carcass : There was a positive and highly significant relationship between carcass lipid and glycogen but a negative and highly significant relationship between carcass protein and lipid. There was a weak positive yet significant relationship between carcass protein and glycogen.

In the liver and gonad : Liver lipid and gonad glycogen, liver glycogen and gonad lipid, liver lipid and gonad protein were all positively correlated (highly significantly so). There was no relationship between liver protein and gonad glycogen, liver protein and gonad lipid, but there was a significant but weak positive relationship between liver glycogen and gonad protein.

In the liver and carcass : Liver lipid and carcass glycogen, liver glycogen and carcass lipid were all positively correlated and highly significant. There was a weak positive relationship between liver protein and carcass glycogen but a weak negative relationship between liver protein and carcass lipid. There was no relationship between liver lipid and carcass protein but there was a very weak negative relationship between liver glycogen and carcass protein.

In the gonad and carcass: Gonad lipid and carcass glycogen, gonad glycogen and carcass lipid, gonad protein and carcass glycogen were all positively correlated and highly significant. Gonad protein and

carcass lipid were negatively correlated and highly significant. There were no relationship between gonad glycogen and carcass protein, gonad lipid and carcass protein.

Thus, a strongly positive and highly significant relationship exists between glycogen and lipid reserves in all body compartments. The relationship between glycogen and protein levels was less marked and more variable being positive in the gonad and carcass and weakly negative in the liver. Lipid and protein levels were strongly negatively related in the carcass, positively related in the gonad, and there was no relationship between them in the liver.

6.3.1.2 During the breeding season (June and July)

During the breeding season the relationship between glycogen, lipid and protein levels in the body compartments were as follows (See Table 6.1 B and C).

In the liver: There were no significant relationships between lipid, glycogen and protein in the liver during June and July.

In the gonad: Gonad lipid and glycogen, gonad protein and glycogen were significantly positively correlated in June and July. Gonad protein and lipid were positively correlated (highly significantly so) in June but in July they were not significantly related.

In the carcass: There were no relationships between carcass lipid and glycogen in June and July. There was a weak negative relationship between carcass protein and lipid in June but in July there was no

TABLE 6.1 B Correlations between biochemical components in in the liver, gonad and carcass during the breeding season (June)

Abbreviations:

R = Product-moment correlation coefficient

P = Levels of significance

*** = P <0.001

** = P <0.01

* = P <0.05

+ = P <0.1

NS = Non-significant

N = 22 (Number of samples)

	LIPID			PROTEIN			
	LIVER	GONAD	CARCASS	LIVER	GONAD	CARCASS	
LIVER	R=0.100 P=NS	R=0.193 P=NS	R=0.197 P=NS	R=-0.075 P=NS	R=-0.079 P=NS	R=-0.187 P=NS	
GONAD	R=0.130 P=NS	R=0.648 P=**	R=-0.185 P=NS	R=-0.165 P= NS	R=0.644 P=**	R=-0.073 P=NS	GLYCOGEN
CARCASS	R=0.105 P=NS	R=-0.162 P=NS	R=0.045 P=NS	R=-0.035 P=NS	R=-0.272 P=NS	R=-0.087 P=NS	
LIVER				R=0.267 P=NS	R=0.016 P=NS	R=-0.507 P=*	
GONAD				R=-0.316 P=NS	R=0.763 P=***	R=-0.151 P=NS	LIPID
CARCASS				R=0.319 P=NS	R=-0.170 P=NS	R=-0.488 P=*	

TABLE 6.1 C Correlations between biochemical components in in the liver, gonad and carcass during the breeding season (July)

Abbreviations:

R = Product-moment correlation coefficient

P = Levels of significance

*** = P <0.001

** = P <0.01

* = P <0.05

+ = P <0.1

NS = Non-significant

N = 18 (Number of samples)

	LIPID			PROTEIN			
	LIVER	GONAD	CARCASS	LIVER	GONAD	CARCASS	
GLYCOGEN	R=0.215 P=NS	R=0.162 P=NS	R=0..085 P=NS	R=-0.204 P=NS	R=0..835 P=***	R=-0.566 P=*	LIVER
	R=0.168 P=NS	R=0.532 P=*	R=-0.129 P=NS	R=-0.137 P= NS	R=0..532 P=*	R=-0.480 P=*	GONAD
	R=0..270 P=NS	R=0.288 P=NS	R=0.058 P=NS	R=-0.200 P=NS	R=0.613 P=**	R=-0.017 P=NS	CARCASS
LIPID				R=0.170 P=NS	R=0.177 P=NS	R=-0.242 P=NS	LIVER
				R=0.036 P=NS	R=0.153 P=NS	R=-0.584 P=**	GONAD
				R=0.161 P=NS	R=0.017 P=NS	R=-0.365 P=NS	CARCASS

relationship. There were negative relationships between carcass protein and glycogen in June and July (but not significantly so).

In the liver and gonad: There were no relationships between the biochemical components in the liver and gonad during the breeding season, other than between liver glycogen and gonad protein in July which was positively correlated (highly significant).

In the liver and carcass: There were no relationships between glycogen, lipid and protein levels in the liver and carcass during the breeding season, other than weak negative relationships between liver lipid and carcass protein in June and liver glycogen and carcass protein in July.

In the gonad and carcass: In June there were no relationships between the biochemical components in the gonad and carcass. In July there were no relationships between lipid and glycogen reserves, and gonad protein and carcass lipid. The relationships between gonad lipid and carcass protein, gonad glycogen and carcass protein were negative (significant). There was a positive (significant) relationship between gonad protein and carcass glycogen in July.

Thus there were no relationships between the biochemical components in the liver and carcass in June but in the gonad they were all positively correlated (significant). In July there were no relationships between glycogen and lipid reserves in the liver and carcass but these were positively correlated and weakly significant in the gonad. The relationships between glycogen and protein levels were strongly negative (highly significant) in the carcass and were

positive in the gonad but there was no relationship between their levels in the liver. There were no relationships between protein and lipid levels in all body compartments during July.

6.3.1.3 Differences in the relationship between biochemical components during the non-breeding season and breeding season.

Many of the significant relationships between glycogen, lipid and protein levels in the different body compartments (of the male three-spined stickleback) disappeared once the breeding season started. In some cases the relationship disappeared because both biochemical components dropped to uniformly low levels. In other cases the relationship disappeared because one of the biochemical component was differentially depleted. This section indicates the specific case and gives scattergrams (see Fig 6.1 A-B and 6.2 A-B) to illustrate representative examples.

During the non-breeding season the relationships between glycogen and lipid reserves in all body compartments were strongly positive (highly significantly so). This indicates that lipid and glycogen reserves were accumulated parallely during the non-breeding season. The strong positive correlation between glycogen and lipid reserves in the liver (see Fig 6.1 A) and in the carcass (see Fig 6.2 A) disappeared during the breeding season. The disappearance of the strong positive correlation between glycogen and lipid stores in the liver was a result of equal degrees of depletion of both reserves to uniformly low levels. In July, about 98% of glycogen and 95% of lipid reserves were depleted from the liver (on wet weight basis) (see Fig

Figure 6.1 A Scattergram showing the strong positive correlation between glycogen and lipid reserves in the liver of the male stickleback during the non-breeding season (viz. August to May)

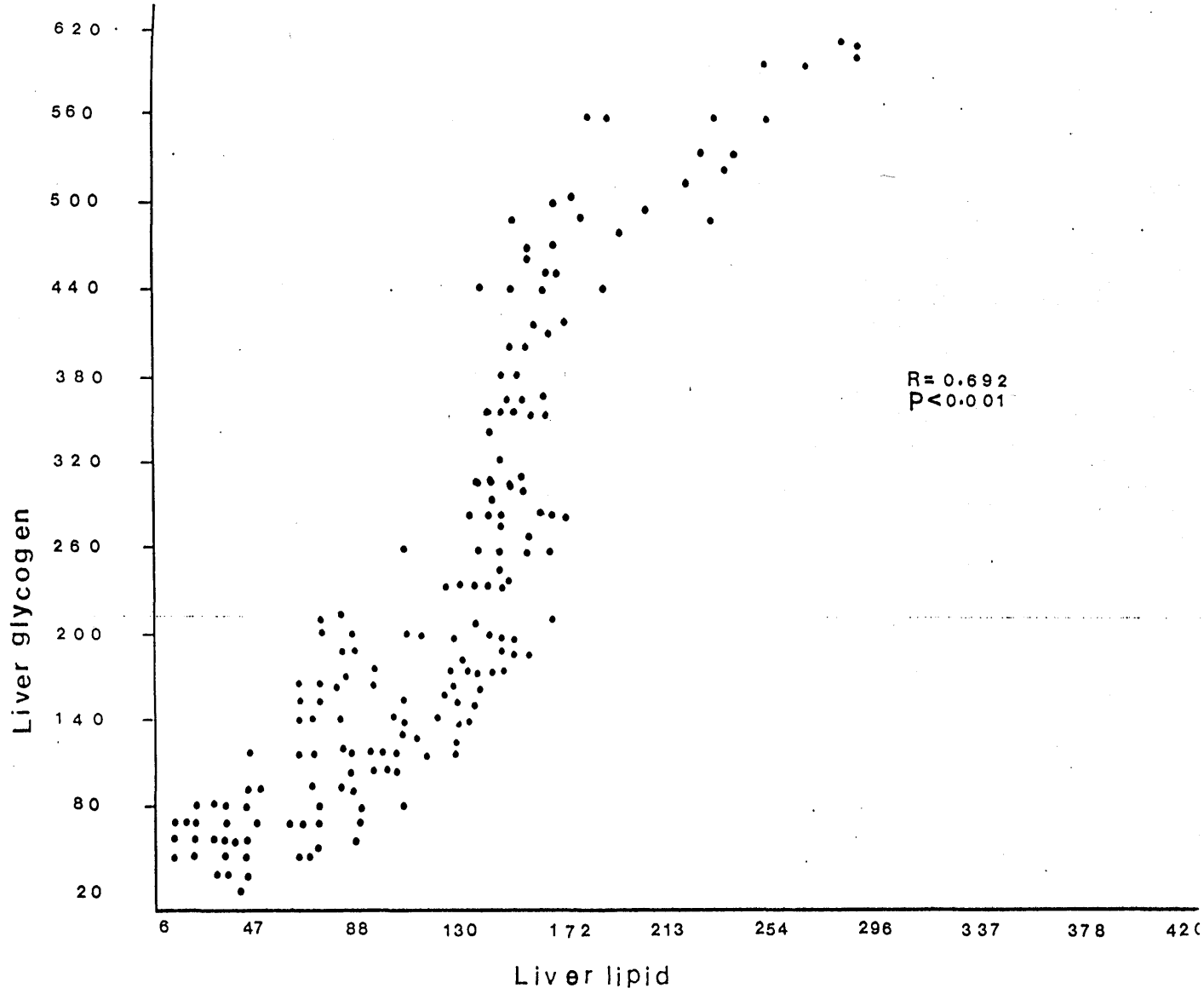

620
560
500
440
380
320
260
200
140
80
20
Liver glycogen
6
47
88
130
172
213
254
296
337
378
420
Liver lipid
R= 0.692
P<0.001

Figure 6.2 A Scattergram showing the strong positive correlation between glycogen and lipid reserves in the carcass of the male stickleback during the non-breeding season (viz. August to May)

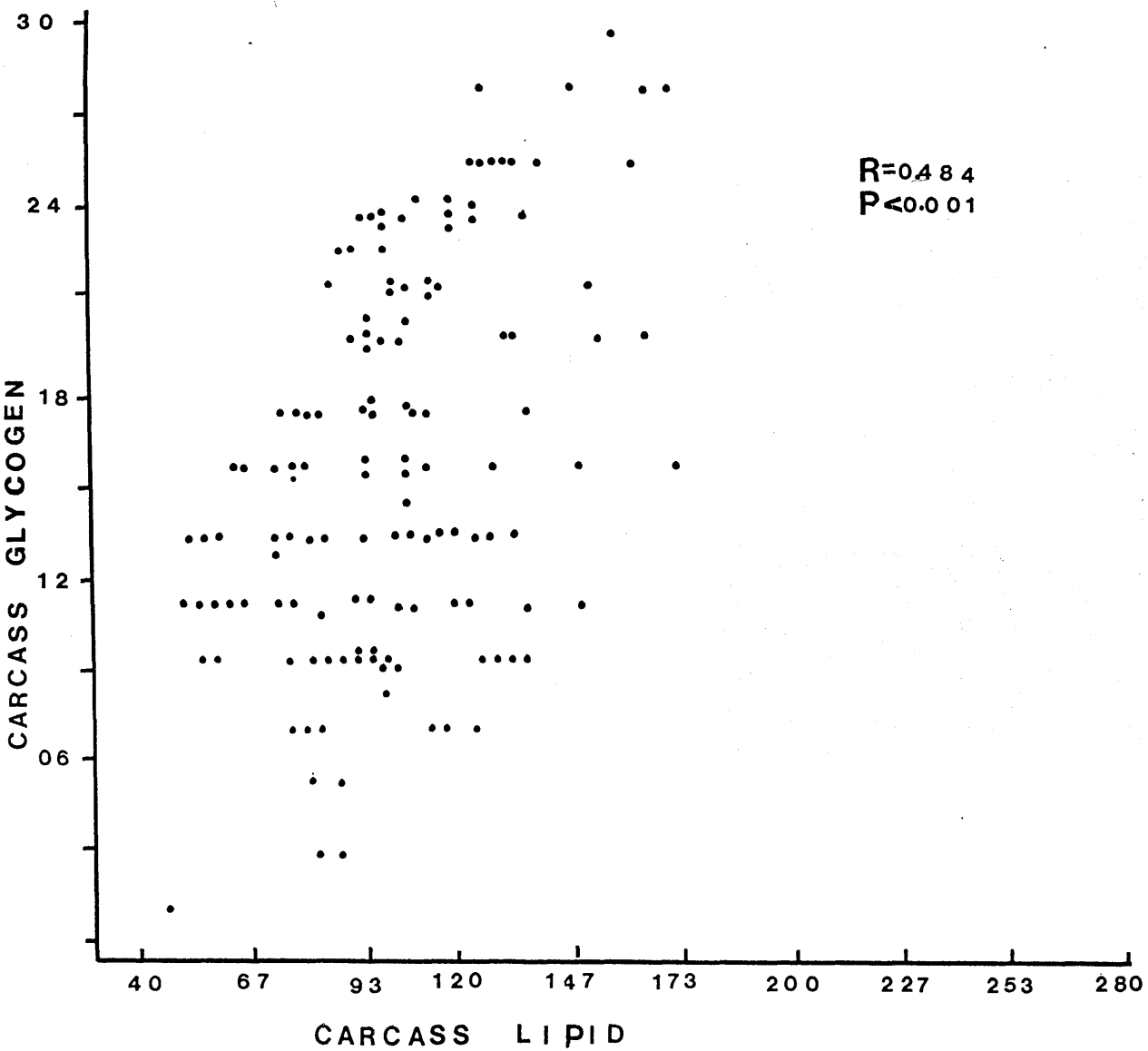

30
24
18
12
06
CARCASS GLYCOGEN
R=0.484
P<0.001
40
67
93
120
147
173
200
227
253
280
CARCASS LIPID

Figure 6.1 B Scattergram showing depletion of glycogen and lipid reserves in the liver of the male stickleback in July

Figure 6.2 B Scattergram showing differential depletion of glycogen and lipid reserves in the carcass of the male stickleback in July

Figure 6.1B

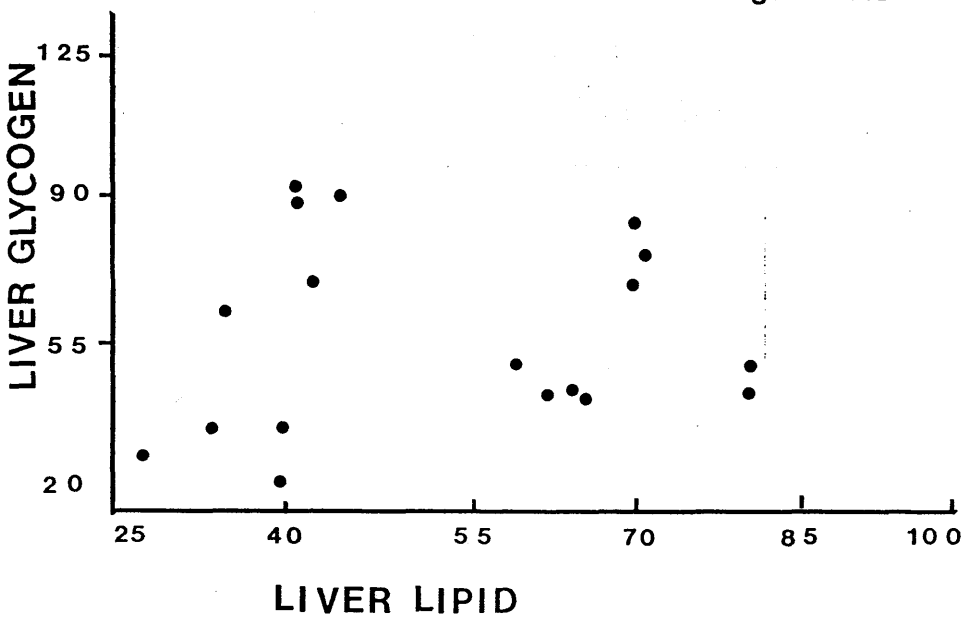
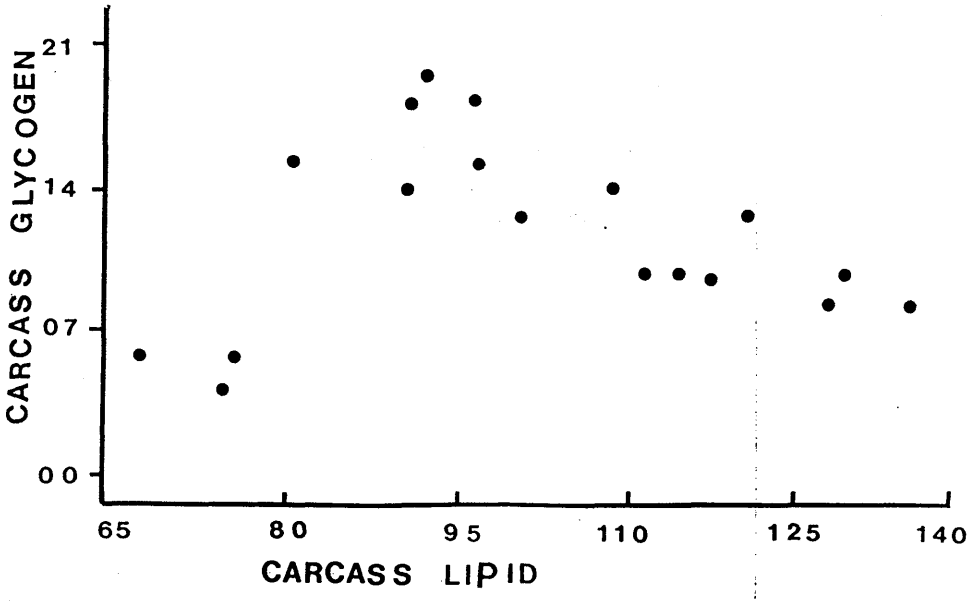


Figure 6.2B



6.1 B). On the other hand the positive correlation between glycogen and lipid stores in the carcass disappeared due to differential depletion of these reserves (carcass glycogen having depleted more markedly than carcass lipid). In July, about 67% of glycogen and 30% of lipid stores were depleted from the carcass (on wet weight basis) (see Fig 6.2 B). There were no relationships between glycogen and lipid levels in the liver and carcass during the breeding season, but these were positively correlated (weakly significant) in the gonad. As a result of depletion of energy stores the fish had very low glycogen and lipid reserves by the end of the breeding season which explains the disappearance of strong positive correlation.

The relationships between glycogen and protein levels were not clearly marked during the non-breeding season. Levels were weakly negatively related in the liver but were positively correlated in the carcass and gonad. During the breeding season there were no relationships between glycogen and protein levels in the liver due to depletion. In June there was a weak negative correlation between glycogen and protein levels in the carcass and in July this relationship was strongly negative (highly significant).

There were no relationships between lipid and protein levels in the liver either outside or during the breeding season. The relationships between lipid and protein in the gonad were positive (highly significant) outside the breeding season and in June but there was no relationship in July. Lipid and proteins had a negative (highly significant) relationship in the carcass during the non-breeding

season but this relationship was weaker in June and there was no relationship in July.

6.3.2 Percentage composition of body.

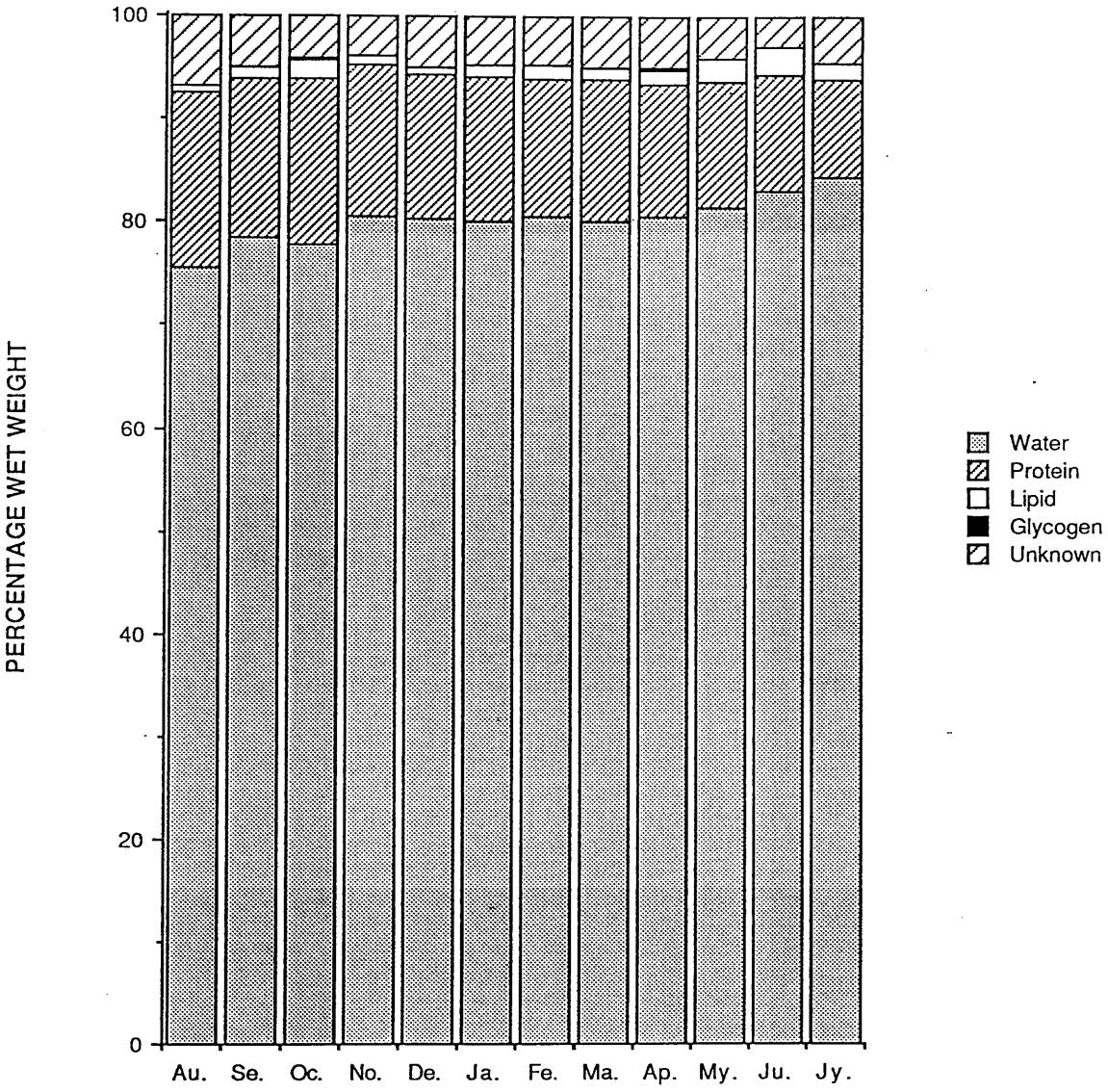
6.3.2.1 Percentage composition of body wet weight.

Figure 6.3 depicts percentage composition of body wet weight in young of the year over a period of twelve months.

In the young of the year the percentage of water was 75.5% in August; this increased steadily to 80% by November and went up to 84.3% during the breeding season. Protein made up 17% of wet weight in August; this decreased to 14.8% by November and declined sharply to 9.7% in the following July. There was an approximate inverse relationship between water and protein levels. About 0.65% of the weight of the young of the year consisted of lipid reserves; this steadily increased to 2% by October but declined during the winter to 0.63%. Thereafter the lipid reserves increased to 2.69% but were depleted to 1.5% by July. The glycogen reserves constituted a small proportion of the total biochemical components of the body. In the young of the year the percentage of glycogen reserves was 0.03% in August which increased to 0.11% by the beginning of the breeding season. By July there was only 0.02% of glycogen reserves due to heavy depletion. The percentage represented as unknown in the stack-columns was presumably ash (which was not estimated).

Figure 6.3 Stack-columns showing the annual changes in percentage composition of body wet weight

% Composition of body wet weight



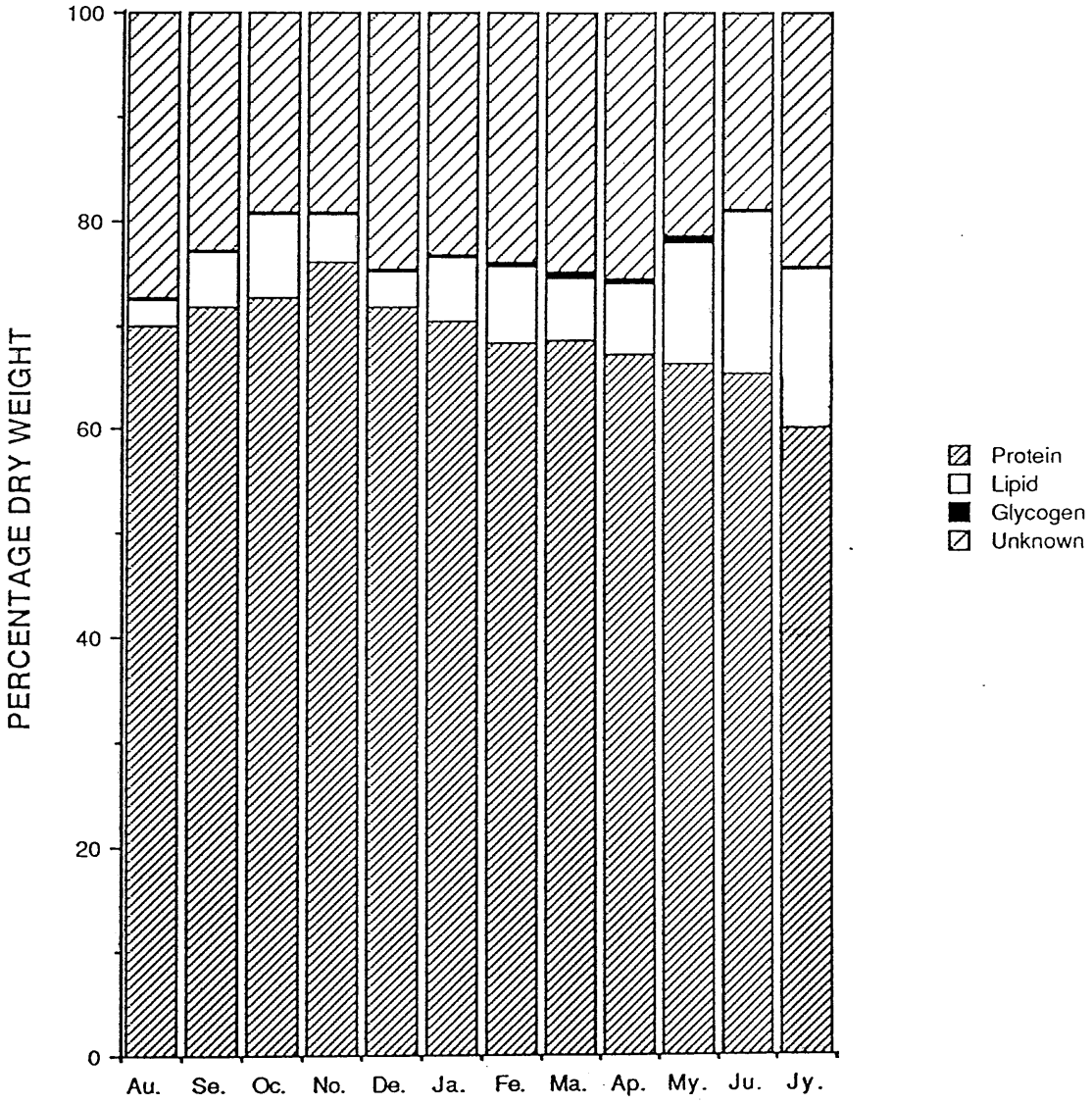
6.3.2.2 Percentage composition of body dry weight.

Figure 6.4 depicts percentage composition of body dry weight in young of the year over a period of twelve months.

Protein made up 69.8% of the dry weight of the young of the year in August, when it formed bulk of the dry matter. The percentage of protein increased steadily up to 75.3% in November followed by a gradual decline and in July protein constituted only 58% of the dry matter. In August, lipid constituted 2.6% of the dry matter in young of the year. There was a gradual increase in the lipid reserves up to October which was checked during winter. From January the lipid reserves increased until the start of breeding season when it constituted 15.5% of dry matter. In July the liver lipid reserves were completely depleted but those of the carcass was not. In August the glycogen reserves constituted 0.13% of dry matter and by April this had increased to 0.6%. By July the glycogen reserves had been depleted to a level of 0.2% of the dry matter. The remaining fraction of the dry matter probably represents the ash conponent.

Figure 6.4 Stack-columns showing the annual changes in percentage composition of body dry weight

% COMPOSITION OF BODY DRY WEIGHT



MONTH

6.3.3 Depletion of energy sources during the breeding season

The extent of depletion of energy sources due to reproductive activity was estimated by expressing the biochemical component levels in June and July as a percentage (wet weight) of the peak levels (in April/May) prior to the breeding season. The results are shown in Table 6.2 A-B.

Levels of all energy sources fell from peak levels during the breeding season, and in each case the fall was more marked in July than in June. By the end of the breeding season liver lipid and glycogen reserves were severely depleted. Carcass glycogen was also heavily depleted by two thirds. Carcass protein and carcass lipid were depleted by one third. The depletion in carcass glycogen was greater in the period up to June than between June and July. On the other hand, the drastic depletion on liver glycogen occurred in July. Liver lipid was depleted equally in June and July, as was carcass protein. Carcass lipid stores were not depleted in June (instead there was a slight increase), but were depleted by approximately 30% in July.

Table 6.2 Relative degrees of depletion of various energy sources during the breeding season.

6.2 A June

Variable	% Depletion
Liver lipid	57
Carcass glycogen	56
Liver glycogen	33
Carcass protein	15
Carcass lipid	2

6.2 B July

Variable	% Depletion
Liver glycogen	98
Liver lipid	95
Carcass glycogen	67
Carcass protein	38
Carcass lipid	30

6.4 DISCUSSION

Glycogen and lipid reserves

In simple chronological terms, there is a clear parallelism in the trends of accumulation of glycogen and lipid stores in all body compartments during the non-breeding season (see chapters 3 and 4). This is reflected in the strong positive correlations between glycogen and lipid reserves in the liver, gonad and carcass outside the breeding season. Dramatic changes occur in these relationships during the breeding season, essentially due to depletion of either one or both energy reserves. The strong positive correlations between glycogen and lipid reserves in the liver disappear altogether in June and July due to equal degrees of depletion of both reserves to uniformly low levels. In July, about 98% of glycogen and 95% of lipid reserves are depleted from the liver (on wet weight basis). On the other hand the positive correlations between glycogen and lipid stores in the carcass disappear due to differential depletion. About 67% of glycogen but only 30% of lipid stores are depleted from the carcass (on wet weight basis) in July. In the gonads, glycogen and lipid stores are strongly positively correlated from August to May but during the breeding season the relationship becomes weaker as the reserves are gradually depleted (22% of glycogen and 25% of lipid reserves).

Glycogen and protein levels

The relationship between glycogen and protein levels are not pronounced in any of the body compartments. This is probably because

protein constitutes bulk of the dry matter (about 72% to 76%) whereas glycogen constitutes only a fraction of it (about 0.03% to 0.05%). However, if glycogen and lipid reserves tend to increase during the non-breeding season then it is expected that the relative protein level should decrease to maintain a balanced body composition. This expectation is fulfilled by the negative correlations between glycogen and protein levels in the liver during the non-breeding season.

Lipid and protein levels

Levels of these two biochemical components are strongly negatively related in the carcass (but not in the liver) during the non-breeding season. This is expected because when the fish grow there is a shift from protein growth to lipid storage associated with attainment of a larger body size (Deegan, 1986). The inverse relationship between lipid and protein levels in the carcass reflect the increase in lipid stores and a decrease in relative protein level during the non-breeding season. During the breeding season, the strong negative relationship between protein and lipid stores in the carcass becomes weak in June and disappears in July due to gradual depletion of both components.

Energy sources

The relative degrees of depletion of the various energy sources during the breeding season of the mature male three-spined stickleback, suggests that energy for reproductive activities comes from liver lipid, liver glycogen, carcass glycogen, carcass protein and carcass lipid. Energy reserves are markedly depleted by June, but have even lower levels in July (see Table 6.2).

The differences in the levels of depletion of energy reserves during the months of June and July may be due to the following reasons: a) There may be two different categories of males breeding during these months. Those males which accumulate sufficient energy reserves and are bigger at the beginning of the breeding season breed actively in June and do not breed again. Those males which are smaller and with low energy reserves in May, grow up and breed in July and their poor body condition is reflected in the relative degrees of depletion in July. A third category of male exists, namely those males which fail to reach a sufficient size in their full summer of life and do not breed at all. These may be the late hatched fry of the previous season (Ukegbu, 1986).

b) Alternatively, those males which breed in June may breed again in July thus depleting their reserves still further. The fish that breed in July have therefore already used more of their carcass protein than those fish that breed in June. Carcass protein is depleted by 15% (on wet weight basis) in June but is depleted by 38% in July. Since the fish that breed in June deplete their energy reserves in the liver (33% of glycogen and 57% of lipid) and carcass (56% of glycogen), in July they deplete their carcass protein to meet the additional energy expenditure.

Observations in the field (during sampling) and in the laboratory indicate very high mortality in post-spawning males. This suggests that males breed just once and therefore favours the first explanation. However, further studies should be carried out to clarify this point.

6.5 CONCLUSIONS

In the young male sticklebacks protein forms the bulk of the dry matter. An increase in body size is accompanied by a shift from protein to lipid accumulation. This is reflected in the strong negative relationship between lipid and protein concentration in the carcass. Glycogen and lipid reserves accumulate in all body compartments during the non-breeding season. This results in the strong positive correlation between glycogen and lipid stores in the liver, gonad and carcass. Dramatic changes occur in these relationships during the breeding season, due to different degrees of depletion. The strong positive correlation between glycogen and lipid reserves in the liver disappears during the breeding season due to drastic depletion of both reserves. On the other hand the positive correlation between glycogen and lipid stores in the carcass disappears due to differential depletion, carcass glycogen having depleted more markedly than carcass lipid.

CHAPTER 7

THE RELATIONSHIPS BETWEEN ENERGY RESERVES, BODY SIZE, CONDITION AND GONADAL MATURATION

CHAPTER 7 THE RELATIONSHIP BETWEEN ENERGY RESERVES, BODY SIZE, CONDITION AND GONADAL MATURATION

7.1 INTRODUCTION

Changes in body composition of fish in relation to growth and reproduction have been studied by many workers (Love, 1970; Craig, 1977; Wootton et al., 1978; Fernandez and Planas, 1980; Dawson and Grimm, 1980; Gill and Weatherley, 1984; Boetius and Boetius, 1985, Deegan, 1986). Most of their observations were described in the previous chapter. Work in this field is done by determining the biochemical composition of various body compartments from wild fish population or fish reared under laboratory conditions. The results of such studies are related to seasonal changes in body composition and reproductive cycles of fish.

A study on the perch (Perca fluviatilis) describes in detail the length-weight relationship, changes in gonad weight and body condition of perch population in Windermere Lake (Le Cren, 1951). Body composition in the perch (P. fluviatilis) has been studied with reference to seasonal changes and reproduction (Craig, 1977). The seasonal change in lipid and glycogen reserves of the different body compartments (viz. liver, gonad and carcass) in female three-spined sticklebacks from a Welsh upland and a lowland river population has been related to seasonal changes and breeding. The somatic condition factor, the hepatosomatic index and gonadosomatic index have been studied in addition to the overall condition factor of the body (Wootton et al., 1978).

7.1.1 Condition factor

The condition factor is a traditional way of estimating the body condition in fish and is calculated as a ratio between the weight and length. The relationship between the weight and length of fish is expressed mathematically by the following equation:

$$W = a \ L^{n}$$

where W is weight, L is length, a is a constant and n an exponent. A linear relationship between weight and length is given by the equation:

$$\log W = \log a + n \log L$$

which indicates that a plot of the logarithm of weight against the logarithm of length yields a straight line whose slope is n which usually lie between 2.5 and 4.0 for various species of fish. The relative condition factor not based on the ideal length-weight relationship is calculated from the following equation :

$$Kn = \frac{W}{a \ L^{n}}$$

The relative condition factor is designated by Kn (Le Cren, 1951). If the fish maintains the same shape throughout its growth, then n = 3, so the equation can be written as follows:

$$W = a\ L^{3}$$

The length-weight relationship in three-spined stickleback is generally cubic. For stickleback weighing more than 0.1 gm the value for n ranges from 2.945 (Pennycuick, 1971) to 3.01 (Wootton, 1976). The constant a in the above equation can be used as an indicator of the condition factor of the fish :

$$a = \frac{W}{L^{3}} = \text{condition factor.}$$

This is known as the condition factor based on the cube law thus distinguished from the relative condition factor.

Differences in condition factor have been used to compare the 'fatness' of fish, and are based on the hypothesis that the heavier fish of given length are in better condition. The overall condition of two Welsh populations of female three-spined stickleback has been measured by the following equation (Wootton et al., 1978)

$$CF = \frac{W}{L^{3}} \times 10^{6}$$

where W is wet weight in gms, L is total length in mm and CF is the condition factor. For both populations there is a decline in the CF in autumn and a sharp increase in spring. In both populations the maximum condition factor occurs early in the breeding season viz. May (Wootton et al., 1978).

7.1.2 Somatic condition factor

The effect of the enlargement of gonads (particularly in female fish) and the liver on the condition factor can be seen when the somatic condition factor (SCF) is calculated using the following equation:

$$SCF = \frac{CW}{L^3} \times 10^6$$

where CW is the carcass wet weight in gms, and L total length in mm (Wootton et al., 1978).

7.1.3 Hepatosomatic index

There are marked changes in the relative size and dry matter content of the liver in the male stickleback during the breeding season. In Autumn, the liver forms about 5% dry matter but as the breeding season approaches both the size and the dry matter content of the liver declines (Immers, 1953). The relative size of the liver is measured as the hepatosomatic index (HSI) and is calculated using the following equation:

$$HSI = \frac{\text{wet weight of liver}}{\text{total wet weight of fish}} \times 100$$

In fish the highest HSI is usually found in the pre-spawning season and the lowest during the breeding season. For example, in the female three-spined stickleback HSI is highest in spring and drops sharply

after the breeding season (Wootton et al.,1978). The livers of male haddock (Melanogrammus aeglefinus) from the North Sea accumulate lipid prior to breeding and deplete it during the breeding season (Campbell and Love, 1978). The hepatosomatic index (HSI) and the gonadosomatic index (GSI) are closely related to each other negatively in the Blennid fish (Blennius pavo). During the pre-spawning period when the gonad weight rises steadily, HSI diminishes because the energy required for the gonad growth is made available by energy reserves from the liver. At the end of the spawning period, when gonad weight decreases, HSI begins to rise because food uptake offers more energy than is needed (Podroschko et al., 1985).

7.1.4 Gonadosomatic index

The relative size of the gonad is measured as the gonadosomatic index (GSI) and is calculated using the following equation:

$$GSI = \frac{\text{wet weight of gonad}}{\text{total wet weight of fish}} \times 100$$

The paired testes at their maximum size account for only about 1.5% of the total body weight in mature male stickleack (Borg, 1982 a). In females of the Rheidol stickleback population, there is a slow increase in the GSI over the autumn and winter months but there is a rapid increase in the GSI between March and August, with a peak in May. This indicates that the breeding season of the Rheidol stickleback population lasts for three to four months. On the other hand, in the females of the Frongoch stickleback population the

breeding season lasts for only one month (viz. May) during which the GSI is high (Wootton et al., 1978).

The aims of this chapter

The results discussed in the previous chapters (3, 4, 5 and 6) indicate that energy reserves are accumulated during the pre-spawning period and that there is a considerable drain on the energy stores during the breeding season. In fact, the death of spawned males may be attributed to the large amount of energy depleted from the body for behavioural activities associated with breeding (see chapter 6). Data collected on body, liver and gonad size of the male stickleback across the year were analysed to investigate the relationship between energy reserves, body size, condition factor and gonadal maturation.

Therefore the specific aims of this chapter are as follows:

a) To find out how body size, condition factor (CF), somatic condition factor (SCF), hepatosomatic index (HSI) and gonadosomatic index (GSI) of the male stickleback vary across the year.

b) A subsidiary aim is to investigate the relationship between CF, HSI, GSI and energy reserves in the male sticklebacks.

7.2 MATERIAL AND METHODS

Male stickleback were collected regularly as described in chapter 2. The methods used to estimate the biochemical components of the body compartments are described in chapters 3, 4 and 5.

Condition factor (CF) was calculated using the following equation:

$$CF = \frac{W}{L^3} \times 10^6$$

where W is the total wet weight of the fish in gms. and L is the total length in mm. The length-weight relationship in the three-spined stickleback is generally cubic as described in the previous section of this chapter (Pennycuick, 1971; Wootton, 1976).

The length-weight relationship

A logarithmic plot of the relationship between length and weight of the male three-spined stickleback from the River Kelvin yields a straight line (see Fig 7.1). In the equation $\log w = \log a + n \log L$ (where n is the slope) the value for n was found to be 3.09. This agrees with the earlier observations where n ranges from 2.945 (Pennycuik, 1971) to 3.01 (Wootton, 1976). Hence the equation can be written as $W = a L^3$ and the constant a can be used as an indicator of the condition factor of the body in the present context for the male stickleback.

The somatic condition factor (SCF) was calculated using the following equation:

$$SCF = \frac{CW}{L^3} \times 10^6$$

where CW is the wet weight of carcass in gms and L is the total length in mm. The hepatosomatic index was calculated using the equation:

$$HSI = \frac{\text{wet weight of liver (gms)}}{\text{total wet weight of fish (gms)}} \times 100$$

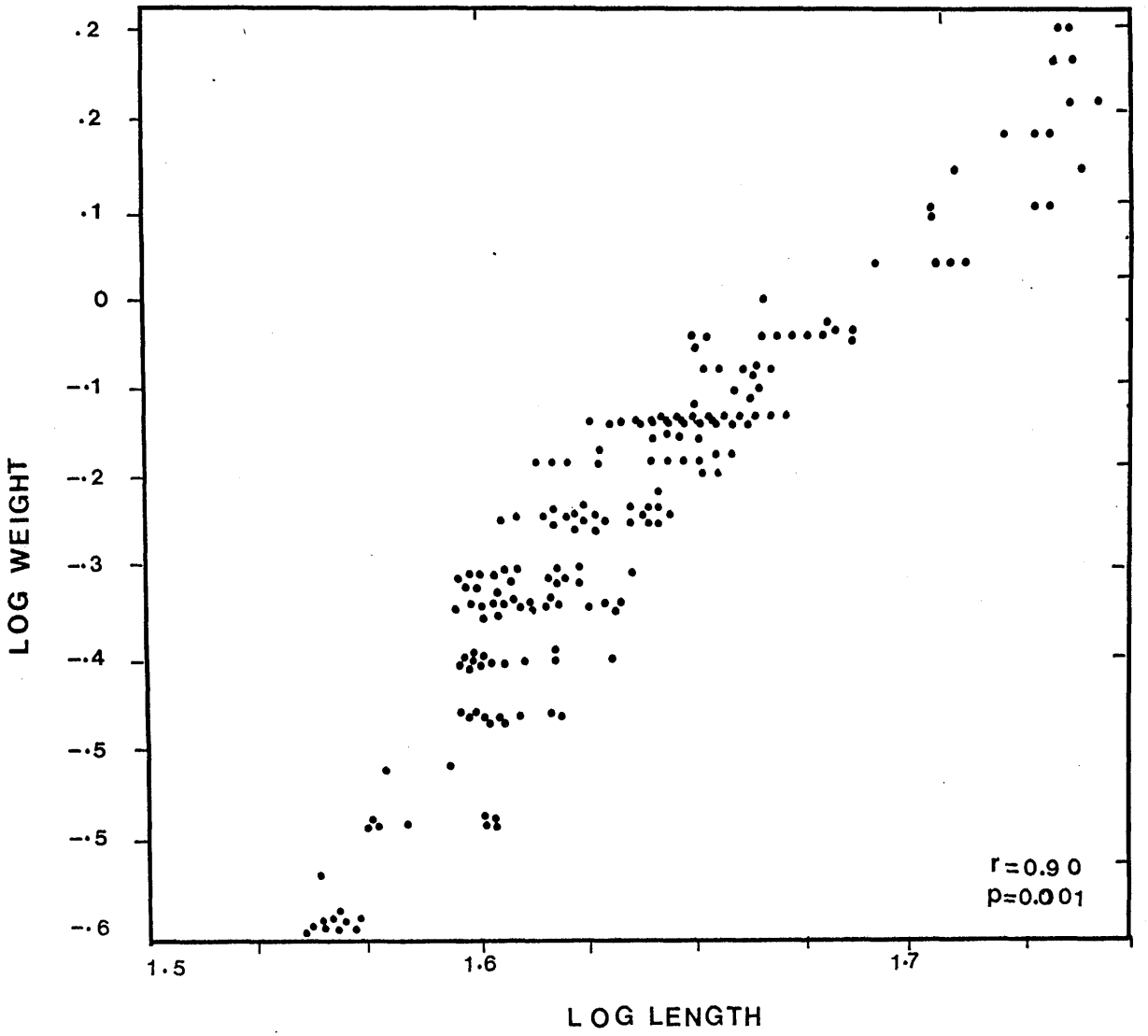
The gonadosomatic index was calculated using the equation:

$$GSI = \frac{\text{wet weight of gonad (gms)}}{\text{total wet weight of fish (gms)}} \times 100$$

These equations were adopted from Wootton et al.,(1978).

FIGURE 7.1 Logarithmic plot of the relationship between weight and length of male three-spined stickleback from the River Kelvin

$Y = \log_e$ (weight); $X = \log_e$ (length)


.2
.2
.1
0
-.1
-.2
-.3
-.4
-.5
-.5
-.6
LOG WEIGHT
1.5
1.6
1.7
LOG LENGTH
r=0.90
p=0.001

7.4 DATA ANALYSIS

The data were checked for normal distribution and logarithmically transformed when necessary. Oneway analysis of variance (ANOVA) was used to check for a seasonal variation in length, weight, CF, SCF, HSI and GSI. In order to investigate the relationship between CF, HSI, GSI and energy reserves, stepwise multiple regression analyses were done. Product-moment correlation coefficients were calculated between condition factor, somatic condition factor, hepatosomatic index and gonadosomatic index to investigate the relationship between them during the non-breeding season and the breeding season. Condition factor, hepatosomatic index and gonadosomatic index were regressed on all the independent variables (viz. energy sources in different body compartments) using stepwise multiple regression analysis.

Changes in mean length of the male stickleback over a period of one year are shown in Fig 7.2 and changes in mean wet weight are shown in Fig 7.3. The annual variation in condition factor is shown in Fig 7.4 and the annual variation in somatic condition factor is shown in Fig 7.5. The annual changes in hepatosomatic and gonadosomatic indices of the male stickleback are shown in Figs. 7.6 and 7.7.

Table 7.1 summarizes the results of oneway analysis of variance (ANOVA). Tables 7.2 7.3 and 7.4 summarize the results of stepwise multiple regression analyses of condition factor, hepatosomatic index, gonadosomatic index (dependent variables) against energy sources in different body compartments (independent variables). Table 7.4 A to C

TABLE 7.1 ONEWAY ANALYSIS OF VARIANCE BY MONTH

VARIABLE	SOURCE	D.F.	SUM OF SQUARES	MEAN OF SQUARES	F RATIO	F PROBABILITY
LENGTH	Between groups	11	0.1307	0.0119	5.0920	0.000
	Within groups	271	0.6324	0.0023	—	—
WEIGHT	Between groups	11	1.8341	0.1667	6.9540	0.000
	Within groups	271	6.4976	0.0240	—	—
C F	Between groups	11	1.3578	123432	6.5086	0.000
	Within groups	271	5.1394	189645	—	—
S C F	Between groups	11	1.5956	145050	7.2032	0.000
	Within	271	5.4571	20137	—	—
H S I	Between groups	11	0.1298	0.0118	5.8936	0.000
	Within groups	271	0.8132	0.0030	—	—
G S I	Between groups	11	0.1276	0.0116	5.0821	0.000
	groups	271	0.6024	0.0022	—	—

CF = Condition factor SCF = Somatic condition factor

HSI = Hepatosomatic index GSI = Gonadosomatic index

shows product-moment correlations between condition factor, somatic condition factor, hepatosomatic index and gonadosomatic index.

7.4 RESULTS

7.4.1 Annual changes

The samples collected for energy reserve estimations excluded the young of the year which were less than 30 mm in total length, as they could not be sexed without histological analysis and the delay involved in these analyses could have resulted in glycogen breakdown prior to biochemical assays. Data collected from samples were analysed to find out how body size, condition factor, somatic condition factor, hepatosomatic index and gonadosomatic index of the male stickleback vary across the year.

Length

Few recently hatched young fish (which were about 30 mm in total length) were encountered in July, but such fish were collected in sufficient numbers for analysis from August onwards, when they measured 31 mm in length. Figure 7.2 shows the changes in mean length over a period of twelve months. After an initial period of slow growth, in October there was a sharp increase in length and the average length was 35 mm. The rate of increase in length slowed down during the winter months. Low growth in winter may be due to either lack of food, or low rates of food consumption due to low winter temperatures. From January to March there was a steady increase in length followed by another rapid increase from May to July. The adult

FIGURE 7.2 ANNUAL CHANGES IN MEAN LENGTH

Mean and 95% confidence intervals of length (mm)

(The months are arranged on the X-axis to depict the complete life cycle of the male three-spined stickleback from the River Kelvin)

Y = young of the year

A = non-breeder adult

FIGURE 7.2

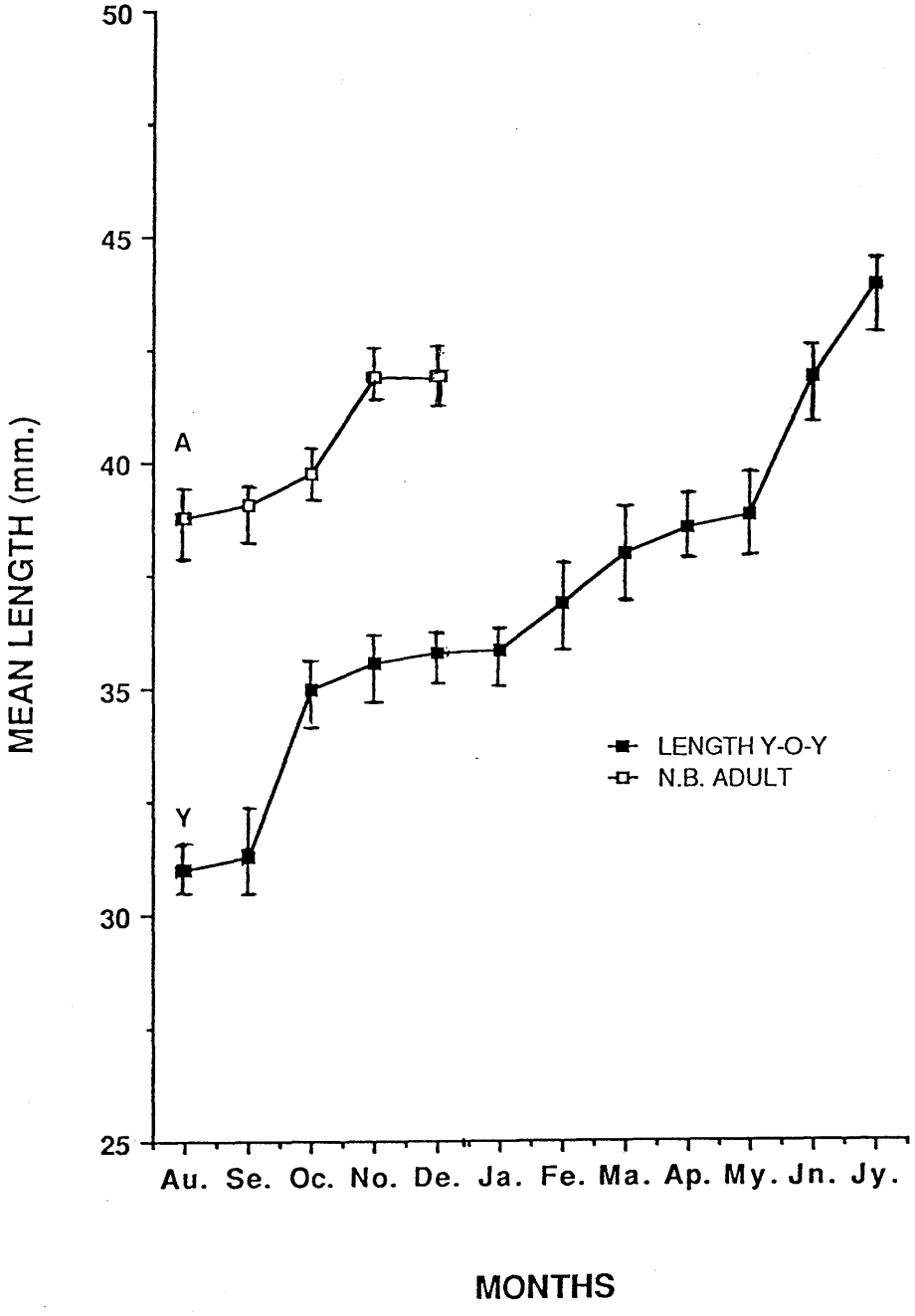

50
45
40
35
30
25
MEAN LENGTH (mm.)
A
Y
LENGTH Y-O-Y
N.B. ADULT
Au. Se. Oc. No. De. Ja. Fe. Ma. Ap. My. Jn. Jy.
MONTHS

FIGURE 7.3 ANNUAL CHANGES IN MEAN WEIGHT

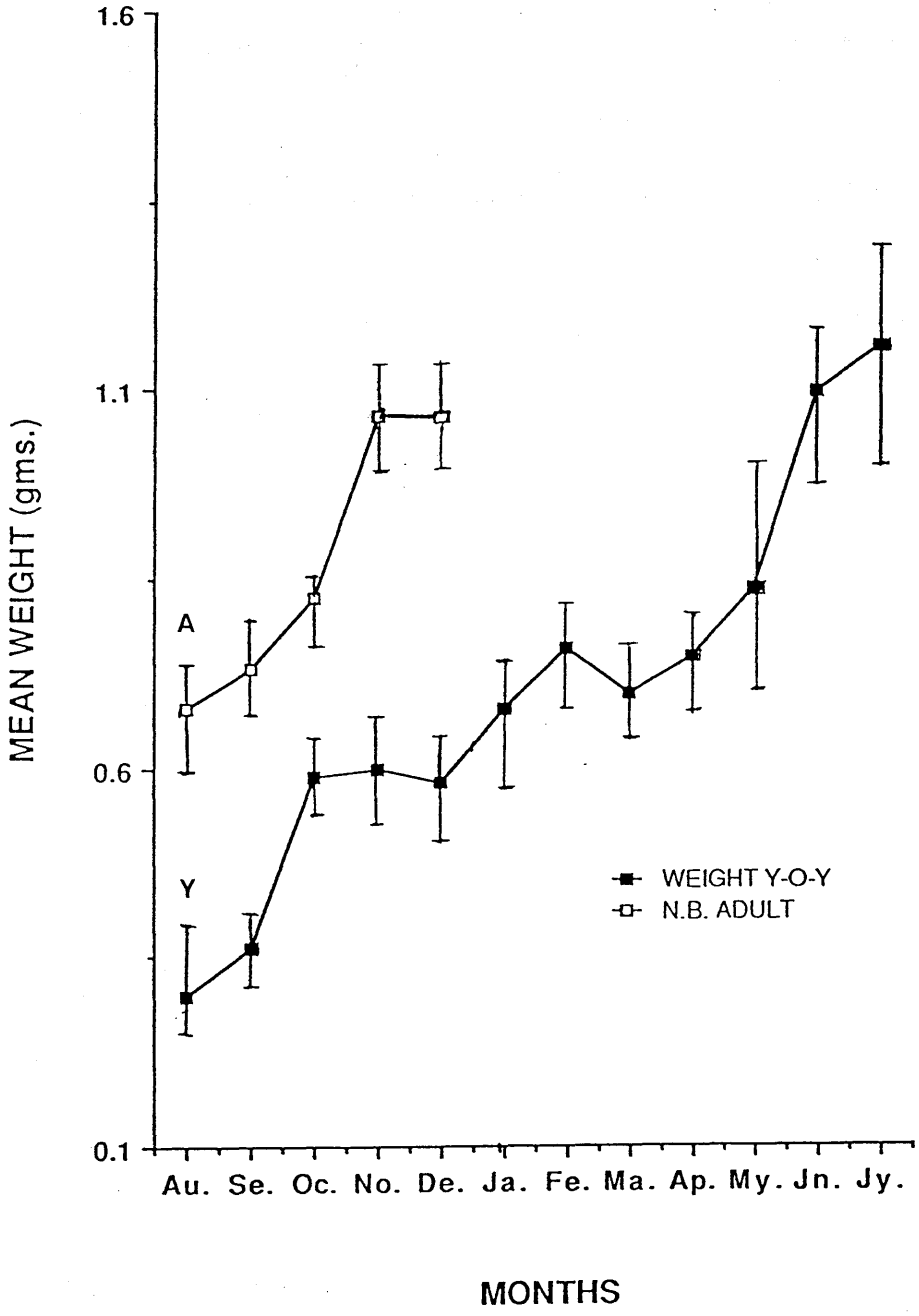
Mean and 95% confidence intervals of weight (gms)

(The months are arranged on the X-axis to depict the complete life cycle of the male three-spined stickleback from the River Kelvin)

Y = young of the year

A = non-breeder adult

FIGURE 7.3


1.6
1.1
0.6
0.1
MEAN WEIGHT (gms.)
A
Y
WEIGHT Y-O-Y
N.B. ADULT
Au. Se. Oc. No. De. Ja. Fe. Ma. Ap. My. Jn. Jy.
MONTHS

fish measured 44 mm in July. The greater average length of the fish collected in June and July compared to those collected in May is not the result of a growth spurt in male sticklebacks. Instead it is an artefact of the collection procedure in June and July, when the sample was deliberately biassed towards breeding male sticklebacks which are relatively larger.

Samples collected between August and December consisted of fish of two different age categories (viz. the recently hatched young of the year and adult fish which probably hatched from the later broods in the previous year). Such males measured about 38.8 mm in August and 42.6 mm in December after which no further fish of this age class were encountered in the sample.

Weight

Figure 7.3 shows a plot of mean wet weight against months for the male three-spined stickleback. In July the recently hatched fish (of 30 mm length) weighed about 0.30 gms. Weight increased slightly between August and September but in October the mean weight of the young of the year increased sharply to 0.588 gms. Increase in weight slowed down during the winter months but from January onwards there was a steady increase in weight other than from February to March. In June and July the adult fish weighed 1.10 gms and 1.16 gms respectively.

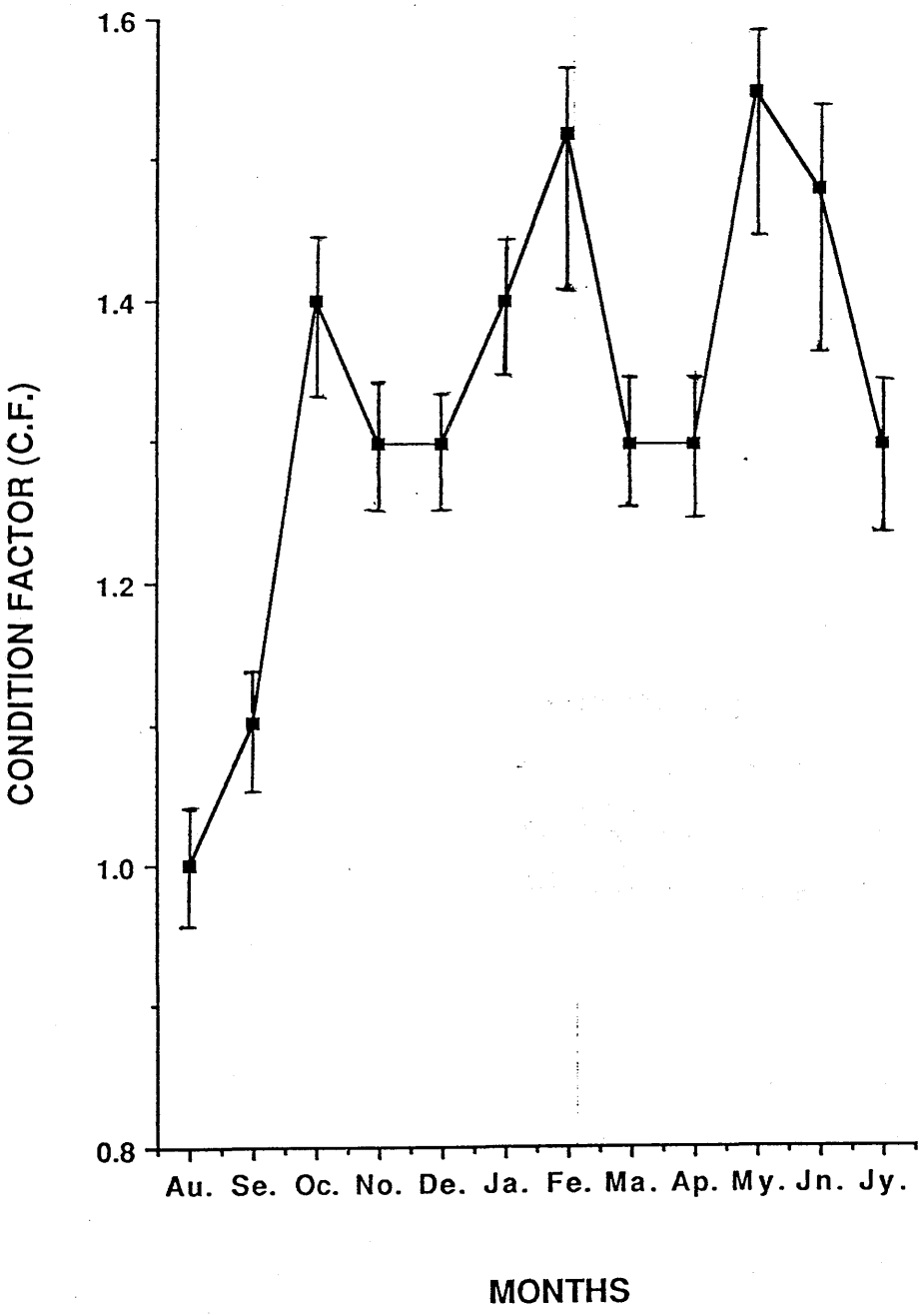
Adult fish collected in August (along with the young of the year) weighed about 0.68 gms. Their weight increased during the Autumn but slowed down in December. Such fish did not survive beyond their second winter.

FIGURE 7.4 ANNUAL CHANGES IN CONDITION FACTOR

Mean and 95% confidence intervals of condition factor in young of the year

(The months are arranged on the X-axis to depict the complete life cycle of the male three-spined stickleback from the River Kelvin)

FIGURE 7.4


1.6
1.4
1.2
1.0
0.8
CONDITION FACTOR (C.F.)
Au. Se. Oc. No. De. Ja. Fe. Ma. Ap. My. Jn. Jy.
MONTHS

Condition factor

Figure 7.4 shows the annual changes in the mean condition factor of the body of the male three-spined stickleback. There were marked variations in condition factor across the months of the year. In August, the young of the year had a low condition factor which increased sharply in October. Condition factor declined during the winter months but was followed by a sharp increase in early spring coinciding with increased body size. There was another decline between February and March (coinciding with a decrease in body weight). The maximum condition factor occurred in May, early in the breeding season after which there was a decline in body condition.

Somatic condition factor

Figure 7.5 shows the annual changes in the mean somatic condition factor of the male three-spined stickleback. The effect of gonad development and increase in the size of the liver can be seen when the somatic condition factor is examined. The overall pattern of the annual changes in somatic condition factor was similar to that of condition factor of the body apart from the fact that any spring recovery was much less pronounced for the somatic condition factor than for the condition factor. This difference was most marked in the month of May, when condition factor was at its maximum due to the effect of the enlarged liver and gonad. The maximum somatic condition factor occurred in February.

FIGURE 7.5 ANNUAL CHANGES IN SOMATIC CONDITION FACTOR

Mean and 95% confidence intervals of somatic condition factor in young of the year

(The months are arranged on the X-axis to depict the complete life cycle of the male three-spined stickleback from the River Kelvin)

FIGURE 7.5

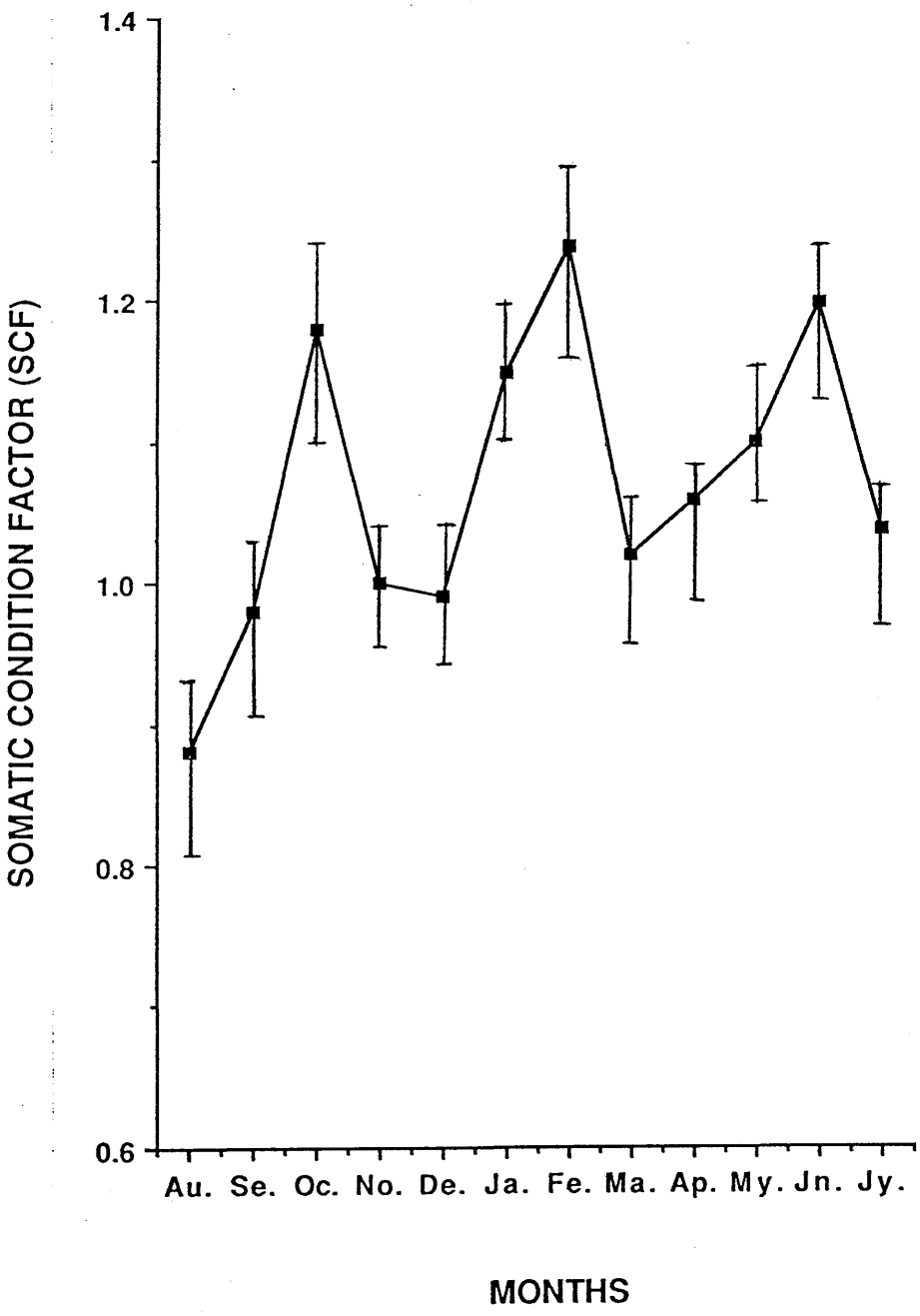
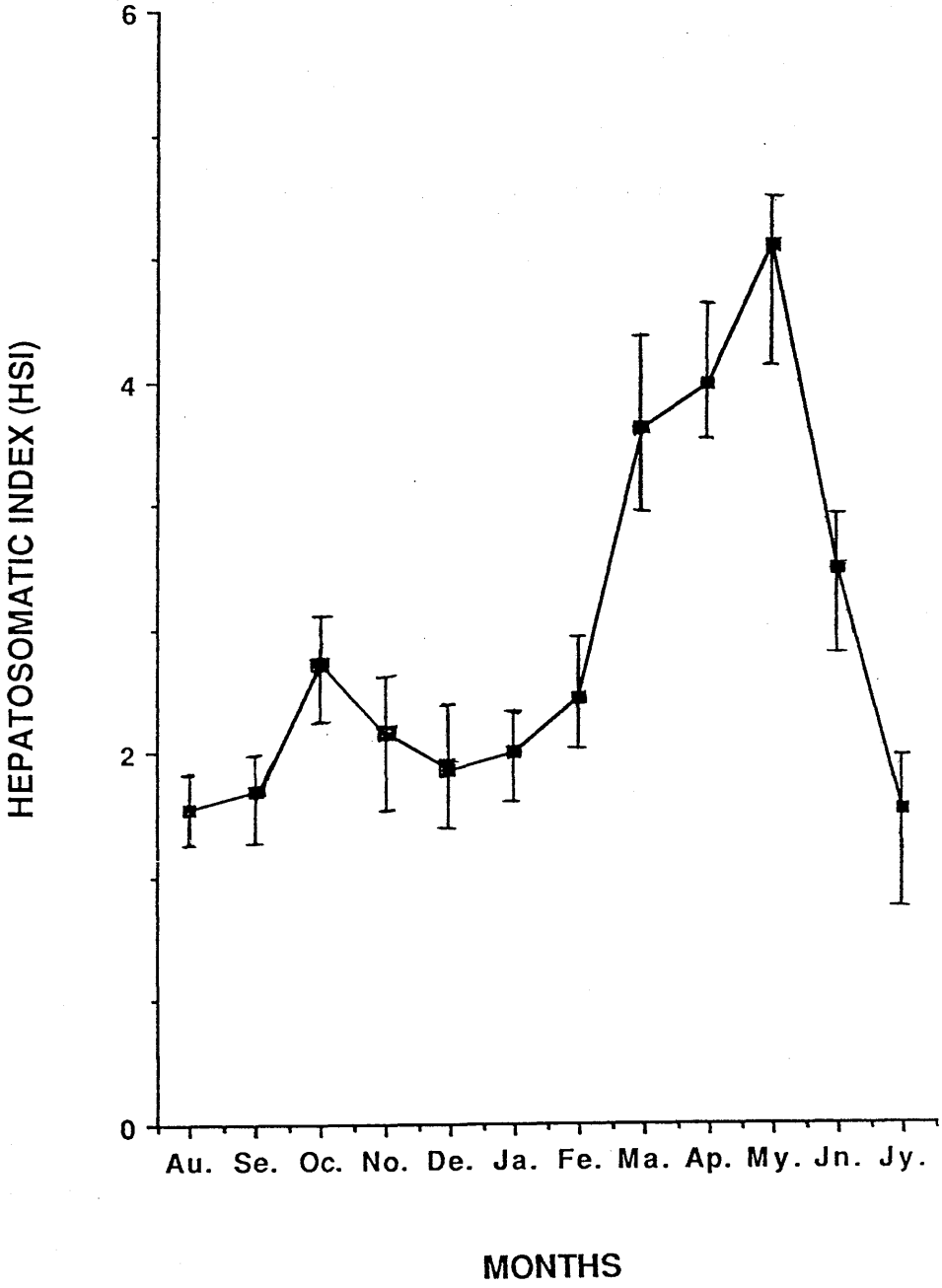

SOMATIC CONDITION FACTOR (SCF)
1.4
1.2
1.0
0.8
0.6
Au. Se. Oc. No. De. Ja. Fe. Ma. Ap. My. Jn. Jy.
MONTHS

FIGURE 7.6 ANNUAL CHANGES IN HEPATOSOMATIC INDEX

Mean and 95% confidence intervals of hepatosomatic index in young of the year

(The months are arranged on the X-axis to depict the complete life cycle of the male three-spined stickleback from the River Kelvin)

FIGURE 7.6


6
4
2
0
HEPATOSOMATIC INDEX (HSI)
Au. Se. Oc. No. De. Ja. Fe. Ma. Ap. My. Jn. Jy.
MONTHS

Hepatosomatic index (HSI)

The hepatosomatic index reflects the relative size of the liver. Figure 7.6 shows a plot of the mean hepatosomatic index against the months of the year. In the young of the year, there was a slight increase in the hepatosomatic index until October; this was followed by a drop during the winter. With the onset of the spring, the HSI increased sharply and reached a peak in May, followed by a drop during the breeding season.

Gonadosomatic index (GSI)

Figure 7.7 shows the annual changes in the mean gonadosomatic index of the male three-spined stickleback. From August to February there was slow increase in the gonadosomatic index. This period of slow increase over the autumn and winter months was followed by a period of rapid increase in the gonadosomatic index from March onwards. The maximum GSI was reached in May which was followed by a decline.

7.4.2 Condition factor and energy reserves

To examine whether the condition factor is useful as an index of energy reserves and to investigate the relationship between CF and energy stores in the different body compartments of the male stickleback, stepwise multiple regression analysis was done (see Table 7.1). Condition factor (dependent variable) was regressed against energy sources in the different body compartments. The results summarized in Table 7.2 show that while some of the biochemical

FIGURE 7.7 ANNUAL CHANGES IN GONADOSOMATIC INDEX

Mean and 95% confidence intervals of gonadosomatic index in young of the year

(The months are arranged on the X-axis to depict the complete life cycle of the male three-spined stickleback from the River Kelvin)

FIGURE 7.7

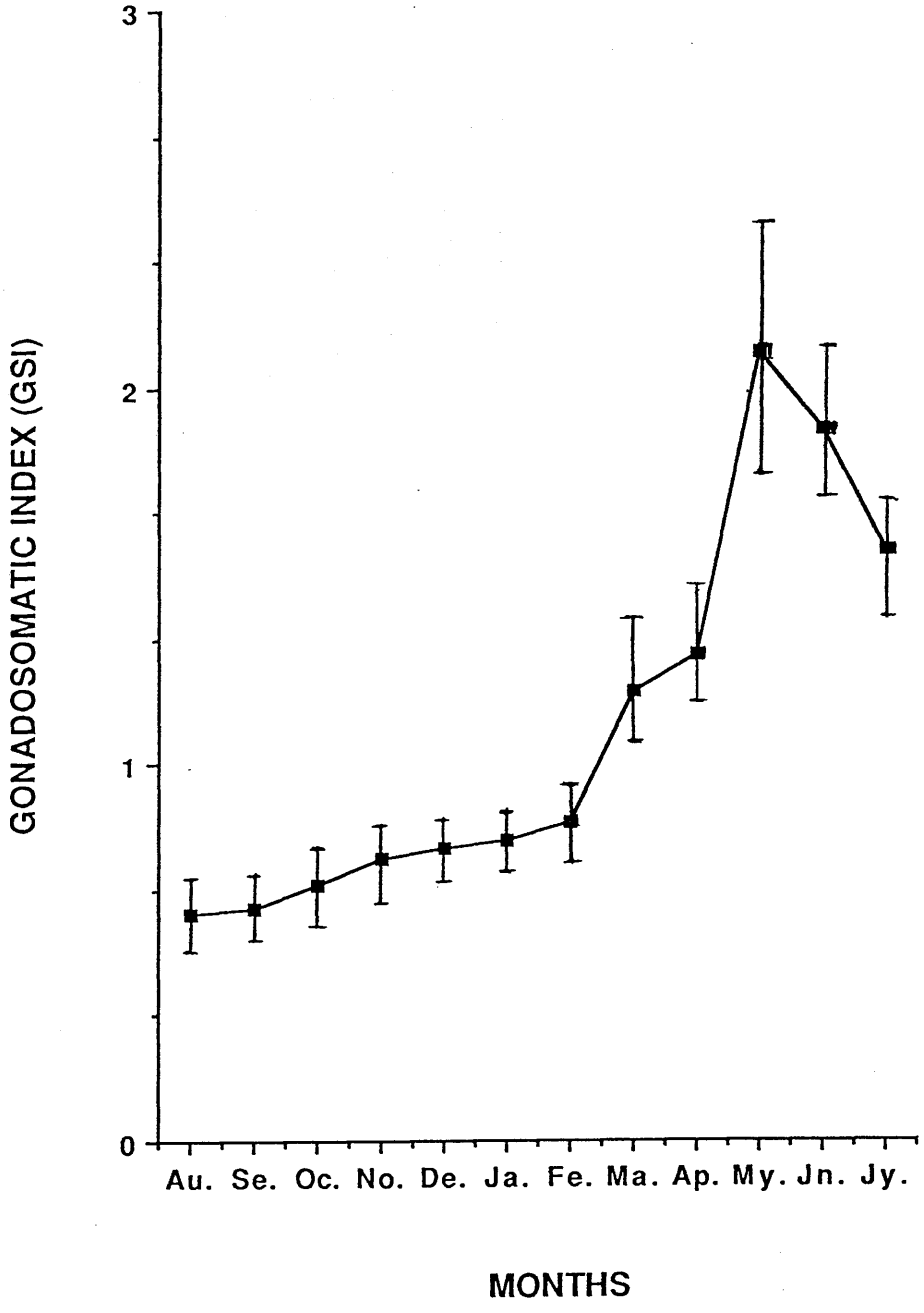

GONADOSOMATIC INDEX (GSI)
3
2
1
0
Au. Se. Oc. No. De. Ja. Fe. Ma. Ap. My. Jn. Jy.
MONTHS

TABLE 7.2 Summary of stepwise multiple regression analysis of condition factor (dependent variable) against 9 independent variables. According to linear model

$$Y = BX + C$$

where Y is the dependent variable which is logarithmically transformed

R = The multiple correlation coefficient, is given along with the standard error of the estimate (S.E) which measures the closeness with which the predicted value agree with the observed values.

R^2 = Coefficient of multiple determination which indicates the proportion of variance in the dependent variable accounted for by the independent variables.

Abbreviations : LIVPRO = liver protein, CARPRO = carcass protein, LIVLIP = Liver lipid, LIVGLY = liver glycogen, CARLIP = carcass lipid, CARGLY = carcass glycogen, GONGLY = gonad glycogen, GONPRO = gonad protein, GONLIP = gonad lipid

P = level of significance

B = coefficient in the equation

7.2 A = Summary of stepwise multiple regression analysis of condition factor

7.2 B = Variables in the equation

7.2 C = Variables not in the equation

Table 7.2 Summary of stepwise multiple regression analysis of condition factor

A.

	STEP 1	STEP 2	STEP 3	STEP 4	STEP 5
VARIABLES	CARLIP	CARLIP LIVPRO	CARLIP LIVPRO CARPRO	CARLIP LIVPRO CARPRO LIVLIP	CARLIP LIVPRO CARPRO LIVLIP CARLIP
R	0.22321	0.27571	0.33294	0.37450	0.37303
S.E.	0.07625	0.07533	0.07403	0.07292	0.07284
R^2	0.04982	0.07602	0.11085	0.14025	0.13915
ANOVA	F = 14.733 P < 0.001	F = 11.518 P< 0.001	F = 11.594 P < 0.001	F = 11.337 P < 0.001	F = 15.033 P < 0.001

R^2 = 0.13915 ie. 13.91% of variance in condition factor is explained by multiple regression equation

7.2B VARIABLES IN THE EQUATION

VARIABLE	B	T	P
LIVPRO	2.83630-04	4.370	< 0.001
CARPRO	-3.15630-04	-5.531	< 0.001
LIVLIP	2.59387-04	3.785	< 0.001
(constant)	1.14507	28.245	< 0.001

PREDICTION EQUATION

CF = (0.000284 x LIVPRO) - (0.000316 x CARPRO) + (0.000259 X LIVLIP) + 1.14507

7.2C VARIABLES NOT IN THE EQUATION

VARIABLE	B	T	P
LIVGLY	0.107116	1.330	0.184
CARLIP	0.042011	0.596	0.551
CARGLY	-0.058923	-0.733	0.464
GONGLY	0.053584	0.832	0.406
GONLIP	0.039177	0.581	0.562
GONPRO	-0.034500	-0.488	0.626

components (viz. liver protein, carcass protein and liver lipid) do relate significantly to condition factor, together they account only for 13.91% of total variance in condition factor. This suggests that condition factor is not a reliable index of energy reserves in the body of the male stickleback.

7.4.3 Hepatosomatic index and energy reserves

In order to check whether hepatosomatic index is a valid measure of energy reserves in the male three-spined stickleback, a stepwise multiple regression analysis was carried out. HSI was the dependent variable regressed against 9 independent (biochemical) variables. The results (see Table 7.3) show that the most important single predictor of HSI is liver glycogen which accounts for 25.63% of total variance in hepatosomatic index. Liver lipid accounts for only 2.07% of total variance in HSI. A cluster of other variables (viz. carcass glycogen, carcass protein, carcass lipid and gonad protein) relate negatively to hepatosomatic index. Together these variables account for 41.15% of total variance in hepatosomatic index. This indicates that hepatosomatic index is a good measure of mobilisable liver energy reserves in the male stickleback.

TABLE 7.3 Summary of stepwise multiple regression analysis of hepatosomatic index (dependent variable) against 9 independent variables. According to linear model

$$Y = BX + C$$

where Y is the dependent variable which is logarithmically transformed

R = The multiple correlation coefficient, is given along with the standard error of the estimate (S.E) which measures the closeness with which the predicted value agree with the observed values.

R^2 = Coefficient of multiple determination which indicates the proportion of variance in the dependent variable accounted for by the independent variables.

Abbreviations : LIVPRO = liver protein, CARPRO = carcass protein, LIVLIP = Liver lipid, LIVGLY = liver glycogen, CARLIP = carcass lipid, CARGLY = carcass glycogen, GONGLY = gonad glycogen, GONPRO = gonad protein, GONLIP = gonad lipid

P = level of significance

B = coefficient in the equation

7.3 A = Summary of stepwise multiple regression analysis of hepatosomatic index

7.3 B = Variables in the equation

7.4 C = Variables not in the equation

7.3 A

	STEP 1	STEP 2	STEP 3	STEP 4	STEP 5	STEP 6
VARIABLES	LIVGLY	LIVGLY CARGLY	LIVGLY CARGLY CARLIP	LIVGLY CARGLY CARLIP GONPRO	LIVGLY CARGLY CARLIP GONPRO CARPRO	LIVGLY CARGLY CARLIP GONPRO CARPRO LIVLIP
R	0.50628	0.58333	0.60258	0.62515	0.63463	0.64149
S.E.	0.12564	0.11855	0.11668	0.11432	0.11340	0.11277
R^2	0.25632	0.34027	0.36311	0.39081	0.40275	0.41151
ANOVA	F=96.850 P < 0.001	F=72.209 P < 0.001	F=53.021 P < 0.001	F=44.585 P < 0.001	F=37.358 P < 0.001	F=32.166 P < 0.001

R^2 = 0.41151 IE 41.15% VARIANCE IN HEPATOSOMATIC INDEX IS EXPLAINED BY MULTIPLE REGRESSION EQUATION.

Table 7.3 Summary of stepwise multiple regression analysis of hepatosomatic index

B. VARIABLES IN THE EQUATION

VARIABLE	B	T	P
LIVGLY	7.43319-04	9.067	< 0.001
CARGLY	-0.006415	-3.508	< 0.001
CARLIP	-0.001133	-5.360	< 0.001
GONPRO	-3.09450-04	-2.768	< 0.006
CARPRO	-2.84889-04	-2.704	< 0.007
LIVLIP	3.39118-04	2.027	< 0.043
(Constant)	0.708643	8.956	< 0.001

PREDICTION EQUATION

HSI = (0.000743 x LIVGLY) - (0.00642 x CARGLY) - (0.00113 x CARLIP) - (0.0003 x GONPRO) - (0.000285 x CARPRO) + (0.000339 x LIVLIP) + 0.7086

C. VARIABLES NOT IN THE EQUATION

VARIABLE	B	T	P
LIVPRO	0.022506	0.391	0.696
GONGLY	0.045007	0.546	0.585
GONLIP	0.177613	1.900	0.058

7.4.4 Gonadosomatic index and energy reserves

A stepwise multiple regression analysis was done in order to to examine how gonadosomatic index relates to energy reserves (see Table 7.4). Results show that the most important predictor is gonad glycogen which forms 33.65% of total variance in GSI. Carcass glycogen and gonad protein account for 1.7% and 1.1% respectively of total variance in GSI. A cluster of variables (viz. gonad lipid, liver lipid and liver protein) relate negatively to gonadosomatic index. Together these variables account for 43% of total variance in gonadosomatic index. This indicates that GSI is a reasonably good index of energy reserves in the gonad.

TABLE 7.4 Summary of stepwise multiple regression analysis of gonadosomatic index (dependent variable) against 9 independent variables. According to linear model

$$Y = BX + C$$

where Y is the dependent variable which is logarithmically transformed

R = The multiple correlation coefficient, is given along with the standard error of the estimate (S.E) which measures the closeness with which the predicted value agree with the observed values.

R^2 = Coefficient of multiple determination which indicates the proportion of variance in the dependent variable accounted for by the independent variables.

Abbreviations : LIVPRO = liver protein, CARPRO = carcass protein, LIVLIP = Liver lipid, LIVGLY = liver glycogen, CARLIP = carcass lipid, CARGLY = carcass glycogen, GONGLY = gonad glycogen, GONPRO = gonad protein, GONLIP = gonad lipid

P = level of significance

B = coefficient in the equation

7.4 A = Summary of stepwise multiple regression analysis of gonadosomatic index

7.4 B = Variables in the equation

7.4 C = Variables not in the equation

Table 7.4 Summary of stepwise multiple regression analysis of gonadosomatic index

A.

	STEP 1	STEP 2	STEP 3	STEP4	STEP 5	STEP 6
VARIABLE	GONGLY	GONGLY CARGLY	GONGLY CARGLY GONLIP	GONGLY CARGLY GONLIP LIVLIP	GONGLY CARGLY GONLIP LIVLIP LIVPRO	GONGLY CARGLY GONLIP LIVLIP LIVPRO GONPRO
R	0.58013	0.59471	0.62007	0.63598	0.64711	0.65546
S.E.	0.16698	0.16510	0.16141	0.15905	0.15742	0.15622
R^2	0.33655	0.35368	0.38449	0.40447	0.41875	0.42962
ANOVA	F=142.542 P < 0.001	F=76.611 P < 0.001	F=58.094 P < 0.001	F=47.202 P < 0.001	F=39.911 P < 0.001	F=34.648 P < 0.001

R^2 = 0.42962 ie 42.96% VARIANCEIN IN GONADOSOMATIC INDEX IS EXPLAINED BY MULTIPLE REGRESSION EQUATION.

7.4 B VARIABLES IN THE EQUATION

VARIABLE	B	T	P
GONGLY	0.011135	6.922	< 0.001
CARGLY	0.014414	5.383	< 0.001
GONLIP	-0.008127	-3.795	< 0.001
LIVLIP	-6.76077-04	-3.256	< 0.001
LIVPRO	-5.15144-04	-3.423	< 0.001
GONPRO	3.53396-04	2.294	0.022
(constant)	-0.125213	-1.549	0.122

PREDICTION EQUATION

GSI= (0.011135xGONGLY) + (0.014414xCARGLY) - (0.008127xGONLIP)
- (0.000676xLIVLIP) - (0.000515xLIVPRO) + (0.000353xGONPRO)
-(0.125213)

7.4C VARIABLES NOT IN THE EQUATION

VARIABLE	B	T	P
LIVGLY	0.034756	0.352	0.7253
CARPRO	-0.008426	-0.144	0.8853
CARLIP	-0.042814	-0.780	0.4358

Relationship between condition factor, somatic condition factor, hepatosomatic index and gonadosomatic index

In order to investigate the relationship between condition factor, somatic condition factor, hepatosomatic index and gonadosomatic index, product-moment correlation coefficients were calculated. Table 7.4 A to C show correlations between CF, SCF, HSI and GSI during the non-breeding season (viz. August-May) and the breeding season (June and July).

During the non-breeding season

The relationship between hepatosomatic index and gonadosomatic index was strongly positive (highly significantly so) indicating that gonad development was most pronounced in sticklebacks with good energy reserves. There was also a strong positive correlation between condition factor of the body and somatic condition factor (highly significantly so). There was no relationship between condition factor and gonadosomatic index. Similarly there was no relationship between somatic condition factor and gonadosomatic index. There was no relationship between somatic condition factor and hepatosomatic index, or between condition factor and hepatosomatic index.

During the breeding season

The strongly positive relationship between hepatosomatic and gonadosomatic indices that existed during the non-breeding season gradually disappeared during the breeding season. In June there was a non-significant, weak positive correlation between HSI and GSI and by

July the relationship between HSI and GSI was weakly negative (but not significantly so). The size of the liver declined during the breeding season the decline being most marked in those fish with high gonadosomatic index. This decline parallels the severe depletion of energy reserves in the liver (see chapters 3 and 4) and presumably occurs because of the energetic drain for behavioural activities during the breeding season. The strong positive relationship which existed between condition factor of the body and somatic condition factor during the non-breeding season persisted in June and July. This supports the view that the overall pattern of the annual changes in somatic condition factor is similar to that of condition factor (see section 7.4.1).

There were no significant correlations between either condition factor or somatic condition factor and gonadosomatic and hepatosomatic indices. In June, the negative relationship between somatic condition factor and gonadosomatic index approached significance suggesting the possibility of a trade-off between gonad development and body condition.

TABLE 7.5 A Correlation coefficients and levels of significance between CF, SCF, HSI and GSI during the non-breeding season (August to May)

Abbreviations:

R = Product-moment correlation coefficient

P = Levels of significance

*** = P <0.001

** = P <0.01

* = P <0.05

+ = P <0.1

NS = Non-significant

N = 241 (Number of samples)

CF = condtion factor

SCF = somatic condition factor

HSI = hepatosomatic index

GSI = gonadosomatic index

Table 7.5 A Correlations between CF, SCF, HSI and GSI (August - May)

	CF	SCF	HSI	GSI
CF		R = 0.710 P = ***	R = 0.016 P = NS	R = 0.017 P = NS
SCF			R = 0.052 P = NS	R = 0.062 P = NS
HSI				R = 0.709 P = ***
GSI				

N = 241

TABLE 7.5 B-C Correlation coefficients and levels of significance between CF, SCF, HSI and GSI

B = during the breeding season (month of June)

C = during the breeding season (month of July)

Abbreviations:

R = Product-moment correlation coefficient

P = Levels of significance

*** = P <0.001

** = P <0.01

* = P <0.05

+ = P <0.1

NS = Non-significant

N = Number of samples

CF = condtion factor

SCF = somatic condition factor

HSI = hepatosomatic index

GSI = gonadosomatic index

Table 7.5 B (June)

	CF	SCF	HSI	GSI	
		R = 0.970 P = ***	R =-0.011 P = NS	R =-0.277 P = NS	CF
			R =-0.068 P = NS	R =-0.366 P = +	SCF
N = 22				R = 0.204 P = NS	HSI
					GSI

Table 7.5 C (July)

	CF	SCF	HSI	GSI	
		R = 0.828 P = ***	R =-0.054 P = NS	R =-0.029 P = NS	CF
			R =-0.277 P = NS	R =-0.096 P = NS	SCF
				R =-0.245 P = NS	HSI
N = 18					GSI

7.5 DISCUSSION

One of the aims of this study was to find out how body size of the male sticklebacks varies across the year. In natural populations the stickleback can attain a length of about 17 mm within thirty days of hatching (Mullem, 1967). In the River Kelvin, recruitment of young stickleback measuring 15 mm and above was observed in small numbers from June onwards. Young fish sized 30 mm were present in small numbers in July and August samples. The earlier broods of young fish grew fast in their first months of life but their growth was checked during the winter months. Some fish in the present study measured about 42 mm in length and weighed just under a gram when they were ten months old, but at the same time there were some small fish which measured only 32 mm in length and weighed 0.396 gms. The possible explanation for this disparity in size is that those fish which were hatched towards the end of the season did not have an abundant food supply unlike those hatched earlier in the breeding season.

At the end of their first year of life the adult male fish had a mean length of 44 mm and weighed about 1.16 gms. The sticklebacks of the River Kelvin therefore had a growth rate comparable to that of sticklebacks living in Bere stream in Southern England (Mann, 1971). Those fish which had reached sexual maturity and had sufficient energy reserves bred when they were about a year old. Most adult males died at the end of the breeding season due to heavy depletion of energy reserves and hence had a maximum life span of a year. On the other hand, those males that were small in April (probably hatched from the

later broods in the previous year) did not attain sexual maturity. Such males failed to breed in their first year and only survived until their second winter and perished thereafter. These observations are in agreement with a study of the age structure of Scottish stickleback populations based on length frequency histogrammes and otolith analysis (Ukegbu, 1986).

The condition factor of the body showed marked variations across the months of the year. Young of the year had a low condition factor in the month of August. The high rate of size increase during early Autumn and a check in size increase during the winter months were paralleled by the condition factor of the body. Stepwise multiple regression analysis suggests that condition factor is not a good index of energy reserves in the body. The annual changes in the somatic condition factor of the body closely followed the pattern of the condition factor apart from the fact that it indicated to some extent the increase in the size of the liver and gonad particularly at the beginning of the breeding season.

In the young fish the liver formed about 2% of body weight but as the breeding season approached it formed about 5% of body weight. The relative size of the liver declined during the spawning season. The changes in the relative size of the liver paralled the changes in energy reserves (see chapter 3 and 4). Stepwise multiple regression analysis indicates that hepatosomatic index is a good measure of liver energy reserves. Together liver glycogen and liver lipid account for 28 % of total variance in hepatosomatic index.

The paired testes of the male stickleback account for about 1.5% to 2% of the total body weight when they were at their maximum size. The gonadosomatic index clearly reflects the development of the testes in the stickleback. Stepwise multiple regression analysis suggests that the most important predictor of gonadosomatic index is gonad glycogen which forms about 34% of total variance in GSI.

The relationship between hepatosomatic index and gonadosomatic index was strongly positive during the non-breeding season but this disappeared during the breeding season. Thus up to the breeding season males with higher energy reserves had relatively well developed gonads. However, during the breeding season fish with the highest gonadosomatic index were those that bred most actively and therefore experienced the greatest depletion in energy reserves.

7.6 CONCLUSIONS

Work described in this chapter gives information on how the condition factor, somatic condition factor, hepatosomatic index and gonadosomatic index vary across the year in relation to energy reserves in male sticklebacks from the River Kelvin. The condition factor and somatic condition factor were strongly positively correlated throughout the year, indicating that the overall pattern of changes in somatic condition factor is very similar to that of condition factor. The relationship between hepatosomatic index and gonadosomatic index is strongly positive from August to May, indicating that gonad development is prominant in male sticklebacks

with good energy reserves. This relationship disappeared during the breeding season, as the size of the liver declined and this was most marked in those fish with with high gonadosomatic index. Stepwise multiple regression analyses suggest that condition factor is not a reliable index of energy reserves of the body in the male stickleback. On the other hand, hepatosomatic index is a good measure of liver energy reserves and gonadosomatic index is a reasonably good index of energy reserves in the gonad.

CHAPTER 8

REPRODUCTIVE AGGRESSION AND ENERGY RESERVES

CHAPTER 8 REPRODUCTIVE AGGRESSION AND ENERGY RESERVES

8.1 INTRODUCTION

8.1.1 Factors influencing decisions during animal disputes

When animals compete with one another the costs and benefits of adopting a particular pattern of behaviour depend on what other individuals in the population do. The techniques of game theory have been used to investigate the evolutionary consequences of this fact (Maynard Smith, 1982a; Krebs and Davies, 1984; Huntingford and Turner, 1987). This approach treats evolution as a game in which the players adopt different patterns of behaviour (or strategies) and works out how the frequencies of different strategies will change from one generation to the next. In particular, such analyses attempt to identify the evolutionarily stable strategy (ESS). A strategy is an ESS if, when adopted by most members of a population, it cannot be invaded by any rare alternative strategy in the game.

Game theory has been used extensively in the study of agonistic behaviour, where it represents a source of clear predictions about how animals should behave. One class of model investigates the consequences of asymmetries between contestants both in their relative fighting ability and in the value of disputed resource. A number of insights have emerged:

1) Where there is a difference in the fighting ability of the animals (usually size-related), decisions about whether or not to engage in a contest should be based on this asymmetry.

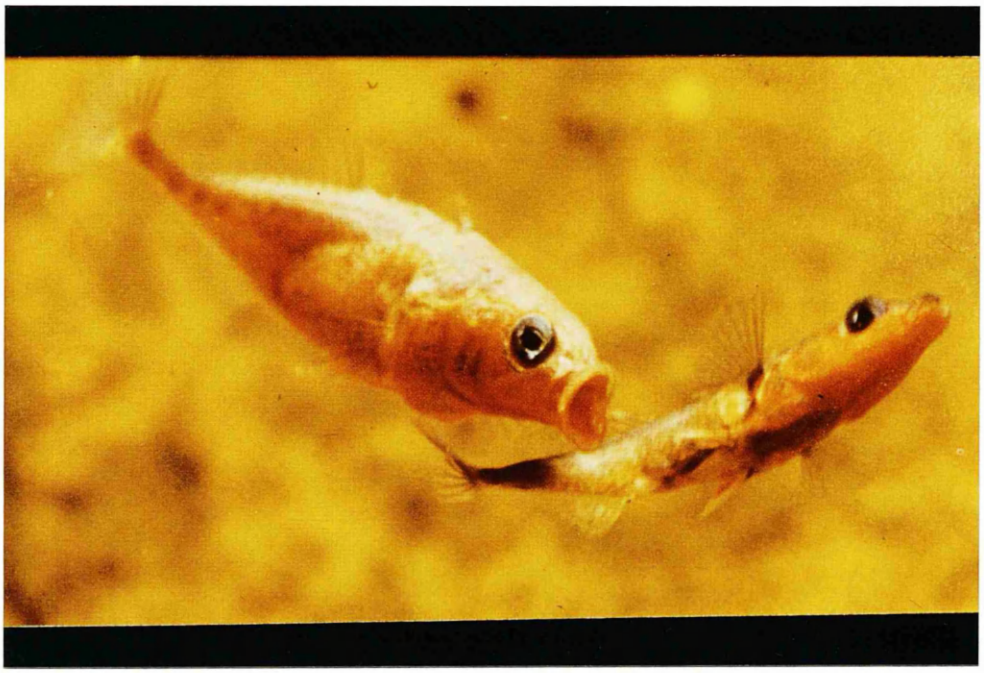
2) Where contestants are evenly matched, escalation of contests is more likely to occur.

3) The greater the value of the disputed resource, the more animals should invest in contesting it and where one animal stands to gain more by winning it should fight more fiercely than its opponent.

A number of empirical studies have demonstrated that these predictions are met. Thus, larger individuals win fights in many cases (eg beetle, Podischnus agenor; Eberhard, 1979 and cockroach, Nauphoeta cinerea; Breed et al., 1980), smaller or weaker individuals withdraw early in many cases (eg toad, Bufo bufo; Davies and Halliday, 1978 and red deer, Cervus elephas; Clutton-Brock and Albon, 1979) and fights are more intense between evenly matched opponents (eg Spiders; Riechert, 1982 and swimming crabs, Glass and Huntingford, 1987)

However, not all empirical studies have confirmed the predictions of Game Theory. For example, although the larger of two male swimming crabs (Liocarcinus depurator) or mouthbrooder cichlid fish (Oreochromis mossambicus) almost always win fights, smaller crabs are just as likely as larger ones to initiate fights (Glass and Huntingford, 1988) and smaller cichlid fish escalate fights just as often as the larger fish do (Turner and Huntingford, 1986). The importance of the value of a disputed resource in determining the

PLATE 8.1 An agonistic encounter between male three-spined sticklebacks.



course and outcome of fights has also been demonstrated for a number of species, for example in spiders (Austad, 1983; Riechert, 1982).

8.1.2 Dispute between sticklebacks

Breeding male sticklebacks restrict most of their activities to a restricted area around their nest. The male fish persistently attack any intruder that comes near their nest and chase the intruder up to the edge of the territory (see plate 8.1). As discussed above, Game theory predicts that decisions during conflicts will depend both on the relative resource holding power of the participants and on the value of the resource in question.

Colour and size

A factor which influences the outcome of agonistic encounters between sexually mature male stickleback is the red nuptial colouration. Brighter coloured males are more aggressive than duller male fish (Bakker and Sevenster, 1983), and red models are attacked less than silver ones (Rowland, 1984). In pairs of male sticklebacks deliberately mismatched for colour and size, dominance decisions are made rapidly without any test of strength of the rival male. Larger sized and to a lesser extent, more brightly coloured males have an advantage over smaller and duller male fish. Decisions are usually made after a single bite and the loser flees and exhibits a subordinate display (Whoriskey and Wootton, 1986).

Value of resource

The presence of a nest increases the value of the territory to the occupier male stickleback and makes him defend the territory more intensely (Stanley and Wootton, 1986). The value of a territory to a male stickleback also depends on whether he is able to exploit the territory, i.e. to retain possession and to obtain and rear successfully a brood of young. This ability will depend in part on his current nutritional reserves, since both defence of the territory and rearing a brood are energetically costly. The role of nutritional state on agonistic behaviour was investigated experimentally by Stanley and Wootton (1986). In similar-sized mature male sticklebacks maintained on different rations (viz. 2%, 6% and 18% of total body weight ration per day for twelve days) it was found that males on low rations have significantly smaller kidneys than those on higher rations, indicating some inhibition of nest building at low food levels. Those males that received the highest ration establish and maintain large territories at the expense of neighbouring males on a lower ration (Stanley, 1983). This supports the prediction that decisions are based on current energy levels.

The experimentally-induced differences in nutrition levels in that study were large, which leads to the question whether natural variations in energy reserves in sexually mature male sticklebacks also influence decisions during territorial conflicts. The main aim of the present study was therefore to see whether natural variation in energy reserves among breeding male stickleback is reflected in their

behaviour during territorial disputes. This was investigated by comparing energy reserves in the winners and losers of short territorial disputes between freely interacting breeding male stickleback matched for size and colour. A preliminary set of experiments showed that winners had higher glycogen levels than losers, but did not differ in lipid reserves (see Table 8.1). Since glycogen is rapidly mobilised in response to stress it is possible that this difference is a consequence and not a cause of the behaviour of the male sticklebacks during territorial fights.

8.1.3 Effects of fighting

It is well established that engaging in a fight can have complex physiological consequences. Fighting activities rapidly modulate hormonal levels both in winners and losers (for details see chapter 1). For example, during a pairwise encounter between adult male swordtail fish (Xiphophorus helleri), testosterone levels increase in the winner and decrease in the loser. At the same time the glucocorticoids increase dramatically in both participants but this is more pronounced in the loser. The increased glucocorticoid levels show that fighting is stressful, especially so to the loser. It remains rather unclear whether these hormonal changes influence decisions during agonistic encounters or are mere biochemical by-products of fighting without any biological consequences (Hannes et al., 1984).

The difference in liver glycogen levels between winners and losers stickleback described above could therefore be a cause of the

different experience during fighting but could also be a consequence, mediated via differential increase in catecholamines in winners and losers. In order to distinguish between these two possibilities, a second series of experiments was designed in which the length and intensity of interactions between winners and losers were manipulated experimentally. If the association between high liver glycogen levels and victory is a causal one, then the longest fight should be between fish whose energy reserves are similar. On the other hand, if a low level of liver glycogen in losers is a consequence of differential depletion of liver glycogen stores in winners and losers, then the greatest difference in liver glycogen reserve should be seen in fish that have taken part in the longest agonistic interaction.

8.1.4 Aims of the experiment

The specific aims of this part of the study are as follows:
1) To compare the major energy reserves (viz. liver glycogen, liver lipid, carcass glycogen and carcass protein) in the winners and losers of short territorial fights.

2) To manipulate the length and intensity of territorial fights and to relate these to the degree of difference in energy reserves between the eventual winners and the eventual losers of territorial conflicts.

8.2 MATERIAL AND METHODS

Sexually mature male sticklebacks were collected from the River Kelvin from May to July in 1985 and 1986 and were maintained in the laboratory.

Each aquarium tank (1000 X 280 X 360 mm) was divided into two equal sized compartments by two removable partitions, of which one was transparent and the other a opaque. Two similar sized male sticklebacks were housed in a tank, one in each compartment. A constant aeration system was employed in the tanks and precautions were taken to minimise disturbance to the fish. The light regime in the laboratory was maintained at 16 light hours and 8 dark hours. The temperature in the laboratory varied from 18 ^{o} C to 20 ^{o} C. The fish were fed daily ad libitum with live Tubifex worms. As the male stickleback settled down in the tanks, most of them started to establish territories and build nests with aquatic vegetation provided in the tank.

Experiment 1 (in 1985) Free interaction

Seven pairs of breeding male sticklebacks were tested. In this and the later experiment, the males were size-and colour-matched since these are known to influence agonistic behaviour (see above) and might obscure any effect of energy reserves. In each experiment, males which were housed in the two compartments of the tank for two weeks were allowed to interact freely by removing an opaque partition which separated them. The fish were observed for a period of five minutes, after which they were killed in liquid nitrogen. Total glycogen and lipid reserves of each fish were estimated using whole body extracts.

Experiment 2 (in 1986) Manipulation of fight length and intensity

Twenty pairs of similar size-and colour-matched breeding male sticklebacks were tested to measure their reproductive aggression. In each experiment, males which had been housed in two compartments of the tank (for two weeks during which they usually built nests) were allowed to fight. Three different categories of tests were carried out. During each test, the male fish was presented with a rival in the form of another male conspecific in breeding condition. Males housed in the two compartments of the tank could only see each other when an opaque partition was lifted for specific periods during the experiment. The fish were able to interact freely when both partitions were removed.

In the first test category seven pairs of fish were observed for a period of five minutes, after removing an opaque partition in the tank but retaining the transparent partition. In this test there was no physical contact between the fish. In the second test category nine pairs of fish were observed for a period of five minutes, after removing both partitions in the tank. In the third test category four pairs of fish were observed for a period of ten minutes, after removing both partitions in the tank.

The original design of this experiment was to test breeding male sticklebacks using all three fight categories in each month (June and July). However, due to unavoidable circumstances this was not possible, and few five minutes tests were conducted in July. Sexually

mature male stickleback collected in the wild (also from the River Kelvin and at the same time as the experimental fish) were used as non-experimental breeding male fish for comparison of energy reserves corrected for body weight.

Behavioural observations

During the tests the behaviour of the fish was recorded as continuous verbal descriptions dictated into a tape-recorder.

The following categories of behaviour were noted:

1) Facing : Beginning and end of the periods of facing the rival fish. The fish was said to be facing while it remained motionless with its head pointing towards the rival.

2) Chasing : Beginning and end of periods of chasing the rival fish.

3) Inactive : Beginning and end of periods of remaining stationary (but not facing the rival or near the nest).

4) Sustained attack : Beginning and end of periods of sustained attack. Sustained attack was defined as an approach to the rival fish, followed by a period of continuous, violent swimming movements including biting and lunging.

a) Bites : Bites were defined as a contact with the rival, with the mouth of the fish being opened and closed once.

b) Lunges : Lunges were defined as rapid movement from a facing position towards the rival fish.

5) Near nest : Beginning and end of periods of nest oriented behaviour viz. facing the nest (within 50 mm of the nest) and creeping through the nest.

Among the behaviour patterns recorded sustained attack (bites and lunges) was coupled with chasing to define total sustained attack.

Assessment of energy reserves

After each experiment the fish were killed by immersing them in liquid nitrogen, were labelled and stored in a deep freezer (-70^{o} C). Glycogen, lipid and protein contents of these fish were estimated (see chapters 3, 4 and 5 for details of estimation). Since the hypothesis is that agonistic behaviour depends on total energy reserves, analyses were carried out on absolute rather than relative levels of energy reserves. Hepatosomatic and gonadosomatic indices were calculated using the following equations (adopted from Wootton et al., 1978)

$$HSI = \frac{\text{wet weight of liver (gms)}}{\text{total wet weight of fish (gms)}} \times 100$$

$$GSI = \frac{\text{wet weight of gonad (gms)}}{\text{total wet weight of fish (gms)}} \times 100$$

8.3 DATA ANALYSIS

Behaviour observations recorded in a tape-recorder were analysed using a BBC Microcomputer program. Data were analysed using parametric tests. Table 8.1 gives mean and 95% confidence intervals of size and overall energy reserves in winners and losers of territorial fights from the 1985 tests. Table 8.2 gives mean behaviour scores for winners and losers in 1986 tests. Table 8.3 gives mean and 95% confidence intervals of size and energy reserves in winners and losers from all the 1986 tests. Table 8.4 gives mean and confidence intervals of size and energy reserves in fish participating in the different fight categories of 1986 tests. Table 8.5 shows the relative energy reserves in winners of all the different fight categories. Table 8.6 gives mean and confidence intervals of energy reserves corrected for body weight in non-experimental breeding male fish caught in the wild from the River Kelvin in 1986.

8.4 RESULTS

8.4.1 Behaviour of winners and losers of territorial fights

When size-and colour-matched, sexually mature male three-spined sticklebacks were allowed to participate in brief territorial disputes, in each case a clear winner emerged rapidly. Fourteen fish were tested in 1985 and in all seven pairs clear winners and losers were identified. Of the twenty pairs of fish tested in 1986, in eighteen cases winners and losers were identified and two encounters ended in draws. It was observed that decisions of territorial disputes

were made quickly, usually after a few bites and /or chases. The loser usually fled and exhibited a submissive posture (i.e. in the corner of the tank at the water's surface in a head-up position; Whoriskey and Wootton, 1986). Table 8.2 summarizes the behavioural distinction between winners and losers of the 1986 tests, in which detailed behavioural observations were made.

8.4.2 Hepatosomatic and Gonadosomatic indices and the outcome of territorial fights

As discussed in the earlier chapter (chapter 7), the hepatosomatic index is a good measure of energy reserves of the liver in the male stickleback, particularly liver glycogen (as it accounts for 25.6% of total variance in hepatosomatic index). In the present experiment, all winners had significantly higher hepatosomatic indices than those of the losers (see Table 8.3). However, winners and losers did not differ in their gonadosomatic indices (see Table 8.3).

8.4.3 Energy reserves and the outcome of territorial fights

1985 data

The winners had significantly higher glycogen reserves than the losers (Table 8.1). Absolute levels in the winners averaged 134 μ mol of glycogen compared to 72 μ mol of glycogen in losers. Winners and losers did not differ significantly in their total lipid reserves.

Test category	Sample size	Length (mm)	L CI	Wet weight (gms)	W CI	Liver Glycogen	L.G. CI	Liver Lipid (mg)	L.L. CI
All winners	7	40.14	± 5.7	1.05	± .15	134	± 19	220	± 31
All losers	7	40.42	± 5.7	1.07	± .15	72	± 10	215	± 30
		t = 0.140 P = NS		t = 0.164 P = NS		t = 3.51 P < 0.02		t = 0.305 P = NS	

TABLE 8.1 SIZE AND OVERALL ENERGY RESERVES IN WINNERS AND LOSERS OF TERRITORIAL FIGHTS OF THE 1985 TESTS. (MEAN AND 95% C.I.)

Test category	Number of first attack	Total sustained attack(sec)	Nest activity (sec)	Face (sec)	Stationary (sec)
All Winners	18	36	109	34	160
All Losers	0	07	42	28	150

Table 8.2 Behaviour of winners and losers in the 1986 tests

Test category	Sample size	Length (mm)	L CI	Wet weight (gms)	W CI	Liver Glycogen	L.G. CI	Liver Lipid (mg)	L.L. CI	Carcass Protein (mg)	C.P. CI	HSI	HSI CI	GSI	GSI CI
All winners	18	41	± 2.9	1.01	±.19	123	± 77	69.6	± 27	622	± 35	2.81	±.20	1.78	± .08
All losers	18	41	± 3.7	1.06	± .29	93	± 46	71.3	± 24	642	± 36	2.65	± .19	1.77	± .06
				t = 0.357 P <0.01 >0.002		t = 2.26 P <0.05 >0.02		t = 0.22 P = NS		t = 1.847 P <0.10		t = 6.930 P <0.02		t = 1.620 P = NS	

TABLE 8.3 SIZE AND ENERGY RESERVES IN WINNERS AND LOSERS OF ALL THE 1986 TESTS. (MEAN AND 95% C.I.)

1986 data

The winners had significantly higher liver glycogen reserves than did the losers. On an average, the winners had 123 μ mol of glycogen in the liver whereas the losers averaged 93 μ mol of liver glycogen (see Table 8.3). In contrast, winners and losers did not differ in their carcass glycogen ,liver lipid reserves or carcass protein concentration.

8.4.4 Energy reserves and the content of territorial fights

Table 8.4 shows that liver glycogen reserves were depleted more (and hepatosomatic index dropped more) in longer/more intense fights. The other energy sources were not significantly different between the three fight categories, although liver lipid levels were lower in the fish that had participated in 10 minutes of free interaction. However, the extent of these changes in glycogen levels is different for winners and losers. Fig 8.1 A and B show the scatter plots of liver glycogen reserves for winners and losers against the total duration of sustained attack given/received by winners/losers in the territorial fights. The more sustained attack that the eventual winner gave in a fight, the lower the liver glycogen reserves were at the end of the test period in both winners and losers (see Table 8.4). The relative depletion in the losers (expressed as the ratio of winner's level to loser's level) increases with the duration of sustained attack (Fig 8.4 A and Table 8.5).

Time (min)	Test Category	Sample Size	Length (mm)	L S.D.	Wet Weight (gms)	W S.D.	Liver Glycogen (μ mol)	L.G. S.D.	Liver Lipid (mgs)	L.L. S.D.	Carcass Protein (mgs)	C.P. S.D.	Hepato-somatic Index	HSI S.D.
05	with transparent partitoin	14	41.6	± .25	1.02	± .04	140	± 46	68	± 9.7	650	± 20	2.73	± .12
05	free interaction	18	41.8	± .24	1.11	± .04	111	± 40	74	± 8.0	619	± 16	2.59	± .09
10	free interaction	8	41.3	± .25	1.08	± .04	49	± 08	55	± 6.2	656	± 18	2.47	± .10
Total		40	F = 0.036 P = NS		F = 0.430 P = NS		F = 11.344 P < 0.001		F = 0.823 P = NS		F = 0.024 P = NS		F = 10.30 P < 0.05	

TABLE 8.4 SIZE AND ENERGY RESERVES OF ALL FISH PARTICIPATING IN THE DIFFERENT FIGHT CATEGORIES IN THE 1986 TESTS. (MEAN AND S.D.)

TABLE 8.5 Relative energy reserves (winners vs losers) for different fight categories in the 1986 tests.

TEST CATEGORY	W:L LIVER GLYCOGEN	W:L LIVER LIPID	W:L CARCASS PROTEIN	W:L CARCASS GLYCOGEN
With transparent partition (5 mins)	1.61	0.80	1.00	0.80
Free interaction (5 mins)	1.21	1.76	0.94	0.88
Free interaction (10 mins)	1.00	0.91	1.00	1.40
	t = 0.339 P = NS	t = 0.396 P = NS	t =-0.067 P = NS	t = 0.850 P = NS

Month	Sample size	Length (mm)	L CI	Wet weight (gms)	W CI	Liver Glycogen	L.G. CI	Liver Lipid (mg)	L.L. CI	Carcass Protein (mg)	C.P. CI	HSI	HSI CI	GSI	GSI CI
June	22	42	± 3.9	1.10	±.28	146	± 66	62	± 38	582	± 61	3.01	±.09	1.82	± .06
July	18	44	± 6.4	1.16	± .06	53	± 29	54	± 23	500	± 47	1.75	± .09	1.64	± .08

TABLE. 8.6 ENERGY RESERVES CORRECTED FOR BODY WEIGHT IN NON-EXPERIMENTAL BREEDING MALE STICKLEBACKS CAUGHT IN THE WILD.
(MEAN AND 95% C.I.)

The scatter plots of liver lipid reserves against total sustained attack (see Figures 8.2 A and B) show that, as in the case of liver glycogen, liver lipid levels are negatively correlated with the duration of sustained attack in both winners and losers, although these relationships are only marginally significant. Similarly the relative depletion in the loser (measured by winner's level/loser's level, see Fig 8.4 B) is positively related to duration of sustained attack, but again, this fails to reach significance. Fig 8.3 A-B and 8.4 C show that there were no relationships between carcass protein levels and the content of territorial fights.

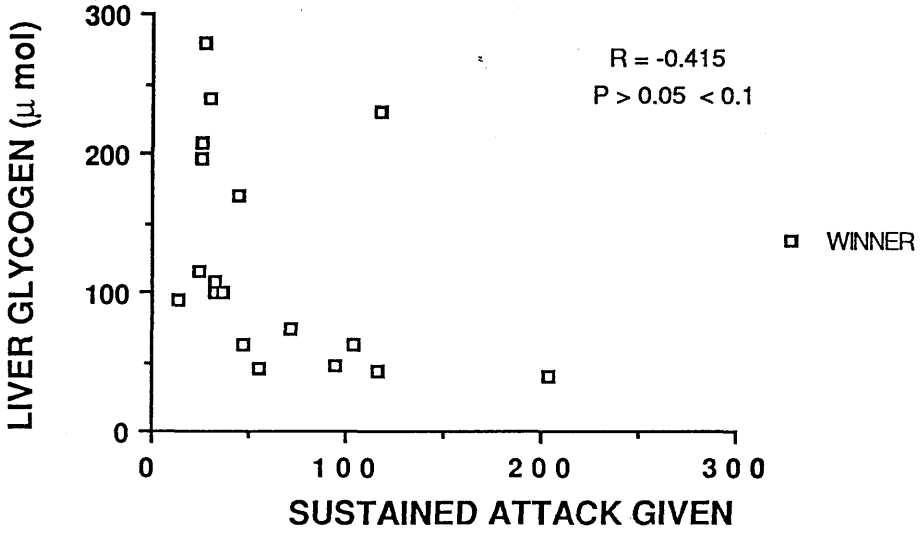
These results suggest that experience of a fight depletes liver glycogen stores (and possibly liver lipid stores too) in both participants. Winners and losers did not differ in their carcass glycogen reserves. They also suggest that depletion is more marked in longer/more intense fights and that losers suffer a more severe depletion of liver energy reserves.

8.4.5 Energy reserves in non-experimental fish

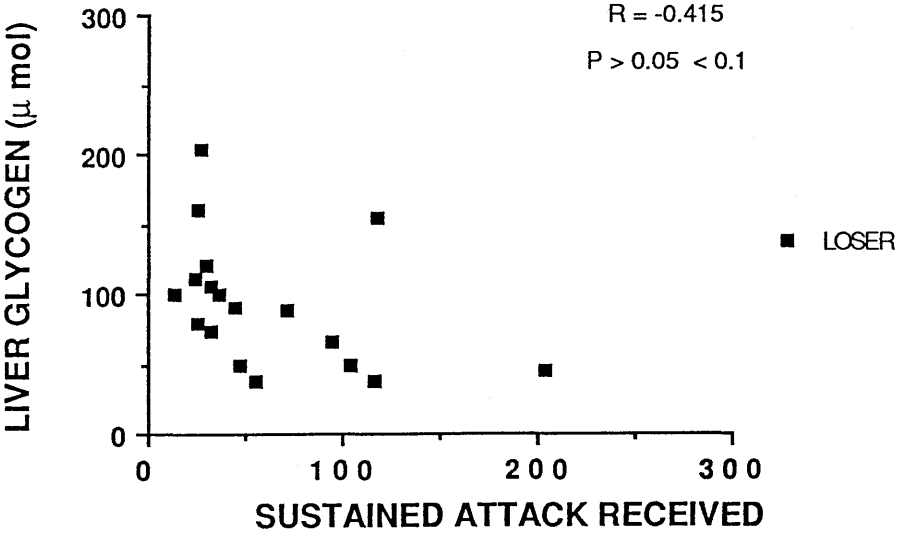
Mean values for energy sources corrected for body weight in non-experimental breeding male sticklebacks caught in the wild in 1986 (see Table 8.6) compared well with those of the test fish but illustrate that average energy sources are lower in non-experimental fish in July than in June.

Figure 8.1 A Scatter plots of liver glycogen reserves against the total duration (in seconds) of sustained attack given by the winners

Figure 8.1 B Scatter plots of liver glycogen reserves against the total duration (in seconds) of sustained attack received by the losers.


LIVER GLYCOGEN (μ mol)
300
200
100
0
0
100
200
300
SUSTAINED ATTACK GIVEN
R = -0.415
P > 0.05 < 0.1
WINNER

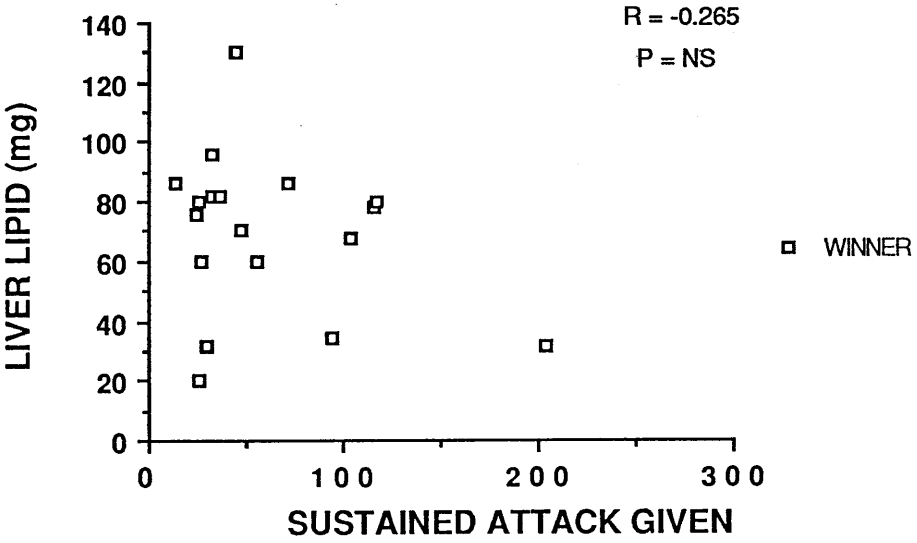
8.1 A


LIVER GLYCOGEN (μ mol)
300
200
100
0
0
100
200
300
SUSTAINED ATTACK RECEIVED
R = -0.415
P > 0.05 < 0.1
LOSER

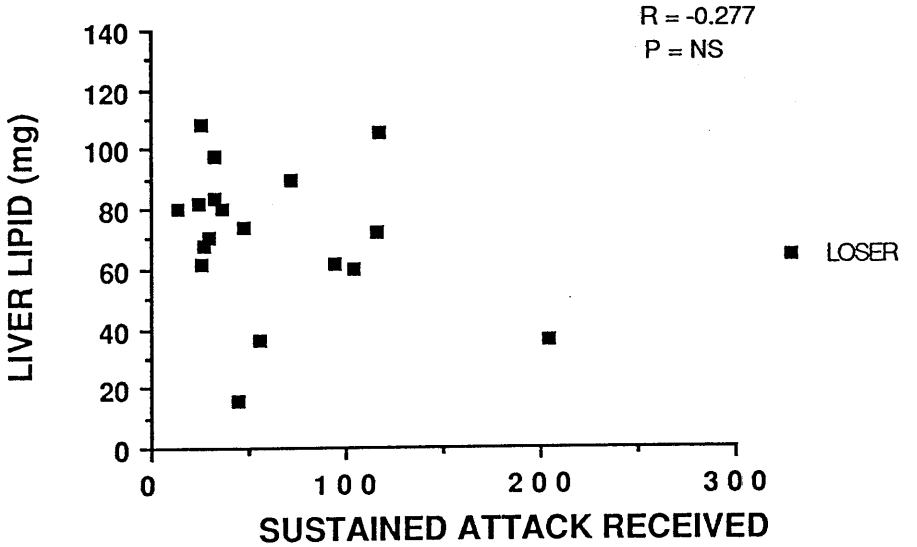
8.1 B

Figure 8.2 A Scatter plots of liver lipid reserves against the total duration (in seconds) of sustained attack given by the winners

Figure 8.2 B Scatter plots of liver lipid reserves against the total duration (in seconds) of sustained attack received by the losers.


R = -0.265
P = NS
LIVER LIPID (mg)
140
120
100
80
60
40
20
0
0
100
200
300
SUSTAINED ATTACK GIVEN
WINNER

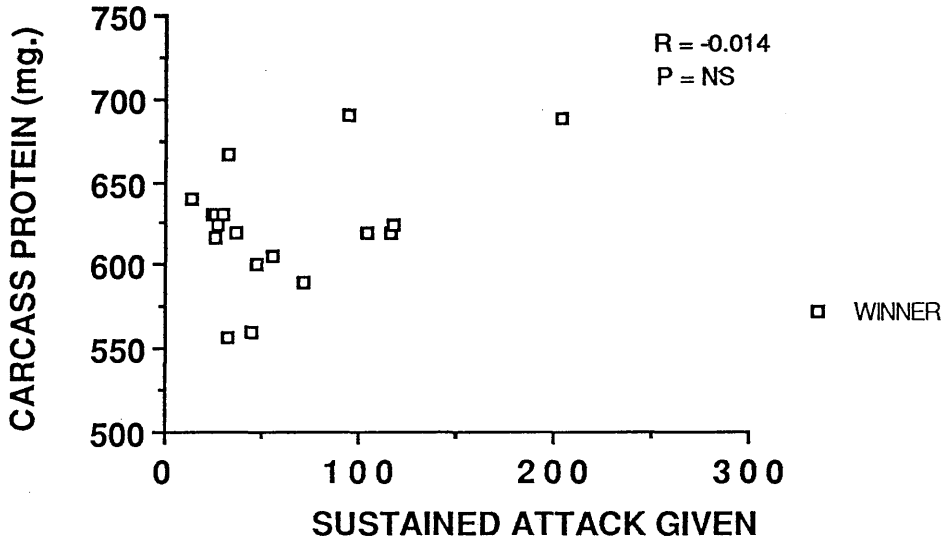
8.2 A


R = -0.277
P = NS
LIVER LIPID (mg)
140
120
100
80
60
40
20
0
0
100
200
300
SUSTAINED ATTACK RECEIVED
LOSER

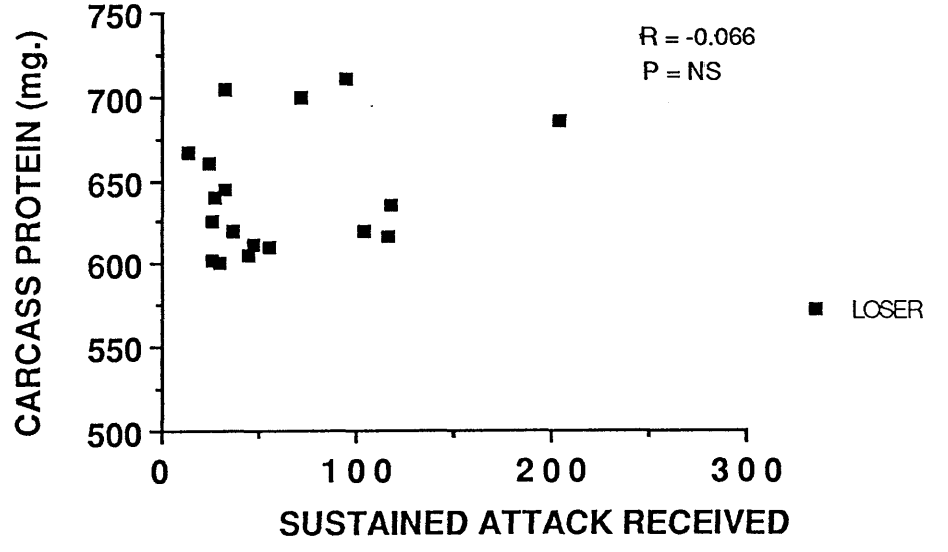
8.2B

Figure 8.3 A Scatter plots of carcass protein concentration against the total duration (in seconds) of sustained attack given by the winners

Figure 8.3 B Scatter plots of carcass protein concentration against the total duration (in seconds) of sustained attack received by the losers.


CARCASS PROTEIN (mg.)
750
700
650
600
550
500
0
100
200
300
SUSTAINED ATTACK GIVEN
R = -0.014
P = NS
WINNER

8.3 A


CARCASS PROTEIN (mg.)
750
700
650
600
550
500
0
100
200
300
SUSTAINED ATTACK RECEIVED
R = -0.066
P = NS
LOSER

8.3 B

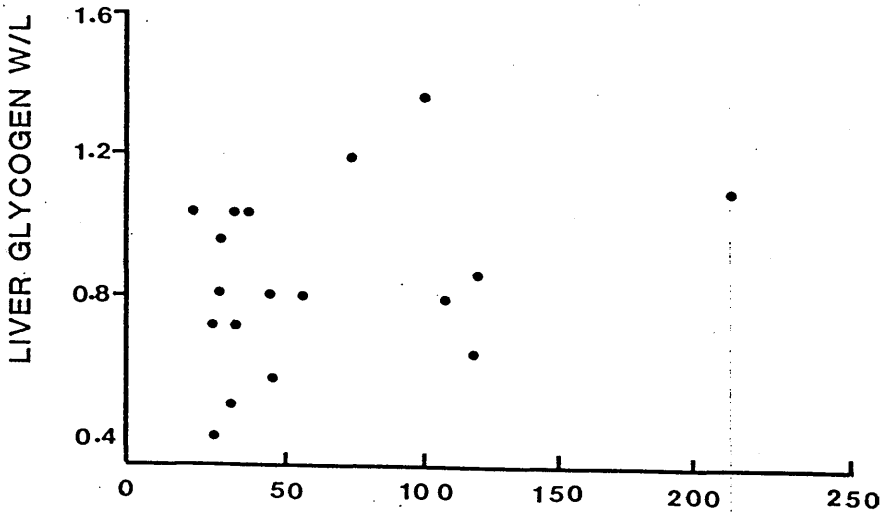
Fig 8.4 Relative depletion of energy sources (W/L ratio) against the total duration (in seconds) of sustained attack

W = winners L = losers

A = Liver glycogen W/L

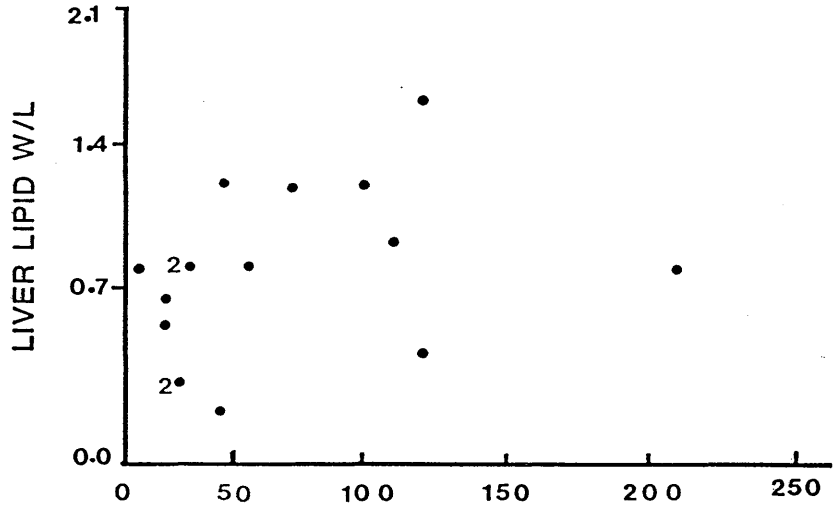
B = Liver lipid W/L

C = Carcass protein W/L



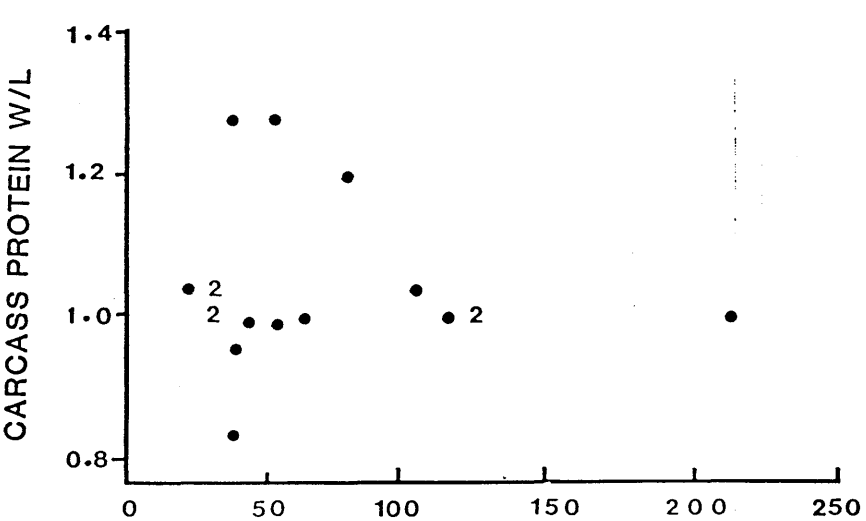
8.4 A

SUSTAINED ATTACK



8.4B

SUSTAINED ATTACK



8.4C

SUSTAINED ATTACK

8.5 DISCUSSION

The tests described in this chapter were designed to see whether natural variation in agonistic behaviour, as reflected in the ability to win fights, can be related to natural variation in energy reserves. In fights between size and colour matched male three-spined sticklebacks with similar gonadosomatic indices, a clear winner emerged after a very short time. From the start of the encounter the winners consistently attacked the loser, who did not retaliate. Victorious males did not have higher levels of either liver lipid or carcass protein (two important long term energy sources for these fish). Nor did they differ in carcass glycogen levels. However, levels of liver glycogen were markedly higher in the winners of territorial fights than the losers.

A number of lines of evidence suggest that difference in liver glycogen is a consequence of experience during the fight and not a cause of the behavioural differences that allowsone fish to win. First of all, carcass glycogen reserves are not different in winners and losers. In addition, if the difference in liver glycogen was a cause of the differential behaviour, then one would expect energy reserves to be more similar in winners and losers of longer and/or more intense fights, but this is not the case. Both winners and losers experience greater liver glycogen depletion in long/intense fights and differential depletion in the loser increases with the amount of attack he receives.

A problem arises in interpreting these results from the fact that tests using the three fight categories could not be carried out in each month as originally planned, because of unavoidable constraints of time. Thus the effect of fight category are confounded with the effects of month. As Table 8.6 shows, non-experimental fish caught at the same time as the experimental fish, had lower energy reserves in July than in June. The low liver glycogen levels in the fish experiencing ten minutes free interaction may therefore reflect the fact that these tests were carried out in the month of July. However, differential depletion of liver glycogen in the loser is still observed, so this result is a direct effect of experience and not an artefact of the month of testing.

This result therefore suggests that decisions during these territorial encounters are not dependant on the long-term energy reserves of the fish, contrary to prediction arising from a consideration of Stanley and Wootton's experimental study (1986). However, selection of colour-matched males may inadvertantly have resulted in selection of males with broadly similar energy reserves. It therefore, remains possible that more extreme differences in energy reserves may influence territorial decisions.

The greater depletion of liver glycogen (a rapidly mobilized energy reserve in response to stress) in both participants when fights are long (if this is accepted as a genuine effect) and differential depletion of liver glycogen in losers, can be related to the results of studies of experience-mediated hormone changes during fights.

Catecholamines promote glycogen mobilisation (Ross and Ross, 1984) so if similar hormone-dependant changes occur during agonistic encounters between male sticklebacks this could generate the observed differences in liver glycogen levels between winners and losers.

8.6 CONCLUSIONS

Higher liver glycogen reserves and victory in territorial fights between breeding male sticklebacks go hand in hand. This seems to be a consequence of fighting experience and not a cause of the outcome of territorial fight between breeding male sitcklebacks. The greatest difference in liver glycogen reserves was seen in those fish that took part in the longest territorial fights.

CHAPTER 9

GENERAL DISCUSSION

CHAPTER 9 GENERAL DISCUSSION

This thesis describes a study of annual variation in major energy sources (i.e. protein, lipid and glycogen) in the male three-spined stickleback (*Gasterosteus aculeatus* L.) collected from the River Kelvin in Scotland. The major aims of this study are as follows:

1) To estimate the energy reserves in the different body compartments (viz. liver, gonad and carcass) in male three-spined stickleback, on a monthly basis over one complete year.

2) To relate the annual variation in energy reserves to changes in (a) body size (b) condition factor of the body (c) somatic condition factor (d) hepatosomatic index and (e) gonadosomatic index.

3) To correlate all these changes to major life cycle events such as gonad maturation and breeding.

4) To examine the relationship between energy reserves and the outcome of territorial fights ie. to examine whether natural variation in energy reserves among breeding male stickleback is reflected in behaviour during territorial disputes.

In this chapter the findings of the research project are summarised and discussed in general terms. More detailed consideration is given in the appropriate sections of the thesis.

Annual variation in energy reserves (aim 1) in relation to body condition and breeding (aims 2 and 3)

Annual variation in the proximate biochemical composition of the body of the male stickleback (chapters 3, 4, 5 and 6) shows that the protein concentration in the dry matter of the body of male stickleback varies from 70 to 76%; Lipid and glycogen reserves constitute 2 to 15% and 0.1 to 1% respectively. An initial increase in body size which occurs from August to October, is essentially due to an increase in protein concentration. Further increase in body size (between October and April) is accompanied by a shift from protein to lipid accumulation. This is reflected in the negative correlation between protein concentration and lipid stores in the carcass which is most marked during the non-breeding season. On the other hand, there is a clear parallelism in the trends of accumulation of lipid and glycogen reserves in all body compartments outside the breeding season. Lipid and glycogen reserves in all body compartments increase from August to October and then level off in the winter. From January until the onset of the breeding season these energy reserves increase markedly. This results in the strong positive correlations between glycogen and lipid stores in the liver, gonad and carcass.

Until the beginning of the breeding season, larger fish have higher lipid and glycogen reserves. This relationship may arise because larger fish hatched early enough to take advantage of the rich summer feeding. The strong positive correlation between body weight and energy reserves indicate that the increase in body size is not

occurring at the expense of energy reserves. On the other hand, the relationship between body weight and protein concentration is predominantly negative during the non-breeding season, reflecting a gradual decrease in protein concentration as the sticklebacks accumulate lipid reserves.

Energy reserves accumulated during the autumn and the spring were mobilized during the breeding season, presumably for reproductive activities. Thus levels of lipids and glycogen in all body compartments, especially in the liver drop to low levels from April/May to July. The relationship between the energy stores in the different body compartments and that between body weights and energy reserves change dramatically during June and July due either to total depletion or to differential depletion of energy reserves. For example, the strong positive correlations between glycogen and lipid reserves in the liver and carcass disappear during the breeding season as a result to total depletion of both these reserves. The positive correlation between glycogen and lipid stores in the carcass also disappears, but this arises because carcass glycogen depletes more markedly than carcass lipid (chapter 6). The relationship between body weight and energy reserves either disappears (as energy reserves become very low in fish of all sizes that breed actively) or reverses (as energy reserves decrease most markedly in bigger breeding fish.

The relative degree of depletion of the various energy sources over the period that male sticklebacks are actively breeding indicates that energy for reproductive activities comes from liver lipid, liver

glycogen, carcass glycogen, carcass protein and carcass lipid. About 33% of liver glycogen, 57% of liver lipid, 56% of carcass glycogen and 15% of carcass protein are used up between May and June. The decline is even more drastic in fish breeding during July, as about 98% of liver glycogen, 95% of liver lipid, 67% of carcass glycogen, 38% of carcass protein and 30% of carcass lipid are depleted.

High mortality was observed in post-spawning male sticklebacks both in the field and in the laboratory, suggesting that males probably breed once and then die. If this is the case, then the very low levels of energy reserves in July can be explained as follows: some males are smaller and with low energy reserves at the beginning of the breeding season. These males increase in size during summer when environmental conditions are favourable and breed in July. Their relatively poor energy reserves are reflected in the very low levels estimated in July. An alternate explanation is that those males which breed in June may breed again in July thus depleting their reserves still further. More studies should be carried out to clarify this point.

Some of the males in the May sample were very small and had very low energy reserves, these are probably late hatched sticklebacks which fail to reach a sufficient size in summer and fail to breed. In June and July, the sample consisted only of mature male sticklebacks with breeding colouration, as they were selectively chosen and due to this

bias in sampling, smaller males were not collected. However, the August sample consisted of such males along with the young of the year. These adult males had no breeding colouration at all and were relatively smaller than the males which bred in June and July. These immature one year old males had higher energy reserves than post-breeding adults and young of the year, accentuating the energetic cost of breeding. Their energy reserves continued to rise during August and September but dropped drastically with the approach of winter, after which this class of fish was not encountered in the samples.

There are marked variations in the condition factor across the months of the year. An increase in condition factor from August to October is followed by a decline in winter. There is a sharp increase in early spring and in May but it declines during the breeding season. Condition factor, and somatic condition factor are strongly positively correlated, indicating that the overall pattern of changes in somatic condition factor is similar to that of condition factor. Stepwise multiple regression analysis suggests that condition factor is not a reliable index of energy reserves of the body in the male stickleback. On the other hand, hepatosomatic index is a good measure of mobilisable liver energy reserves. The important predictors of hepatosomatic index are liver glycogen and liver lipid (which account for 28% of total variance in HSI).

There are no significant correlations between condition factor and gonadosomatic/hepatosomatic indices. The negative relation between somatic condition factor and gonadosomatic index in June approaches

significance suggesting the possibility of a trade-off between gonad development and body condition. The relationship between hepatosomatic and gonadosomatic indices is strongly positive during the non-breeding season, indicating that gonad development is prominant in sticklebacks with good energy reserves. This relationship disappeared in June and July because the size of the liver declined during the breeding season, but this decline was most marked in those fish with a high gonadosomatic index. Male sticklebacks with the highest gonadosomatic index are those that breed actively and consequently experience severe depletion of energy reserves.

These results therefore suggest that male sticklebacks from the River Kelvin are semelparous as they generally have a life span of one year and probably reproduce on one occasion only and then die. Once reproduction is initiated in May/June, most of the energy reserves are utilised for reproductive activities.

Energy reserves and territorial aggression (Aim 4)

Size-and colour-matched, breeding male sticklebacks were allowed to participate in brief territorial disputes to see whether natural variation in energy reserves is reflected in agonistic behaviour during territorial disputes. Winners had significantly higher levels of liver glycogen than the losers but there were no differences between winners and losers in the other energy reserves. The longer and more intense the encounter the greater the differential depletion of liver glycogen reserves in the loser.

The higher liver glycogen reserves in winners are therefore probably a consequence and not a cause of the outcome of territorial fights. Liver glycogen is rapidly mobilised in response to stress-induced catecholamine secretion (Ross and Ross, 1984). Such stress is greater in the losers than the winners (Hannes et al., 1984). If similar hormone-dependant changes occur during agonistic encounters between male sticklebacks, this could generate the differences in liver glycogen levels between winners and losers.

Relation to other studies on energy reserves and reproduction in fish

Existing studies on fish indicate that they suffer a massive depletion of energy sources during spawning migration and reproduction. For example, Atlantic salmon (Salmo salar) lose 99% of lipid, 72% protein and 63% glycogen during spawning migration (Tilik, 1932); Pacific salmon (Oncorhynchus sp) catabolise 60% of body protein during spawning migration (Fontaine, 1975). Northern pike (Esox lucius) lose 5.7% of muscle protein and 16.7% of muscle lipid during the breeding season (Medford and Mackay, 1978). The present study indicates that male three-spined sticklebacks deplete about 45% of glycogen, 57% of lipid and 15% of protein between May and June during the breeding season. Levels of energy reserves are even more drastically reduced in male sticklebacks breeding in July, when about 83% of glycogen, 62% of lipid and 38% of protein have been used.

As predicted by Life History Theory, smaller fishes which have short life expectancies such as the Japanese medaka (Oryzias latipes) give priority to the gonadal development over investment in

the somatic component (Hirshfield, 1980). In male three-spined sticklebacks, increase in body size and gonad maturation takes place in the first year (other than in the smaller sized males which fail to breed at all). In the first few months of their life there is marked increase in body size. During the breeding season, energy sources are mobilised mostly to meet the energy expenditure involved in breeding activities. In June, the negative correlation between somatic condition factor and gonadosomatic index approached significance, suggesting the possibility of a trade-off between gonad development and body condition.

The present study emphasises the fact that the energetic cost involved in the maturation of the testes is lower compared with the cost of ovarian maturation (Wootton, 1985). When males reach maturity between March and May, the energy reserves are not depleted. From May/June onwards, with the development of secondary sexual features and breeding activities (such as territoriality, aggression, courtship, parental care and fanning), the energy reserves are mobilised indicating that this phase is costly. Earlier studies have demonstrated that males in territorial groups lost weight when fed on low rations and parental males that were fed on a low food ration showed significantly less fanning than better fed males (Stanley, 1983). The present study supports this view that breeding activities are energetically expensive and result in massive depletion of energy reserves in the male three-spined stickleback.

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APPENDIX 1

SPECIES LIST

The Gasterosteidae form a monophyletic family containing five genera

Genera/ Species	Common names
1. *Apeltes quadracus*	Four spined stickleback
2. *Culaea inconstans*	Brook stickleback
3. *Gasterosteus aculeatus*	Three-spined stickleback
4. *Gasterosteus wheatlandi*	Black-spotted stickleback
5. *Pungitius pungitius*	Nine-spined stickleback
6. *Pungitius platygaster*	Ukrainian stickleback
7. *Spinachia spinachia*	Fifteen spined stickleback or sea stickleback

(After Wootton, 1976)