Oxygen Consumption and Bioenergetics of the Atlantic halibut (*Hippoglossus hippoglossus* L.): Implications for Culture.

By

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DECLARATION

This declaration certifies that all the research presented in this thesis was conducted by the candidate, and has not been submitted for any other thesis. Where appropriate the work of other researchers has been duly acknowledged.

Signed

Neil Auchterlonie

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ABSTRACT

Aspects of oxygen consumption and bioenergetics of the Atlantic halibut (*Hippoglossus hippoglossus* L.) were studied under laboratory conditions. Resting oxygen consumption rate was monitored over 36 hour periods in Atlantic halibut while held individually in open system respirometers. Routine oxygen consumption rate was determined in small populations of tank held Atlantic halibut over 24 hour periods, through modification of tank systems as open system respirometers. Values for resting and routine oxygen consumption in this species were quantified and models produced enabling the prediction of the energetic cost of homeostasis and spontaneous activity for a range of fish size from 53g to 5861g, at temperatures of 6, 10 and 14°C. These results were further used to form the basis of an energy budget equation for this species. Photoperiod influences on the periodicity of respiratory rhythm in both resting and routine oxygen consumption trials were determined through analysis of data recorded throughout the 24 hour daily period. The results indicated a cyclic respiratory rhythm, with peak oxygen consumption often observed nocturnally. The relevance of these results to culture of this organism are discussed.

Post-prandial oxygen consumption and ammonia excretion were measured in small populations of tank held Atlantic halibut, these results contributing further information to the partitioning of energy within the metabolic and excretory components of the energy budget equation. Values for resting, routine and post-prandial oxygen consumption in the Atlantic halibut were found to be low in comparison to roundfish species, but corresponded closely with literature data produced for other species of temperate marine flatfish.

Activity patterns in small populations of Atlantic halibut were monitored over 24 hour periods in a specially constructed film unit. The results of this work showed dualistic patterns of activity over the diurnal cycle. Further elucidation of the energy budget was achieved through the determination of the metabolic costs of activity in the tank environment. Atlantic halibut were observed to remain at rest for periods of

between 76% and 94% of any 24 hour period, with the time at rest dependent on fish size and stocking density.

One 28 day trial was undertaken in which the components of the energy budget were measured simultaneously, and the balance of the budget investigated. Oxygen consumption, ammonia excretion, growth, feed consumption and faecal production were monitored within a purpose built experimental unit, and these values further applied to the construction of an energy budget model.

Finally, the oxygen consumption data of this study was further applied to produce a model quantifying the water requirements of this species in a single-pass tank system, for the intensive culture of this species in the tank environment. Further development of these figures allowed the quantification of the pumping costs in an Atlantic halibut ongrowing tank system, and these figures were incorporated into a simple economic model.

The results present some of the first bioenergetic data produced for this species, and this is an important step towards the development of an Atlantic halibut farming industry.

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1.1 Atlantic halibut - Natural History and Fisheries

The Atlantic halibut (*Hippoglossus hippoglossus* L.), order *Pleuronectiformes*, family *Pleuronectidae*, subfamily *Pleuronectinae*, tribe *Hippoglossini*, (as classified by Nelson, 1984), is the largest of the flatfishes, and historically has held a position of considerable commercial importance in the fisheries of the North Atlantic (McKenzie, 1946; McIntyre, 1952; McCracken, 1958; Nickerson, 1978; Neilson *et al.*, 1987). This species is distributed over a wide area of the northern Atlantic ocean (Fig.1.1.), and may be found from the Bay of Biscay to the Barents Sea in the east, and in waters north of the state of Virginia in the west (Scott and Scott, 1988). The highest densities of Atlantic halibut occur in the areas along the Norwegian coast, off southern Greenland, and in the waters around Iceland and the Faroe Islands (Haug, 1990a). A preference is shown for cool boreal waters with a temperature range of 2-8°C, and although the lower limit of depth remains unknown the fish does not seem to be present at depths greater than 1460m, with commercially caught fish taken mostly on longlines at a depth of 200-300m (McCracken, 1958; Wheeler 1969).

Annual world catches of *H. hippoglossus* throughout the 1980s were in the region of 7000-8000 mt (F.A.O., 1991). A total of 7509 mt of Atlantic halibut was landed in the world fisheries in 1991, with the countries of Canada, Iceland and the Faroe Islands dominating the catch. In comparison, Nickerson (1978) reports landings in excess of 4000 mt for U.S. east coast fisheries alone in 1886, and with the European catch in 1963 quoted as 12000 mt (Wheeler, 1969), it would seem likely that the overall decrease in catch statistics is a result of fishing pressure. This hypothesis is further strengthened by the observation of Bell and Pruter (1958) that the closure of the Atlantic halibut fisheries over the periods of the two world wars appeared to be associated with a sharp and temporary increase in stock abundance when the fisheries were reopened. The relatively recent introduction of a minimum size limit for the Canadian fishery (Neilson and Bowering, 1989) is a first step towards the management of an Atlantic halibut fishery, and historically fisheries for

this species have not been regulated. With an average length of Atlantic halibut of 50cm, corresponding to an age of 4 years currently being landed at fishing ports (Liewes, 1984), the catch is one in which immature fish predominate, further adding to the fragile nature of these fisheries.

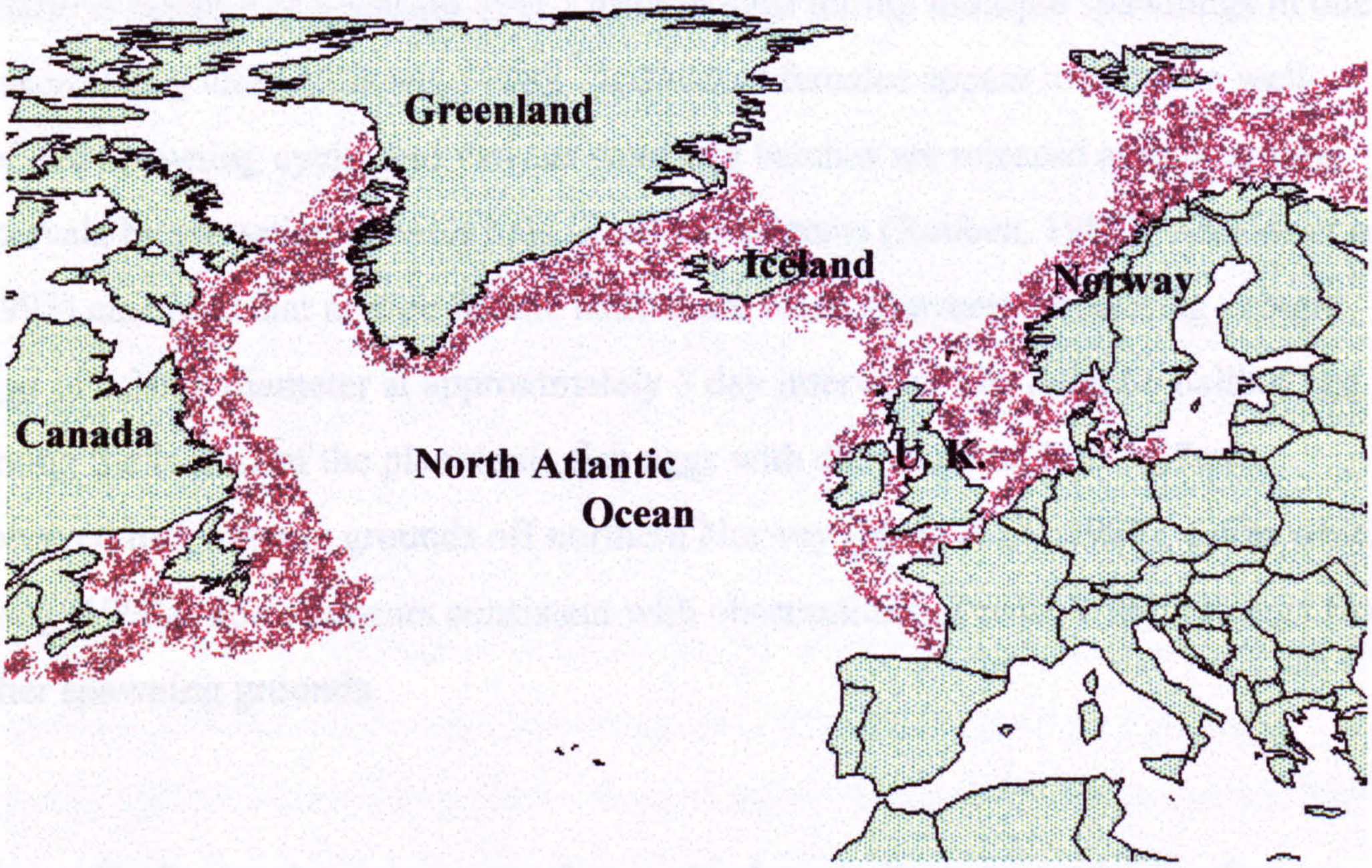


Fig. 1.1. Distribution of *Hippoglossus hippoglossus* (L.) in the north Atlantic ocean (mottled grey area), after Haug (1990a).

It has been deduced from studies on seasonal variation in gonadal maturity of adult wild fish (gonadosomatic index) and on the relative abundance of planktonic eggs on the spawning grounds that peak spawning in northernmost Norwegian waters occurs at the end of January / beginning of February (Kjørsvik *et al.* 1987). In Canadian waters Scott and Scott (1988) report that spawning mainly occurs in the months of February, March and April, although Neilson *et al.* (1993) found spawning peaks in the November/December period. Jákupsstovu (1986) notes an inter-area difference in spawning time between the Faroe Islands and northern Norway, suggesting a possible peak in Atlantic halibut spawning activity during late winter and early spring in Faroese stocks. Although these inter-area spawning differences may suggest the existence of separate stocks of *H. hippoglossus*, studies have shown that

halibut from geographically distinct areas such as the Norwegian coast, Faroes and Greenland, exhibit a large degree of genetic homogeneity between stocks (Mork and Haug, 1983; Haug and Fevolden, 1986; Fevolden and Haug, 1988).

H. hippoglossus is a multiple spawner with a high fecundity, and a single female is capable of releasing over 2 million eggs during multiple spawnings in one season (Haug and Gulliksen, 1988). Individual females appear to follow a well-defined spawning cycle, and various sized egg batches are released at regular time intervals by spawning females held in culture systems (Rabben, 1987). Shields *et al.*, (1993) also note that mature female halibut are batch spawners, producing pelagic eggs of c.3mm diameter at approximately 3 day intervals. The Atlantic halibut egg is among the largest of the planktonic fish eggs with diameters of 3.06-3.49mm observed in spawning grounds off northern Norway (Haug *et al.*, 1984), a size which Haug (1990a) notes appears consistent with observations of pelagic halibut eggs from other spawning grounds.

Studies on the occurrence of spawning have indicated a preference for deepwater locations (Jakupsstovu and Haug, 1988) and various depths have been identified according to geographical location, >180m in Canadian Atlantic waters (Scott and Scott, 1988); 300-700m in Norwegian coastal waters (Haug, 1990a); 300-1000m in North Eastern Atlantic ocean waters (Whitehead *et al.*, 1986); 1000m in the Atlantic waters of the British Isles (Wheeler, 1969). These deep-water locations are all characterised by a very stable physical environment, with temperatures ranging from 5-7°C and salinities of 34.5-34.9‰ (Kjørsvik *et al.*, 1987). The stability of this environment appears to be crucial in the development of halibut eggs.

Differences in spatial distribution between immature and mature Atlantic halibut are widely recognised (Haug, 1990a), and coastal areas exhibiting depths of 20-60m with sandy bottoms appear to be utilised by young halibut as nursery areas. Neilson *et al.*, (1993) report catches of uniformly small (30-50cm) halibut in the

vicinity of Browns Bank, Nova Scotia, and postulate that this area may be the nursery ground for the Canadian north-west Atlantic. Further nursery areas have been identified - between the Faroe Islands, off western Iceland, and in Norwegian coastal areas - and it appears that emigration from these areas commences when the fish are 3-4 years old (Haug, 1990a). Tagging experiments have confirmed the highly migratory nature of these fish, and there is evidence for large distance movements in immature Atlantic halibut. Fish tagged off Browns Bank, Nova Scotia, indicated migrations of distances between 161-968km, although one fish tagged in the Gulf of St. Lawrence in 1946 was recaptured off Iceland in 1953, a distance travelled of 2572km (Scott and Scott, 1988). Outwith the spawning season, when all the fish are feeding, stocks of immature and mature halibut intermix (McCracken, 1958; Bowering, 1986), although as spawning commences only mature fish are found in the spawning areas. Following spawning, mature halibut leave the spawning grounds and migrate in all directions showing little preference for depth, and appear to be actively seeking food after a prolonged starvation during the spawning period. Cyclic annual migrations between spawning and feeding areas have been observed in mature Atlantic halibut, with smaller fish (<75cm) tending to cover greater distances (Stobo *et al.*, 1987). The migration patterns of a group of immature halibut following a mark recapture study in northern Norway are presented in Fig. 1.2.

There is some documentation on growth rates of wild Atlantic halibut, summarised by Haug (1990a). O-group halibut show a large variation in size, this variation becoming more pronounced in later year classes, leading to considerable overlap in size distributions for age groups. Growth rate in immature fish has been shown to vary both with the area of the North Atlantic under survey, and the year of the survey - factors such as temperature, stock abundance, depletion of food competitors, and food supply may have contributed to these differences. Surveys of young Atlantic halibut (I-, II-, and III- group) in Faxa Bay, Iceland, over the period 1936-1950 provide evidence for an annual variation in growth rate which was observed to be almost negligible over the winter/spring period, increasing over

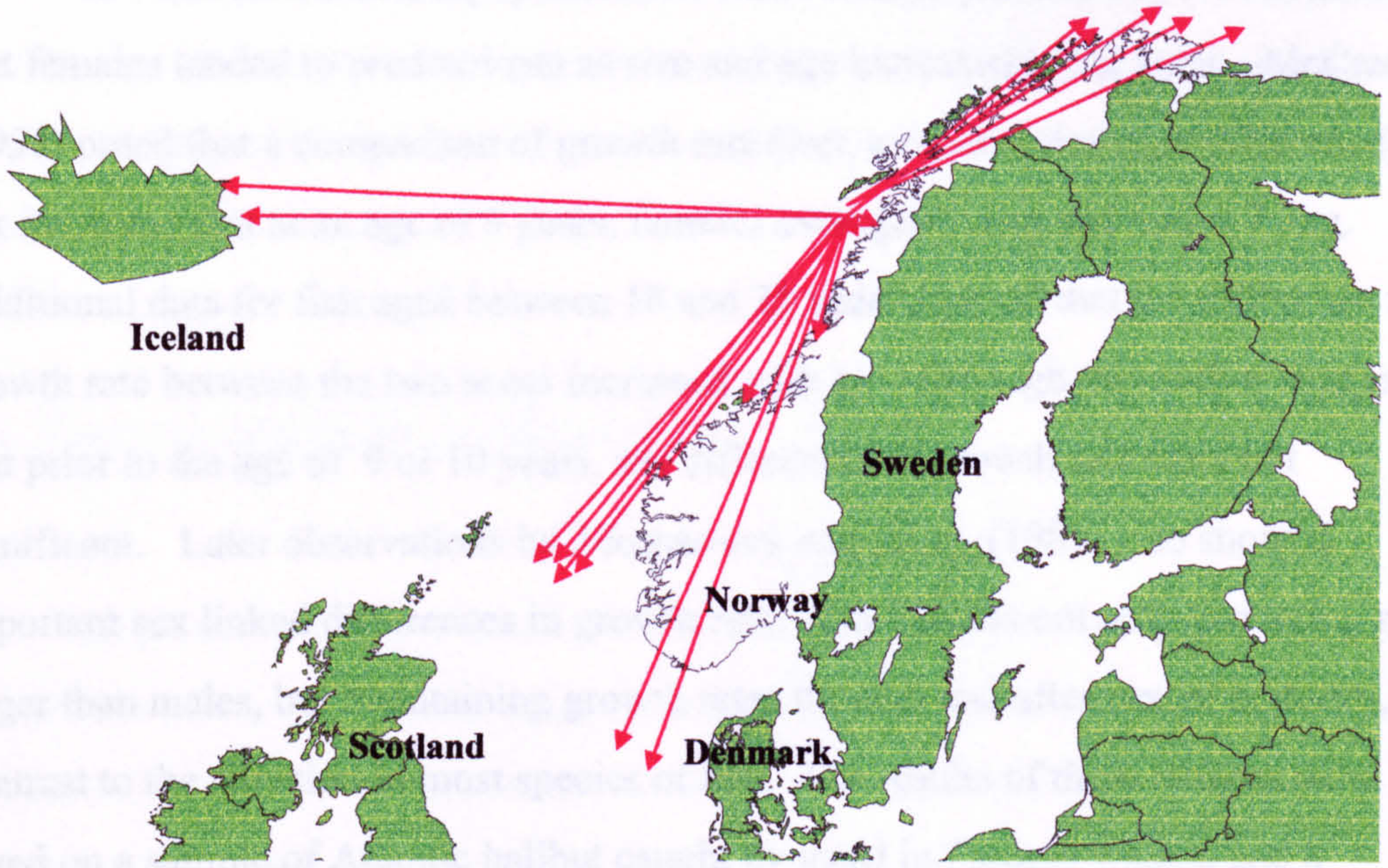


Fig. 1.2. Migration vectors produced for a small population of immature Atlantic halibut tagged off the coast of northern Norway. Data produced by Godø and Haug (1988) for a mark recapture experiment of 300 individual fish tagged in 1961 and 1962 (in Haug 1990a).

summer/autumn. Many field studies (summarised in Haug, 1990a) have also shown a feeding behavioural change in halibut, with fish of <30cm feeding on crustaceans and mysids, fish of 30-60cm feeding on a mixture of crustaceans and fish, and fish of >60cm becoming exclusively piscivorous. In Icelandic waters McIntyre (1952) observed that adult halibut had consumed only 11 species of fish, and the redfish (*Sebastes marinus*) accounted for over 75% of prey items by volume. Leim and Scott (1966) state that *Sebastes marinus* typically inhabit the edges of banks and deep channels, and are generally more plentiful in areas with temperatures between 3 and 8°C and depths of 200 to 500m (110 to 270 fathoms depth). These authors also note that the redfish appears to have a strong behavioural rhythm, and are found on muddy bottoms or around rocks during the day, but become pelagic at night. Adults are generally 8-16 inches (20-40cm) in length. Atlantic halibut are known to be mid-water feeders, at least in the larger size ranges (Nelson, 1984), and this may suggest an element of nocturnal rhythmicity in this species.

In work on a Scottish population of wild Atlantic halibut, Rae (1959) noted that females tended to predominate as size and age increased in the stock. McCracken (1958) noted that a comparison of growth rate (wet, eviscerated weight difference) by sex indicated that at an age of 9 years, females averaged 9.1kg and males 7.3kg. Additional data for fish aged between 10 and 25 years showed that the difference in growth rate between the two sexes increased with age, although this author suggested that prior to the age of 9 or 10 years, sex differences in growth rate were not significant. Later observations by Jákupsstovu and Haug, (1988) also showed important sex linked differences in growth rate, with females not only becoming much larger than males, but maintaining growth rates through and after sexual maturity, in contrast to the situation in most species of fish. The results of these authors were based on a sample of Atlantic halibut caught by trawl in Faroese waters, and a comparison of this data with that produced for halibut in other areas of the North Atlantic showed that Faroese halibut matured slightly earlier (4.5 years for males; 7 years for females) and at lower weights (1.7kg in males; 18kg in females) than in corresponding populations of Atlantic halibut in different regions. Again it was suggested that sex differences in growth rate were not pronounced in immature fish, although average weights of 4.9kg in males and 6.5kg in females are listed for six year old halibut. In a review of wild fish behaviour, Haug (1990a) reports instances of sex ratio in trawled catches deviating from 1:1, and postulates that there may be a seasonal and spatial segregation of the sexes in wild stocks. The same author also hypothesises that there may be a sexual variation in the energetics of this species, evidence for this coming from observed differences in the mobilisation of muscle tissue to reproductive effort in mature males and females. Studies on spawning and post spawning Atlantic halibut of both sexes by Haug *et al.*, (1988) indicated a lower total lipid content in the white muscle of male fish, although lipid and fatty acid compositions showed no significant differences. Although the biological significance of these results is unknown, the authors postulated that there could be a connection with inter-sex physiological differences, particularly the higher growth rates seen in females after maturation.

As discussed by Haug (1990b), the Atlantic halibut appears to encompass the whole range of the r/K continuum in terms of life history strategies, with reproduction more associated with r-selected strategies, while other life history parameters such as size and longevity show greater emphasis on K selection.

1.2. Research Applicable to Atlantic halibut culture.

In his introduction to aquaculture economics, Shang (1990) lists six factors which have driven the increasing importance of fish culture towards the end of the twentieth century. These include the lessening importance of capture fisheries associated with depletion of stock and increasing costs of operating fishing technology, improvements in fish culture technology, and increasing demand for high-priced species in developed countries. In the words of this author "commercial aquaculture is motivated by profit making", and thus the choice of species to culture is vital to the success of any venture. With the collapse of the wild fisheries, an excellent market price, and a rapidly increasing base of culture technology associated with the culture of flatfish species, the Atlantic halibut appears an excellent choice for marine aquaculture in temperate regions. The arrival of this species to commercial fish farming is also timely, enabling the diversification of an industry almost entirely dependent on the culture of the Atlantic salmon.

The value of the wild fisheries indicates the great market potential of Atlantic halibut, and along with the historical significance of this species, the fragile nature of wild populations and the variability in landings often seen with open ocean fish, there is obvious potential for the Atlantic halibut as a cultured fish species. However, in comparison to other farmed fish species, it is only relatively recently that aquaculture technology has reached a sufficient level to allow the production of these fish, and as late as 1990, Tilseth included *H. hippoglossus* as one of four interesting candidates for cold-water aquaculture. Only since the mid-1980s, when the first rearing of Atlantic halibut larvae through to metamorphosis was completed in controlled experiments (Berg and Oiestad, 1986; Rabben *et al.*, 1986; Naas *et al.*, 1987), has

there been a focus of research on the biology of *H. hippoglossus*, a necessary requirement in order to develop rearing techniques for any cultured organism. The culture of the closely related Pacific halibut (*Hippoglossus stenolepis*), is recognised as occupying an equivalent stage in the development of aquaculture technology (Stickney and Liu, 1995).

The early life history is a critical period of development for all flatfish, and procedures involved in rearing from egg, through larval stages to fully metamorphosed flatfish, are dependent on the development of adequate technology to fulfil the requirements of the fish at these vulnerable stages. Liewes (1984) reported the relatively small size and pelagic nature of flatfish eggs, which develop as they float through the aquatic environment, as the greatest obstacle to culture of flatfish species. There is little documentation on the development of halibut larvae in the wild, possibly due to the extremely low larval densities which result from a wide distribution over large ocean areas. Haug (1990a) summarises the literature by noting that 7 authors between the years of 1904 and 1987 recorded only a total of 57 individual *H. hippoglossus* larvae from open Atlantic waters, thus there is very limited information on the early development of Atlantic halibut larvae from studies of wild fish which could be applied to facilitate culture of this species. Much of the recent research has concentrated upon improving larval rearing techniques to improve the yield of juvenile Atlantic halibut from egg through to metamorphosis since these stages, with the early first feeding stage in particular, are widely recognised as the limitation to the production of adequate numbers of juveniles for successful ongrowing (Stickney and Liu, 1993; Brown and Keough, 1994). It may also be observed that, in comparison to other flatfish species, notably turbot (*Scophthalmus maximus*), sole (*Solea solea*) and plaice (*Pleuronectes platessa*), the hatching and larval rearing of Atlantic halibut is more problematical (Liewes, 1984; Ingram, 1987). This has resulted in the allocation of an almost disproportionate amount of research resource towards the areas of early life history, broodstock nutrition and egg quality, emphasising the potential importance of a developing Atlantic halibut farming industry predicted to lessen the dependency on salmonids in marine aquaculture in temperate regions.

Growth studies in culture have indicated higher growth rates and improved condition factors for a population of wild caught fish aged 2-4 years held in tanks, than in a corresponding wild population over a period of 18 months (Haug *et al.*, 1989). The effect of stocking density on the growth rates of two size classes (1.8 and 3.2 kg) was the subject of a further study, the results suggesting that the optimum stocking density of halibut is at a level above 100% of coverage of tank bottom, i.e. between one and two layers of fish on the tank bottom, for these sizes of fish (Björnsson, 1994). For tanks of 1.0m depth these results gave optimum stocking densities of 25-50kg m^{-3} for 2kg halibut, and 50-100 kg m^{-3} for 10 kg halibut. In comparison Atlantic salmon are routinely stocked at a maximum density of approximately 25kg m^{-3}. Growth trials in culture have incorporated the use of various diet types including flying squid (*Todarodes saggittatus*), Iceland scallops (*Chlamys islandica*), herring (*Clupea harengus*), saithe (*Pollachius virens*), redfish (*Sebastes marinus mentella*) (Davenport *et al.*, 1990); frozen capelin (*Mallotus villosus*) and herring (Bjornsson, 1994); fillets and moist pellets based on capelin and herring (Haug *et al.*, 1989).

Nutritional studies for Atlantic halibut ongrowers have also been limited by the supply of adequate numbers of fish for trial populations, however Bjornsson *et al.*, (1991) observed lower growth rates in fish fed salmon diets and a fish silage diet, in comparison to diets containing whole or ground fish. Haug *et al.* (1989) showed the rate of somatic tissue increase in a captive population of Atlantic halibut to be twice that of the corresponding wild population from which the test sample originated. Generally the condition factor of the captive population was also higher than fish at the point of capture. Similar specific growth rates to those observed with Atlantic salmon (*Salmo salar*) at similar water temperatures, were seen in experiments utilising diets containing 58% protein (Hjertnes and Opstvedt, 1990), indicating the potential of *H. hippoglossus* in culture systems. Hallaråker *et al.* (1995) quoted daily growth rates of between 1.7% and 2.0% d^{-1} in juvenile Atlantic halibut of 20g to 90g in weight, and these authors identified the optimum temperature for this size of fish

within the range 10°C to 13°C. Although not statistically significant, an increased growth rate was observed during spring and early summer, which was considered to be related to increasing ambient photoperiod. A further study on optimal temperature for growth in the Atlantic halibut by Björnsson and Tryggvadóttir (1996) indicated that the optimal temperature decreases with increasing fish size, from 14°C for fish of 10g to 60g weight, to 9.7°C for fish of 3kg to 5kg. Specific growth rates were also found to decrease with increasing fish size, from 2.2% d^{-1} for fish of 10g - 60g, to 0.23% d^{-1} for fish of 3kg - 5kg. In a nutritional study of large halibut (0.6-1.5kg) by Berge and Storebakken (1991), dietary fat levels of 8-20% did not significantly affect feed efficiency, chemical composition of fillets and livers or digestibility of dry matter, fat and protein. A comparison of Atlantic halibut and lemon sole (*Microstomus kitt*) satiation meal size by Davenport *et al.*, (1990), revealed a preference for very large meals (mean 11.7 % body weight) with a long gut clearance time (120h) in *H. hippoglossus*, although this work was conducted on fish fed a moist diet. Current practice on ongrowing sites is to feed commercial dry pelleted diet at a level of approximately 1% of body weight per day for fish of greater than 100g.

The principal factor common to many of these studies is the use of wild caught fish which have an unknown biological history - a factor which may have some bearing on the results. Berge and Storebakken (1991) reported a large variation in weight gain within groups of *H. hippoglossus* in a nutrition trial, and hypothesised that these results may be a reflection on the difference in ability of individuals to adapt to the captive environment. In growth and nutrition studies it is usual to compare sibling populations of juvenile fish, though unfortunately the current status of halibut culture technology has not yet provided this opportunity. Mixed sex populations of mature fish have been used in trials, despite documentation on sex differences in growth rate in immature and mature fish. Although there is a great deal of potential for the culture of *H. hippoglossus* and this species does adapt reasonably well to a tank environment, all these factors raise questions over the accuracy of many of the trials in nutrition and growth that have been performed up to this time.

Infestations of the ectoparasite *Entobdella hippoglossi* (Trematoda), have been identified as potential problems associated with the culture of Atlantic halibut (Schram and Haug, 1988), although formaldehyde (repeat treatments every 3-4 months) has proved to be an effective chemotherapeutant in controlling infestations in stocks of broodfish (Haug, 1990). Infectious pancreatic necrosis virus (IPNV), and nodavirus, have recently caused significantly heavy losses of juvenile halibut in intensive culture systems in Norway, however this species does not show the same predisposition to viral haemorrhagic septicaemia virus (VHSV) or to the bacteria *Vibrio anguillarum* and *Aeromonas salmonicida* often seen in cultured salmonids in the marine environment (B. H. A. 1997). Clearly, the potential impact of viral, bacterial and parasitic diseases will not be fully realised until the supply of metamorphosed juveniles allows populations to be maintained at production stocking densities.

One other interesting phenomenon which has been associated with the culture of Atlantic halibut is the occurrence of ocular abnormalities (Williams *et al.*, 1995). This a worrying situation, and although the aetiopathogenesis of the abnormalities is unknown, the incidence of problems is currently greater in broodstock populations than in ongrowing fish. An ophthalmological investigation of a wild-caught, farmed population of Atlantic halibut held in ongrowing tanks at Ardtoe, in Argyll, Scotland, showed ocular abnormalities at an incidence level of 43%, characterised by gross exophtalmus and periorbital cysts (Williams *et al.*, 1995). The exposed position of halibut eyes, especially the right side eye, does make it vulnerable to damage in culture with the associated handling and high stocking densities. Again, the true importance of ocular abnormalities will not be realised until production densities for ongrowing fish are attained.

1.3. Bioenergetics

1.3.1 General Principles

The essential concept of the principle of biological energetics has been incorporated in the phrase "the fire of life" (Kleiber, 1961), emphasising that the basic source of energetic fuel is derived solely from food. The partitioning of energy within the system conforms to the First and Second Laws of Thermodynamics and may be considered to have a profound effect on form and function (Brody, 1945; Calow, 1985; Lucas, 1996). Thus, all energy acquired through the ingestion of food is ultimately lost as waste in faeces or by excretion, used in metabolic processes, or deposited as new body tissue (growth or energy gain), and may be expressed in the form:

$$\mathbf{R = F + U + M + P} \qquad \text{(Jobling, 1994)}$$

Where: **R** = energy gained as food

F = energy lost as faeces

U = energy lost as nitrogenous excretory products

M = energy expended in a range of bodily functions - Metabolism

P = energy storage or growth (occurs when energy intake from external sources (food) is in excess of energy required for maintenance and activity

Jobling (1994) further subdivides M into:

$\mathbf{M_M}$ = minimal costs for maintaining bodily function

$\mathbf{M_A}$ = minimal costs associated with activity

$\mathbf{M_F}$ = minimal costs related to digestion, absorption and processing of food

and P into:

$\mathbf{P_S}$ = somatic (body) growth and $\mathbf{P_R}$ = production of gametes

1.3.2. Fish Bioenergetics

The study of fish bioenergetics provides valuable predictive information for use in minimising costs and maximising production in aquaculture systems (Brafield, 1984; Ross *et al.*, 1988c; Jobling, 1994; Lucas, 1996). The energy budget is of interest in aquaculture, where the theoretical desired maximisation of the factor P is determined through the examination of the inter-relationships of all factors, with complex interactions occurring between abiotic factors (eg. temperature, photoperiod, salinity, dissolved oxygen concentration) and biotic factors (eg. fish size, fish age, competition, ration size, ration composition) affecting the rates of metabolism, M_M, M_A and M_F (Brett, 1979; Dejours, 1988). The partitioning of energy within the system as a whole requires the preferential allocation of energy towards the M factor, thus the factor P may vary according to the energetic costs associated with metabolism (Calow, 1985; Priede, 1985). The metabolic cost of homeostasis must always be met, thus when inputs are limited there are constraints on production.

Jobling (1985) summarises the literature relating growth rates with rates of metabolism, and concludes that there is a fundamental relationship between growth/production and metabolism/respiration. There appears to be a strong correlation between rates of weight gain and metabolism at the level of the organ, individual and community. Steffens (1989) states that there is only a very narrow temperature range over which the resultant energy partitioning may be manifest as growth. This author emphasises the importance of ambient environmental conditions in determining rates of metabolism - a fundamental difference from homeotherms.

Measurement of metabolism (M) in fish, and the relationship of this factor to the many biotic and abiotic factors, is a basic requirement for the understanding of the processes involved within the energy budget equation. Direct calorimetry has been successfully applied to fish metabolism studies (Smith *et al.*, 1978), however it is more usual to measure energy expenditure (M) through indirect calorimetry by

measuring rates of oxygen consumption, and this is a technique which has been used successfully for a number of years (Beamish, 1964a,b; Beamish and Mookherjii, 1964; Brett, 1972; Staples and Nomura, 1976). Mammalian energetics studies utilising indirect calorimetry have encompassed measurements of oxygen consumption, carbon dioxide production and nitrogenous excretion to provide more accurate predictions of M (Brafield and Llewellyn, 1982), and a similar approach to aquatic metabolism studies has been advocated for some time (Brett and Groves, 1979; Brafield, 1985). Simultaneous measurements of oxygen consumption rate and carbon dioxide production rate enable the calculation of the respiratory quotient (R.Q., the ratio of CO_2 produced to O_2 consumed), the value of which changes depending on the substrate metabolised, and to the degree which anaerobic respiration is taking place (Brett and Groves, 1979; Brafield, 1985), thus providing important information on the physiological processes involved. To provide meaningful results, techniques of indirect calorimetry all require that there is no contribution from anaerobic processes, i.e. that respiration is fully aerobic, otherwise an underestimate of the rate of metabolism will ensue (Braaten, 1979). Generally, measurements of carbon dioxide production in terrestrial animals may be made much more conveniently than in aquatic animals, and studies such as those of McGregor and Lee (1995) on metabolism in the rat indicate the value of the application of the respiratory quotient in energetics studies. In aquatic systems the solubility of carbon dioxide gas is considerably enhanced by the reaction with carbonate ($CO_3^{2-} + H_2O + CO_2 \Rightarrow 2HCO_3^-$), and in marine systems the equilibrium constant is so large that much of the carbon dioxide is rapidly converted into bicarbonate (Libes, 1992). As a result, aquatic CO_2 production measurements are difficult, with those in seawater exceptionally so, and this has resulted in a focus of research solely around the calculation of oxygen consumption rates in marine energetic studies. The practicalities associated with the measurement of carbon dioxide production in aquatic studies, has resulted in little published information on R.Q. values in fish. The work of Kutty (1968) on goldfish and rainbow trout, and Tátrai (1980) on the bream produced R.Q. values in freshwater fish species, although generally such work is exceptional. These studies involved the titrimetric determination of dissolved carbon dioxide, and although modern

technology has seen the development of CO_2 electrodes (Brafield, 1985), much remains to be achieved in this field.

The energetic characteristics of aerobic respiration at the cellular level require the presence of oxygen and the production of carbon dioxide during the formation of Adenosine triphosphate (ATP), (Lucas, 1996). This reaction is exergonic, and energy in the form of heat is released into the environment. As previously discussed, the measurement of the levels of oxygen uptake by the organism through indirect calorimetry the metabolic costs of respiration in energy terms may be estimated (Brett and Groves, 1979). When oxygen consumption rates have been calculated, energy equivalents (Q_{ox}) may be applied, and used to estimate values for amounts of energy (J) released per volume O_2 consumed for different respiratory substrates (Elliott and Davison, 1975; Brett and Groves,1979). Energy equivalents have been determined for the metabolism of a variety of energy substrates, measured by bomb calorimetry, and the appropriate factor may be applied to values for oxygen consumption if the energy substrate is known. In this way multiplication of the amount of oxygen consumed by the relevant coefficient allows the estimation of energy lost as heat (Brafield, 1985), thus the energetic costs of various processes may be calculated and used in the construction of an energy budget.

1.3.3. Measurements of M_M, M_A, and M_F

The "metabolic scope" of fish is influenced by such factors as species, age of development, and a range of environmental factors of which temperature is the most important (Priede, 1985).

M_M - Costs of maintaining bodily functions.

This component has variously been described as "standard", "resting", (Beamish, 1964a,b,c), "fasting" (Jobling ,1994), and is equivalent to the "basal"

metabolism of homeotherms (Brett and Groves, 1979) although it should be emphasised that in poikilotherms costs of maintaining bodily functions cover a range of temperatures, while in homeotherms maintenance energy costs are restricted to one temperature. Fry (1957) and Beamish (1964a) used the term routine metabolism to cover standard oxygen consumption under conditions where spontaneous activity was not restricted, although Braaten (1979) notes that measurements of routine metabolism should also involve some measure of the activity level of the fish. The common feature of studies to determine M_M is that the influences of both feed and activity should be minimised by the use of starved fish tested in a thermoneutral environment (Brett, 1962; Jobling, 1994). External stimuli should also be kept to a minimum in the respirometer systems, so that excitation of the test animals does not have an effect on oxygen consumption rates (Fry, 1971).

The effect of temperature on the resting metabolic rate of fish is well documented (reviewed by Fry, 1971), and this factor has been considered the most important variable in determining metabolic energy costs since it exerts a direct effect on rates of reaction at the cellular level (Brett, 1979). In an attempt to clarify the complexity of the physiological processes involved in the M_M component, Jobling (1994) defines the energetic costs of the minimal level of metabolism as "service functions" (energy costs of respiratory and circulatory systems in providing body tissues with oxygen), and "cellular maintenance functions" (cellular energy costs of ion transport, and synthesis and turnover of proteins and lipid), and this author notes that in particular the metabolic costs of maintaining sodium pumps and those associated with protein turnover are significant, and proportionately are likely to vary in energy cost with body tissue type within the organism. Tissue differences in metabolic rate according to acclimation temperature have been observed using techniques which allow the measurement of enzyme activities, and add a further level of complexity to the subject (Johnston and Dunn, 1987). In energetic studies, the metabolic costs at organism level are more usually identified since these results have a greater application to energy budgets and aquaculture. The Q_{10} (temperature coefficient) expression was developed to describe the effect of temperature on physiological processes, and has been widely applied to describe the increase in M_M

observed over a temperature increase of 10°C, although the Q_{10} value is species specific and changes over the temperature range of the test organism (Winberg, 1956). Generally Q_{10} values are high at the lowest temperatures of the organism's range, and decrease as temperature increases. As discussed by Brett and Groves (1979) the Q_{10} value does produce an estimate of the change in level of metabolism over a temperature range without introducing an error of greater than 20% of the observed value. These authors quote from the work of Beamish (1964a) and Brett (1964) on brook trout, bullhead, carp and sockeye salmon, and advocated a Q_{10} value of 2.3 to provide an approximation for temperature effects on metabolism. However, the difficulty in achieving genuine inter-species comparisons was emphasised, since different fish species are adapted to different ranges of temperature. Jobling (1994) stresses the importance of unambiguous detailing of the conditions under which experiments on the temperature effects on metabolism are conducted, and this author also notes that Q_{10} values may approach 1.0 in the mid part of an organism's adaptive temperature range, indicating a degree of independence of the rate of some physiological processes at these temperatures.

Fish size is also viewed as an important factor in determining resting metabolic rate. Historically, the "Surface Rule" had been applied to animal metabolic studies, relating surface area of the organism to metabolic rate. Von Bertalanffy (1957) stated that gill breathing animals appear to follow the Surface Rule, and proposed that it was as valid for fish as it had been for mammalian studies. This statement was questioned by Winberg (1959) who, in reviewing a great deal of data from fish metabolic studies, came to the conclusion that there were discrepancies in predictive models produced according to the Surface Rule in fish, and that the relationship between fish weight and metabolic rate provided a more accurate predictive model. Early work by Beamish (1964a) and Beamish and Mookherjii (1964) showed that increasing size does have the effect of increasing resting metabolic rate in fish, although the rate per unit weight shows a decrease with increasing size. This last phenomenon is attributed by Jobling (1994), to a decrease in proportion of highly metabolically active tissues (e.g. liver, gut) with respect to the relative size of muscle tissue associated with locomotion, as fish increase in size. The

general relation is described by the allometric equation $\mathbf{Y} = \boldsymbol{a}\mathbf{W}^{\boldsymbol{b}}$, where **Y** is the oxygen consumption rate, **W** is the fish body weight, and ***a*** and ***b*** are species specific constants (Brett and Groves, 1979). The weight exponent *b* has been determined in many studies on a large variety of fish species and has been found to range between 0.65 and 0.9, with a value of 0.86 advocated as a broad "cross-species" figure (Glass, 1969; Brett and Groves, 1979; Jobling, 1994). The decline in metabolic rate per unit body weight is considered to reflect proportional changes in the relative size of individual organs to the whole organism, with juvenile fish possessing a larger proportionate mass of highly metabolically active tissues such as the liver and alimentary tract, in comparison to more mature fish in which somatic tissue comprises a greater proportion of total mass (Jobling, 1994).

The situation with respect to larval fish may differ however, and Giguère *et al.*, (1988) show that metabolic rate scales isometrically (ie. b = 1.0) in larval Atlantic mackerel (*Scomber scombrus*), and suggest that the metabolic exponent (b) decreases as body size of the organism increases, as fish enter different stages of their life history. Recent work by Ronnestad and Naas (1993) also confirms an isometric relationship between body dry weight and metabolic rate of Atlantic halibut first feeding larvae.

Stocking density and behavioural interactions may also have an influence on metabolic rate, and Steffens (1989) reports lower oxygen consumption in fish kept in groups rather than as individuals. Umezawa *et al.*, (1983) showed during the measurement of oxygen consumption rate of the ayu (*Plecoglossus altivelis*), that the metabolic rate could either increase or decrease in individuals held within populations, depending on the animal's social history.

In addition to temperature, body size and behavioural interactions, there are several other factors which have also been shown to influence the rate of metabolism, such as the oxygen content of the water (Dejours, 1988; Steffens, 1989); salinity and

the associated metabolic cost of osmoregulation (Rao, 1968; Hettler,1976; Marais, 1987; Gasca-Leyva *et al.*, 1991); photoperiod, diurnal and seasonal rhythms (Beamish, 1964c; Hirata, 1973; Nagarajan and Gopal, 1983; Ross and McKinney, 1988a,b; Chakraborty *et al.*, 1992a); and handling (Marais, 1987).

Along with the stability of test temperatures, great importance has been placed on acclimation of fish to the environmental conditions under which the experimental work will take place, since the physiology of the animal is continually regulated through environmental cues (Fry, 1971). The thermal history of the test animals should coincide with the test temperature (Brett, 1962; Braaten, 1979), and an acclimation period of 4 weeks to test conditions has been generally proposed. By extension, other environmental parameters such as photoperiod and salinity should also be controlled at test condition level during the acclimation period, to avoid undue influence of the test fish response to a change in the environmental parameters under trial conditions.

In summary, it is clear that the results produced in studies of maintenance energy costs in fish may be influenced by a wide variety of factors, and this point is of the utmost importance when the reproducibility of such work is considered. Thus it is vital in fish energetic research that all such factors be adequately controlled, and the limits and range of these factors accurately specified in order that misinterpretation of the results is avoided.

$\underline{M_A}$ - Metabolic cost associated with activity.

It is usual in fish studies to measure active oxygen consumption at a predetermined swimming speed which can be sustained, and oxygen consumption may thus be measured over a range of swimming speeds (Brett, 1972). In recent years it has become more common to measure oxygen consumption at the maximum sustainable swimming speed (Braaten, 1979), and in many studies M_A has been

extrapolated to zero activity to provide an estimate of M_M (assuming a linear relationship between oxygen uptake and swimming speed). The inaccuracies of using this method for the prediction of resting metabolic rate in flatfish have been exposed by Priede and Holliday (1980), who discovered that actual resting rate was somewhat lower than predicted in the plaice, *Pleuronectes platessa*. M_A is also influenced by temperature, although the situation is compounded by the decrease of oxygen solubility in water seen with rising temperatures (Brett and Groves, 1979). Rates of oxygen consumption, and thus rate of metabolism (M_A) is usually found to increase exponentially with an increase in swimming speed, with this relationship derived from experiments with tunnel respirometers in which the fish are forced to maintain a constant swimming speed (Jobling, 1994). Beamish (1970) showed the oxygen consumption of largemouth bass (*Micropterus salmoides*) forced to swim in tunnel respirometers, to increase with weight, and for a given swimming speed, oxygen consumption increased with temperature over the test range (10-34°C). Studies by Brett (1965) on sockeye salmon (*Oncorhynchus nerka*) showed an almost insignificant effect of size on active metabolic rate for a range of temperatures, and in further studies on *O. nerka* by Brett and Glass (1973), the rate of M_A was almost completely independent of size at temperatures below 10°C, although with the combined effect of increasing size and increasing temperature there was found to be an increasing correlation with the rate of M_A.

M_F - Metabolic costs associated with feeding.

Winberg (1956) was one of the earliest workers to recognise that feeding had an effect on metabolic rate in fish, and suggested that the standard rate of metabolism would be doubled under the influence of feeding. The use of the term (apparent) heat increment has been advocated by recent authors (Brett and Groves, 1979; Beamish and Trippel, 1990) as being more descriptive of the processes involved, than the more widely used specific dynamic action (S.D.A.). Jobling (1983) argues that the application of "S.D.A" to ammoneotelic fish may not be wholly justified, since it was originally proposed to describe urea synthesis, nitrogenous excretion and post prandial

metabolic rates in mammalian studies. However, the use of this term remains widespread in fish metabolic studies. More recently, a great deal of research has been undertaken on the effect of feeding on metabolic rate (Staples and Nomura, 1976; Weatherley, 1976; Jobling, 1981a; Jobling, 1983; Beamish and Trippel, 1990; Ross *et al.*, 1992; Chakraborty *et al.*, 1992b), and the increase in rate of post prandial oxygen consumption is considered to reflect the metabolism of dietary protein, carbohydrate and lipid, with the processes of deamination and protein synthesis probably major contributors. The level of the specific dynamic action effect increases with meal size, and body weight , and represents a substantial portion of gross energy intake, with values of 9-15% of calorific content of food reported (Brett and Groves, 1979).

The post prandial increase in oxygen consumption characteristic of the M_F component of metabolism, is associated with quantity and quality of feed, as well as environmental factors, such as temperature (Steffens, 1989) and differences exist between species with respect to the size and duration of the apparent heat increment. Beamish (1974) measured "apparent S.D.A." in swimming largemouth bass (*M. salmoides*) in tunnel respirometers, and found the total increase in oxygen uptake to increase curvilinearly with weight, the rate decreasing with the size of the bass. Studies on the influence of body size and food ration on the energy budget of the rainbow trout (*Oncorhynchus mykiss*) by Staples and Nomura (1976), have shown that the total metabolic expenditure increases with increasing body size and feeding level, and metabolic studies of plaice (*Peuronectes platessa*) and dab (*Limanda limanda*) by Edwards *et al.*, (1969) confirm the effect of increasing rate of metabolism with increased food uptake. Jobling and Spencer Davies (1980) showed from work with plaice (*Pleuronectes* platessa) that the duration of the "S.D.A." effect is reduced with an increase in temperature, and increases with increasing percentage of protein in the diet.

The results of work in this area are directly relevant to aquaculture, allowing the prediction of the duration and the elevation of oxygen consumption peaks following feeding in these systems, information which is useful in the fine tuning of a

farm in order to increase the efficiency of production. The conditions encountered in aquaculture generally do not allow for such precise measurements, since populations of fish on farms are routinely fed within the period of the duration of the S.D.A. effect, thus precise measurements on individuals may be modelled and utilised in the prediction of the effects of feeding on tank populations. The difficulties associated with the extraction of meaningful results from large farmed populations of fish are clear, when the vagaries of water temperature, fish species, fish size, fish activity level, salinity, photoperiod, feeding level, feed composition, timing of data recording and the hydrodynamics of the holding facility are taken into account. Working with such systems it becomes progressively more difficult to ensure that standard trial conditions are maintained as the experimental facility increases in size. Despite these problems, there are studies including those of Mueller-Fuega *et al.*, (1978) on pond reared rainbow trout, Forsberg, (1994) on post-smolt Atlantic salmon, and Graham-Brown *et al.*, (1983) on turbot on commercial sites which provide predictive models for such facilities.

1.4. Research Applicable to Flatfish Metabolism

Much of the literature on fish metabolism and bioenergetics is centred around studies of roundfish species, reflecting both the availability of these species and the comparatively good response of fish such as the goldfish, tilapia and carp to confinement within respirometers. Documentation on the metabolism of flatfish species is limited in comparison, although the commercial importance of species such as the plaice (*Pleuronectes platessa*) in temperate fisheries has diverted some attention to this area, with the practical application of bioenergetic studies as relevant to wild fisheries as it is to aquaculture.

There is some limited information on the oxygen consumption rates of flatfish species, and in general terms the resting metabolic rate of flatfish has been found to be low in comparison to roundfish (Wood *et al.*, 1979; Priede and Holliday 1980, Duthie, 1982), and is considered to reflect a relatively sedentary lifestyle in which low resting

metabolic rates can reduce maintenance costs (Duthie, 1980). Weight specific oxygen consumption rates have been determined in some studies, and work by Duthie (1982) and Jobling (1982), suggests that flatfish conform to the general allometric expression $Y = aW^b$. Generally values which have been identified for the weight exponent in flatfish tend towards the lower end of the general species range, and literature values of 0.78 in plaice and flounder (Fonds *et al.*, 1992); 0.82 in flounder, 0.73 in dab, 0.73 in lemon sole (Duthie, 1982); 0.734 in *Cynoglossus* spp., 0.682 in *Brachirus* spp., 0.682 in *Synaptura* spp. (Edwards *et al.*, 1971) have been determined.

Priede (1985) emphasises that active metabolism and the costs of locomotion in flatfish differs from the situation in roundfish species. Studies on respiratory metabolism of flatfish species swimming in tunnel respirometers, have indicated that extrapolation of oxygen consumption to zero swimming speed results in a figure which is about 60% of the rate of oxygen consumption determined at standard metabolic rate (Priede and Holliday, 1980; Duthie, 1982). A great deal of energy is expended initially in lifting the fish off the bottom, and flatfish appear to swim routinely at a speed very close to the critical swimming speed, taking opportunities to rest as they occur. While swimming at these speeds, there may be some proportion of respiration due to anaerobic processes, and in resting on the bottom flatfish have a good opportunity to repay an oxygen debt (Priede, 1985).

Studies on the effect of feeding on flatfish metabolism have been documented (Edwards *et al.*, 1969; Jobling and Davies, 1980; Jobling, 1982), and there is considerable literature on gastric evacuation rates in the plaice (Jobling and Davies, 1979; Jobling, 1980; Basimi and Grove, 1985), in turbot (Flowerdew and Grove, 1979; Grove *et al.*, 1985) and dab (Jobling *et al.*, 1977), as well as the Atlantic halibut (Davenport *et al.*, 1990). The stomach of the Atlantic halibut is relatively large in comparison to other species of fish within the *Pleuronectidae*. de Groot (1971) classified *Hippoglossus hippoglossus* as a "Type I" pleuronectid, possessing a large oesophagus and stomach, a simple intestinal loop, and large gill rakers, all characteristics of piscivorous fish. In one of the few studies of Atlantic halibut

relevant to the ongrowing of this species, Davenport *et al.* (1990) measured gut transit time following a meal in *H. hippoglossus*. These authors produced a figure of 120h for total gut clearance in this species, although the study fish were of 1.383kg average weight, and the study was conducted at 10°C with the fish fed a moist diet.

There appears to be sufficient differences between the physiology and energetics of flatfish and roundfish species such that the culture of any flatfish species should be regarded as something of a unique situation. The commonly cultured fish species of contemporary intensive aquaculture systems are roundfish, and it may not be assumed that the culture of any species of flatfish can be merely transposed into these systems. Certainly adequate production levels of flatfish may be attained in such systems, but only through a view to the unique energetic characteristics of each individual species may the most efficient production of any flatfish species be attained. The potential culture of the Atlantic halibut is no exception to this, and in many ways the unique position this species holds even amongst flatfish species should indicate the importance of a bioenergetic approach to the farming of this organism.

1.5. Aims of this Study

This review of the biology of *H. hippoglossus* suggests that the farming of this species is likely to present a unique set of circumstances to aquaculturists for a variety of reasons:

1. The temperature range which this species appears to prefer in the wild is considerably lower than that which is likely to be encountered on farm sites on the West coast of Scotland.
2. This species is found in areas of the natural environment characterised by very stable physical parameters.
3. As Atlantic halibut may attain a comparatively large size, it may be hypothesised that resting metabolic rate in this species will be low in comparison to other currently cultured species.

4. Flatfish species are known to be sedentary in habit in relation to roundfish species.
5. The anatomy of the alimentary canal and notes on feeding behaviour suggest an adaptation to single meals of very large size.
6. This species is known to feed in mid-water and at depths of 200-300m in the wild, yet culture tanks and cages are not deeper than 5m.
7. The physiological characteristics of this organism may make it a viable option for pump ashore seawater farm sites present but currently inoperative in Scotland due to the decreasing profit margins observed in the culture of Atlantic salmon.
8. The flesh of the Atlantic halibut is perceived as a luxury product, and possesses a high market value.

Clearly these factors are likely to have some influence on the metabolic rate of this fish in culture situations, and there are likely to be interesting differences in the metabolism of the Atlantic halibut when compared to other currently cultured species. With these considerations in mind, the aim of this study was to investigate the oxygen consumption and the bioenergetics of the Atlantic halibut, as a step towards the calculation of an energy budget for this species under culture conditions. The application of such a budget to halibut culture systems is likely to facilitate the efficient production of this species. A series of experiments was designed to quantify resting metabolic rate in individual Atlantic halibut, covering a range of temperatures likely to be experienced in ongrowing sites in Scotland and the likely production size range of this fish. This data would form the basis of an energy budget equation, facilitating the prediction of the costs of homeostasis. Routine oxygen consumption rates, and oxygen consumption rate following a single meal, were measured in small populations of Atlantic halibut, in order to quantify the energy costs of spontaneous activity and specific dynamic action. This work also covered ammonia excretion rates, in order that a similar model may be constructed for ammonia excretion in this species. In addition, an activity study was undertaken to examine the possibility of activity peaks associated with diurnal periodicity, in particular to investigate any differences between the photophase and the scotophase. Also, the individual

components of the energy budget equation were measured simultaneously in a trial lasting 28 days, to model energy transfer within a single system. The interrelationships of the energy budget components would thus provide information applicable to culture of the Atlantic halibut, and this information could then be used to construct a predictive model, taking into account the economics of operating such a unit.

Chapter 2. General Materials and Methods

2.1. General.

All work in this study was carried out between May 1995 and December 1997, at the Institute of Aquaculture's Marine Environmental Research Laboratory, Machrihanish, Argyll, on the west coast of Scotland. The site at Machrihanish is a pump-ashore, single pass sea-water unit, receiving local sea water through a submerged intake pipe of approximately 3m depth. This water is passed through a 5μm filter before providing a gravity feed to the site tanks. Ambient annual temperature range is 6 - 15°C, and salinity varies between 32 and 35‰.

2.2. Fish

2.2.1. Genetic stock

The Atlantic halibut (*Hippoglossus hippoglossus*, L.) stock used in these trials was comprised of the 1991, 1994, 1995 and 1996 year classes. Fish provided by the Sea Fish Industry Authority's research laboratory at Ardtoe, Acharacle, Argyll, formed the majority of the test animals, although some 1996 year class halibut obtained from Mannin Sea Farms (Isle of Man, U.K.) stock contributed to the experimental work in the latter part of 1997. These fish are all first generation farmed stock, produced from the original wild stock imported from Iceland in 1988 by the British Halibut Association (B.H.A).

2.2.2. Fish Quality

At the time of this study, the Atlantic halibut farming industry was very much underdeveloped, and the production of juveniles was the most important area in which this lack of knowledge was exhibited. As a result, a variation in fish quality was recognised throughout this work, characterised by a range of pigmentation, and a range in the degree of migration of the left side eye. In addition, some fish showed partial or complete loss of the right side eye, thought to be the result of aggressive

social interactions in the hatchery phase. It is important to note that the availability of juvenile halibut over the time course of this study was limited, and as a consequence of this, some fish of less than perfect morphology entered experimentation. An analogy may be drawn with the early years of the turbot farming industry in the 1970s in which early crops also showed a similar wide variation in the quality of intensively reared juveniles (Liewes, 1984). The production of juvenile turbot is no longer affected in this way, and the intensive farming of that species is now an economically viable proposition in countries such as France, Spain, Germany and the U.K., suggesting that an embryonic halibut farming industry given the same research attention can also achieve a commercial level of quality juvenile production.

2.3 General husbandry

Stock halibut of the 1994, 1995 and 1996 year classes were held as small populations of about 10 individuals in $1m^2$ fibreglass tanks with a water depth of 30cm. 1991 stock fish were held on site in a 4m diameter round fibreglass tank with a water depth of 1.5m. The fish were fed daily at 10.00h with a proprietary brand flatfish diet (B.O.C.M. Pauls), with the feed rate determined by manufacturer's tables according to fish weight and temperature (range 0.5-1.0% body weight d^{-1}). Pellet size was 8.0, 13.0 or 18.0mm length, depending on fish weight, with 8.0mm pellets offered to halibut of 40g to 500g, 13.0mm pellets to halibut of 500g to 1000g, and 18.0mm pellets offered to halibut >1000g according to the manufacturer's tables. The 8.0mm pellet possessed a slightly different composition in comparison to that of the 13.0mm and 18.0mm pellets. The manufacturer's analysis of diet composition is represented in Table 2.1.

Stock halibut were weighed monthly and the feed rate adjusted accordingly. Mortality rate was extremely low in all year classes of stock fish over the experimental period, and generally were confined to fish which managed to leap clear from the tanks. The tanks were flushed daily, and checked for mortalities. All tanks were cleaned (brushed) weekly. Prior to, and following fish transfers between tanks, tank surfaces were cleaned, disinfected with iodophor compound ("FAM 30"), and

rinsed. Ambient sea water was supplied to all the stock tanks, with flow rates to individual tanks adjusted and measured within the range 8-15 l min^{-1}. Supplementary aeration was achieved through round (15cm diameter), flat air stones positioned on the floor of each tank. Total tank volume was approximately 75 l.

Table 2.1 Composition of B.O.C.M. Pauls Flat Fish diet.

	Pellet Size		
Analysis %	**8.0mm**	**13.0mm**	**18.0mm**
Protein	**56.0**	**52.0**	**52.0**
Oil	**16.0**	**20.0**	**20.0**
Carbohydrate	**9.0**	**9.0**	**9.0**
Ash	**11.0**	**11.0**	**11.0**
Phosphorus	**1.5**	**1.5**	**1.5**
Nitrogen	**9.0**	**8.3**	**8.3**
Gross Energy kJ g^{-1}	**20.5**	**21.1**	**21.1**

2.4 Weighing of Fish

The calculation of oxygen consumption rate requires the accurate determination of fish mass. Individual halibut were weighed following anaesthesia in a 1:2000 (*v/v*) solution of 2-phenoxy ethanol (Sigma, U.K.), taking care to ensure the removal of any excess water. Weights were recorded to the nearest gram.

2.5 Starvation Period prior to trials

In order to ensure that experiments involving the measurement of resting rate and routine rate metabolism were conducted on halibut which were in the postabsorptive phase, a protocol was devised whereby starvation times were standardised according to fish weight and temperature. Energy content of feed is a principal factor in determining rate of gut evacuation (Pandian, 1987), and it could be hypothesised that gut evacuation rates could be shorter for halibut fed a commercial dry diet in comparison to a moist diet. The published figures of Davenport *et al.* (1994) were used as a base for this work in the absence of data on rates of gut

evacuation in this species when fed commercially prepared dry high energy diets. Gut evacuation time is known to increase with decreasing temperature (Steffens, 1989), and has also been found to increase with increasing fish size in flatfish, for any given ration (Jobling *et al.*, 1977). A scheme determining starvation periods for different sizes of fish at different temperatures was developed (Table 2.2) and adhered to for the duration of the trials. In this manner, all fish entering either resting rate respirometry experiments, or tank trials for determining routine metabolic rate, were considered to have empty alimentary tracts, and be representative of this species in the post absorptive phase.

Table 2.2. Starvation regime applied to test Atlantic halibut prior to entry to respirometry and tank trials, in order to ensure measurements were conducted on fish in the postabsorptive phase.

	Fish Weight (g)		
Temperature (°C)	**<300**	**300 - 1500**	**>1500**
6	5	6	7
10	4	5	6
14	3	4	5

Starvation periods are in days.

2.6 Water Quality - General

Poor water quality and fluctuations of certain water quality parameters are known to influence health, behaviour and metabolic rate in many species of fish (Smart, 1981). In some fish species, dissolved oxygen concentration is known to exert an effect on rate of oxygen consumption outside the "zone of independence" (Ott *et al.*, 1980; Hughes, 1981; Hughes *et al.*, 1983; Martinez-Palacios and Ross, 1986). Consequently, it is of great importance that such environmental parameters are closely monitored during experimental work in this field, and every effort was taken to minimise any possible effect of water quality associated factors in this study. The most important parameters (temperature, dissolved oxygen, total ammonia and pH) were monitored routinely over the course of individual trials and also during acclimation periods.

2.7 Measurement of dissolved Oxygen

2.7.1 Dissolved Oxygen meters

YSI model 57 (analogue) and model 58 (digital) meters were employed for the measurement of dissolved oxygen concentrations in all trials. Meters were connected to YSI 5739 oxygen probes via YSI 5740 - 10 cables. The probes are Clark-type membrane covered polarographic sensors (see Ross, 1985) which possess inbuilt thermistors for temperature measurement and compensation. Both meters have a salinity compensation mechanism whereby externally calculated salinity readings are calibrated into the meter by use of a dial. Site salinity readings were taken by hydrometer as a daily routine, and atmospheric pressure readings were also taken from a site barometer, and these values together with accurate temperature readings were used to calibrate the meters and probes. Oxygen calibration was carried out by the use of tables (Colt, 1984) and Winkler titrations. General maintenance of probes and meters was carried out regularly according to manufacturer's instructions. The electrical zero end point of both meters was regularly compared with a chemical zero end point through immersion of the connected probe in an oxygen depleted sample (25g of sodium sulphite dissolved in 500ml distilled water).

Although the operating instructions for the YSI 5739 dissolved oxygen probes specify the use of high sensitivity (0.5mm thick) teflon membranes when taking dissolved oxygen readings at temperatures below 15°C, in practice the stability of readings was found to be compromised with such membranes. As a result, standard thickness (1.0mm) membranes were used as routine.

In all respirometry experiments (individual fish and tank trials) flow rates were set such that there was an observed reduction in dissolved oxygen concentration of approximately 0.5 - 1.0 mg l^{-1}. Flow rates were set so that the dissolved oxygen concentration of effluent water from respirometers and tanks was not allowed to drop below 75% saturation, in order to minimise changes in oxygen consumption rate due to environmental dissolved oxygen levels.

2.7.2 Winkler Titrations

Winkler titrations were performed according to the methods of Stirling (1985).

2.8 Calculation of Oxygen Consumption Rate

The oxygen consumption rate of fish in individual respirometers was calculated by the following equation:

$$\text{(mg kg}^{-1}\text{ h}^{-1}\text{)} = \frac{[(O_2\text{ inflow} - O_2\text{ test}) \times \text{flow rate (l h}^{-1}\text{)}] - [(O_2\text{ inflow} - O_2\text{ blank}) \times \text{flow rate (l h}^{-1}\text{)}]}{\text{weight of fish (kg)}}$$

Where: **O_2 inflow = Dissolved oxygen content of water from calibration line.**

O_2 test = Dissolved oxygen content of water exiting sample respirometer

O_2 blank = Dissolved oxygen content of water exiting blank respirometer

For the tank trials, the effluent from an identical empty (control) tank was used as the "corrected" inflowing dissolved oxygen concentration, thus the tank trial oxygen consumption rate data was calculated according to the equation:

$$\text{(mg kg}^{-1}\text{ h}^{-1}\text{)} = \frac{(dO_2\text{ blank outflow} - dO_2\text{ test outflow}) \times \text{flow rate (l h}^{-1}\text{)}}{\text{weight of fish (kg)}}$$

2.9 Determination of Total Ammonia

The determination of ammonia in seawater was performed according to the "Alternative Method" of Parsons *et al.* (1984), whereby seawater is treated in an alkaline citrate medium with sodium hypochlorite and phenol in the presence of a catalyzer, sodium nitroprusside. The blue indophenol colour generated is measured for standards, samples and blanks at 640nm.

2.10 Terminology

In the literature there is a wide variety of terms applied to describe the element of metabolism measured, and the units of oxygen consumption rate. In this study, the M_M component has been designated the "resting rate metabolism", the M_A component has been designated "routine metabolism[1]", and the M_F component designated "specific dynamic action" (S.D.A.). Oxygen consumption rate data throughout has been represented as values in mg kg^{-1} h^{-1}.

2.11 Statistical Analysis

Dissolved oxygen, fish weight, and water flow rate data were entered into spreadsheets (Microsoft EXCEL Version 7.0) to facilitate calculation of oxygen consumption rates. The results of these calculations produced time series plots of oxygen consumption rate for individual respirometry and tank trial[1] respirometry which were then exported to a statistical software package (Minitab 10xtra), and processed by a data smoother (4253H, twice) to reveal trends in respiratory patterns. The effect of body weight and temperature on rates of oxygen consumption and ammonia excretion was analysed by regression and analysis of variance (Zar, 1984). The statistical significance of the influence of temperature on oxygen consumption and ammonia excretion rates in temperature groupings was tested through a comparison of individual temperature regressions against a global regression for results produced at all test temperatures.

[1] In this study, the "Activity" component is designated routine metabolism since although the activity of any fish under investigation was limited through confinement in tank systems, spontaneous movement was not restricted.

Chapter 3.

Resting Oxygen consumption rate in the Atlantic halibut (*Hippoglossus hippoglossus* L.)

3.1. Introduction

The application of a bioenergetic approach to maximising the potential of aquaculture systems is a relatively recent phenomenon, and undoubtedly this is an extension of similar approaches to agricultural systems in general. Energetics studies of domestic animals have benefited agriculture for a considerable period (Brody, 1945), and as aquaculture continues to expand with the decline in wild fisheries, the study and application of the bioenergetics of aquatic animals has become an ever more prominent factor in these systems (Lucas, 1996).

Brody (1945) identifies the energetic cost of homeostasis - the "basal" metabolism of homeotherms - as the most convenient starting point for energetic studies since it provides a baseline for measuring various other energy increments, such as those derived from activity and feeding. The same is true of fish studies, and resting metabolism must be evaluated in order that the energetic cost of maintenance may be quantified. Waller (1992) advocates the measurement of routine activity rates in energetic studies of fish, suggesting that such measurements are more applicable to culture systems. While routine rates do incorporate some level of spontaneous movement of fish within the culture systems, coincidental measurements of such activity with metabolic rate are important in order that meaningful data may be produced (Beamish and Mookherjii, 1964). Consequently, inter-specific and intra-specific comparisons of routine metabolic rate may be difficult to make, since it may not be assumed that experimental conditions are the same for different studies. It is likely that more meaningful comparisons will be determined through values for resting rate metabolism, since these measurements are made on fish in the postabsorptive phase and under conditions of zero activity with no external influences. Importantly there are preconditions attached to the comparison of resting metabolic

rates in fish, and only through the adherence to a well defined protocol may such comparisons be made. The study of the energetics of poikilotherms in general, and fish in particular, would benefit greatly from the development of a clearly defined protocol facilitating comparison of data produced from different organisms under investigation. This may be realised in the future, however at this point the comparison of the results of bioenergetic studies necessitates the use of caution in ensuring that experimental conditions do not invalidate such comparisons. (In this study the measurement of routine metabolic rates in combination with resting metabolic rates does give information on the energetic costs of spontaneous activity for a fish species which is a useful application for aquaculture. This is explored further - see Chapter 4.).

Much of the literature on resting rate metabolism in fish derives from studies on roundfish species, in particular those which respond readily to confinement such as *Cyprinus carpio* (Beamish 1964a; Chakraborty *et al.*, 1992), *Carassius auratus* (Beamish and Mookherjii, 1964) and *Oreochromis niloticus* (Ross and McKinney, 1988a,b). In general terms, flatfish species have not been afforded the same attention, although the importance of *Pleuronectes platessa* to wild fish stocks has prompted several studies on this species (Edwards *et al.*, 1969; Priede and Holliday, 1980; Fonds *et al.*, 1992), and data on the commercially important *Platichthys flesus*, *Limanda limanda* and *Microstomus kitt*, has also been produced (Duthie, 1982). These studies reflect the importance of an energetics approach to wild fisheries, which is entirely as well founded as such an approach to aquaculture, providing information on population characteristics which is useful in determining management strategies for exploited stocks (Lucas, 1996). One recent study (Davenport *et al.*, 1995) provides a comparison of resting metabolic rate for cultured *H. hippoglossus* and *Microstomus kitt*.

Determinations of resting metabolic rate have been obtained by extrapolation of the relationship between oxygen consumption rate and induced activity over several swimming speeds, to zero (Brett, 1964, 1965; Webb, 1971; Bushnell *et al.*, 1994). Priede and Holliday (1980) show that such an estimate of resting metabolic

rate is inappropriate for flatfish, leading to overestimates for resting metabolic rate in such species. The resting metabolic rate of flatfish is low in comparison to roundfish (Wood *et al.*, 1979; Priede and Holliday 1980), and this is considered to be representative of a relatively sedentary lifestyle in which low resting metabolic rates can reduce maintenance costs (Duthie, 1980).

Fry (1971) categorises temperature as a "Controlling Factor", and Brett (1979) regards temperature as the principal factor in determining metabolic rate in fish. Temperature most probably determines the metabolic rate of poikilotherms through it's effect on the kinetics of enzymes at the cellular level (Lucas, 1996). For any given fish species, there is a temperature range over which the fish may survive, and a temperature which is optimal for existence in terms of energetic efficiency. Changes in temperature within the organism's functional range require periods of acclimation where the physiological and biochemical cellular processes can adapt to the new thermal regime (Lucas, 1996). For this reason, it is necessary to have prior knowledge of the thermal history of the test fish before experimentation, so that cellular adaptations to the test temperature have been completed, if the effect of temperature is the factor under study (Fry, 1971). An acclimation period of 4 weeks has been generally proposed (Brett, 1962; Braaten, 1979). In conjunction with temperature, nutritional status has been identified as an important factor in acclimation of fish for metabolism studies (Johnston and Dunn, 1987), and since photoperiod is known to exert an effect on metabolic rate (Beamish, 1964c; Hirata, 1973; Nagarajan and Gopal, 1983; Ross and McKinney, 1988a,b; Chakraborty *et al.*, 1992a) it may be concluded that acclimation to test photoperiod cycle is also a requirement for respirometry.

The quantitative relationship between size and metabolic rate has been documented for many species of fish (Brett and Groves, 1979; Jobling, 1994), and is generally described by the allometric equation $Y = aW^b$, where Y is the oxygen consumption rate, W is the body weight, and a and b are constants specific for the species under investigation. Metabolic rate per unit weight decreases with increasing

body weight, and the weight exponent *b* has generally been estimated at 0.86 (Brett and Groves,1979) and 0.79 for marine species (Winberg, 1956).

A photoperiod-mediated variation in resting metabolic rate over 24 hour periods has been confirmed for some species of fish such as *Clupea harengus* (De Silva *et al.*, 1986), *Oreochromis niloticus* (Ross and McKinney, 1988a,b) and *Cyprinus carpio* (Chakraborty *et al.*, 1992). Considerable attention has been devoted to the ryhthmic activity of members of the *Pleuronectidae*, and in many studies activity has been linked to tidal phases (Gibson, 1973; Greer Walker *et al.*, 1978; Weihs, 1978; Harden Jones, 1979, 1980; Ansell and Gibson, 1990). Under tank conditions there is evidence to support an increased activity in the night phase for *Solea solea* (Kruuk, 1963) and for *Pleuronectes platessa* and *Platichthys flesus* (Verheijen and de Groot, 1967). Edwards *et al.* (1971), found evidence for a diel feeding rhythm in some tropical flatfish species, with the greatest feeding activity taking place nocturnally. The increased open water activity seen at night in one of the Atlantic halibut's main prey items *Sebastes marinus* (Leim and Scott, 1966), and the fact that *H. hippoglossus* is considered to be a mid-water piscivore (Nelson, 1984), suggests that wild halibut may share these nocturnal patterns of behaviour.

The phenomenon of diurnal variations in metabolic rate emphasises the importance of obtaining oxygen consumption data over periods encompassing the full photoperiodic cycle. In order to explore such phenomena, it is advantageous to employ open respirometer systems, since such systems are ideal for measuring the physiological responses of aquatic organisms to environmental variables over longer periods of time (Gnaiger, 1983a,b). Importantly, open respirometer systems allow the maintenance of constant conditions over the test period, and are not subject to the effects of fluctuating levels of dissolved gases and metabolites encountered in closed system respirometry.

The aim of this study was to quantify resting metabolic rate in the Atlantic halibut using indirect calorimetry. In this way, values for oxygen consumption in this species would be measured through open system respirometry for animal sizes

covering the potential culture range, and at temperatures most likely to be encountered on farm sites in Scotland. The large size range of *H. hippoglossus* makes it an interesting candidate for this type of study, taking into account size related differences in metabolism and the low values for resting metabolic rate which have been observed in other species of flatfish. The metabolic values obtained in this way facilitate an inter-species comparison of metabolic rate. Information on the life history of this species, and other species of flatfish, suggests the possibility of an increased nocturnal activity in *H. hippoglossus*, and this was also investigated. The data obtained would provide an evaluation of the energetic costs of homeostasis in *H. hippoglossus*, and is the first step towards producing an energetic model for this species under culture conditions.

3.2 Materials and Methods

3.2.1 Fish

Resting oxygen consumption rate was measured in a total of 147 *Hippoglossus hippoglossus* at the test temperatures. Trials were conducted at 6, 10 and 14°C, (50 halibut at both 6°C and 10°C, and 47 halibut at 14°C). Fish were grouped according to respirometer size, the small respirometers for fish of <350g; medium respirometers for fish in the 350-1200g range; large respirometers for fish of >1200g. Over the course of the study, this effectively resulted in 1+ year class fish categorised as "smalls", 2+ and 3+ year class fish categorised as "medium", and 4+ year class fish categorised as "large". Prior to entry to the respirometer system, fish were maintained on the stock diet (see chapter 2) at a feeding rate of 1% body weight, offered daily in a single feed at 10.00h. To ensure that measurements of oxygen consumption were undertaken only on fish in the postabsorptive phase, starvation periods as discussed in chapter 2 were used.

3.2.2 Thermal and photoperiod acclimation

Thermal and photoperiod acclimation was carried out in a purpose built acclimation unit housed in a quiet corner of the tank house at the marine laboratory. The facility was completely enclosed in high density black polythene to ensure no extraneous light penetration. A 12L:12D (light period - 08.00h:20.00h) photoperiod cycle was maintained by using a 100w bulb in a waterproof housing, set 1m above the water surface and attached to a timer. Light levels in the photophase were measured at 140-150lux at the water surface. A 100l header tank received ambient sea water, and supplied two holding tanks in the unit (volumes 75l and 35l). A second pipe leading into the header tank carried chilled sea water from the site cooler and mixing of water from the two pipes, together with the operation of a 1kw heater with thermostatic control sited within the header tank, allowed regulation of the acclimation temperature. Acclimation temperature was within $\pm$ 1.0°C of the temperature at which resting metabolic rates were measured. The temperature in the

acclimation unit was ambient when a group of fish entered, and acclimation temperature was achieved by changing the combination of chilled water and heater settings. The rate of change of temperature in the unit did not exceed 1°C per day. The period of thermal and photoperiod acclimation prior to respirometry was 4 weeks.

3.2.3 Respirometers

The respirometers were purpose-built rectangular perspex chambers with the upper surface removable to allow entry of the test animals. This lid was held in place by stainless steel screws, and the respirometer sealed by a rubber "o"-ring. Respirometer volumes were 2.55l, 10.33l and 34.16l. Small and medium sized respirometers were manufactured from 8mm perspex, and large sized respirometers from 15mm perspex. Sand-filled, sealed PVC spacers of 2.0 and 2.5cm thickness restricted the movement of any individual fish which were small enough to have a degree of freedom within a respirometer. An inlet "baffle" aided dispersion and mixing of the inflowing water within the chamber, confirmed by dye dispersion studies. These studies utilised "Co-op" green food colour, which was injected into flexible tubing at the head of each respirometer at time zero. Sequential water samples were taken from the effluent of the respirometer at time intervals following this, and the dye concentration was measured through light absorption of each sample at 328nm in a spectrophotometer. Extinction curves were determined for the three sizes of respirometer at flow rates corresponding to respirometry work. These curves are represented in Fig. 3.1.

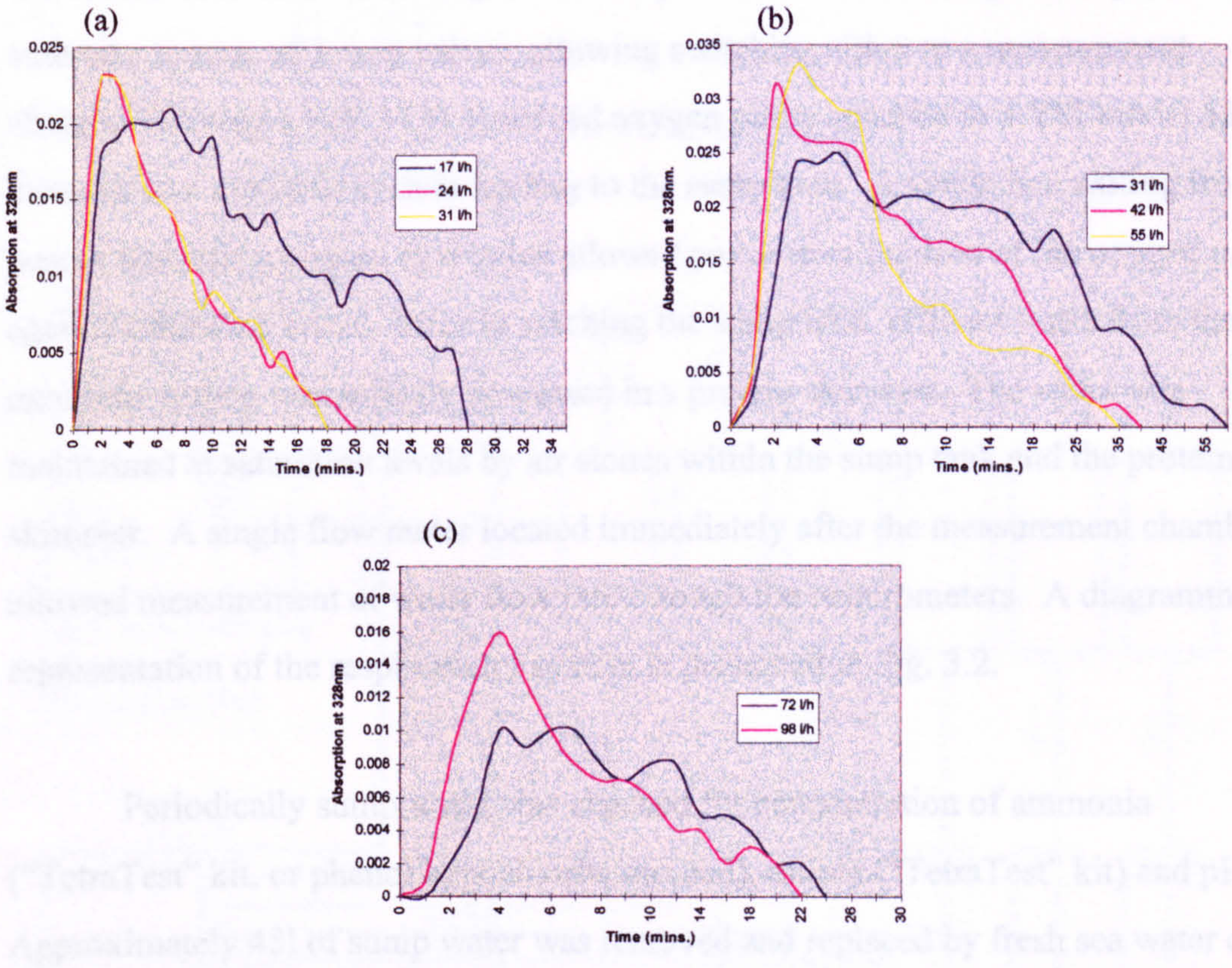


Fig. 3.1 Extinction curves for small (a), intermediate (b) and large (c) respirometers. Flow rates are represented for individual graphs, and corresponded to those used in respirometry experiments. "Co-op" green food dye was used as the marker.

3.2.4 The Respirometer System

The respirometer system was a completely enclosed recirculation system which facilitated temperature control. The whole unit was housed in a quiet corner of the laboratory, separated off by high density black polythene. A 100w bulb maintained a 12L:12D photoperiod as in the acclimation unit, and the photophase light level at the respirometer surface was 150lux, similar to the intensity in the acclimation unit. Excluding respirometers, the total system volume was approximately 140l. The header tank contained the temperature control equipment, a continuously operating Grant C2G cooler and a Haake digital heater with thermostatic control, and recirculating pump. This system allowed the maintenance of test temperature to within $\pm$ 0.2°C. Respirometers were supplied by gravity from the header tank. Sea water from the sump tank was pumped to the header tank by an

Eheim type 1060 pump supplying 38l min^{-1}. A Fluval 4 submerged filter in the sump tank aided in the reconditioning of the sump water. Water exiting the respirometers entered a system of 3-way valves, allowing switching either to a measurement chamber housing a YSI 5739 dissolved oxygen probe attached to a YSI model 58 meter or to a manifold system leading to the sump tank. A single line exiting from the header tank and operated by a valve allowed periodic calibration of the oxygen probe against inflowing water. Prior to reaching the sump tank, effluent water from the manifold system was initially processed in a protein skimmer. The water was maintained at saturation levels by air stones within the sump tank and the protein skimmer. A single flow meter located immediately after the measurement chamber allowed measurement of water flow rate through the respirometers. A diagrammatic representation of the respirometry system is presented in Fig. 3.2.

Periodically sump water was checked for concentration of ammonia ("TetraTest" kit, or phenol hypochlorite method), nitrate ("TetraTest" kit) and pH. Approximately 45l of sump water was removed and replaced by fresh sea water of the same temperature at one point within each 12 hour period. At an early stage in the resting rate experiments, seawater samples from the recirculation respirometer system were taken, and analysed at the Institute of Aquaculture, University of Stirling, for concentrations of the metals copper, iron, nickel and zinc.

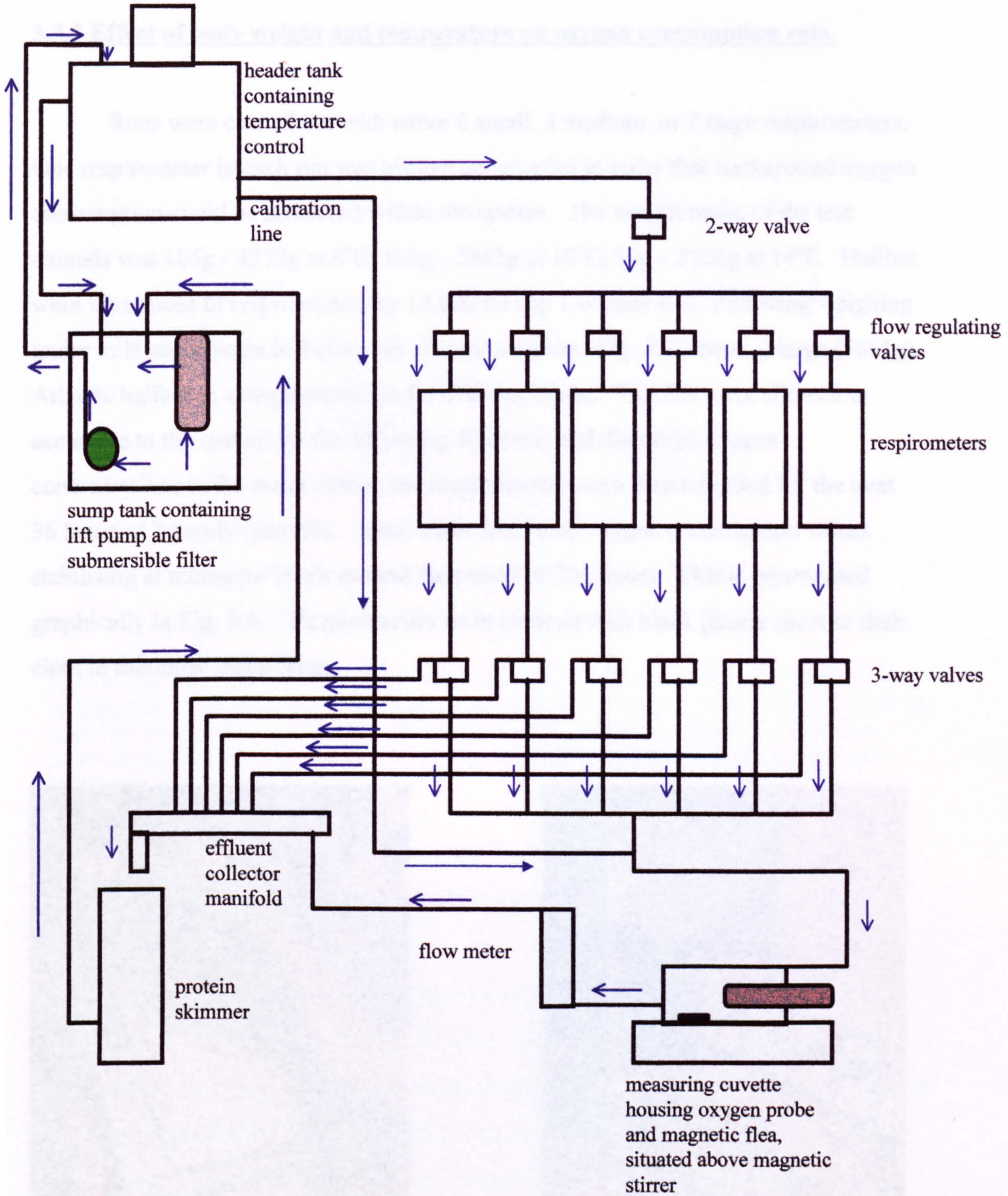


Fig. 3.2. Diagrammatic representation of the respirometry system employed for the measurement of resting oxygen consumption rate in Atlantic halibut. Water flow direction is represented by the blue arrows. (Not to scale)

3.2.5 Effect of body weight and temperature on oxygen consumption rate.

Runs were carried out with either 6 small, 4 medium, or 2 large respirometers. One respirometer in each run was always unoccupied in order that background oxygen consumption could be measured within the system. The weight range of the test animals was 105g - 4372g at 6°C; 128g - 5861g at 10°C; 53g - 2128g at 14°C. Halibut were introduced to respirometers by 18.00h on day 1 of each run, following weighing under mild anaesthesia in 2-phenoxy ethanol solution. Fig. 3.3. shows a large (5861g) Atlantic halibut in a respirometer under trial conditions. The fish were allowed to acclimate to the system for the following 38 hours, and dissolved oxygen concentrations in the water exiting the respirometers were then recorded for the next 36 hours at 2 hourly intervals. Initial trials confirmed oxygen consumption values stabilising at minimum levels around the period of 30+ hours. This is represented graphically in Fig. 3.4. Respirometers were covered with black plastic sheet or dark cloth to minimise disturbance.

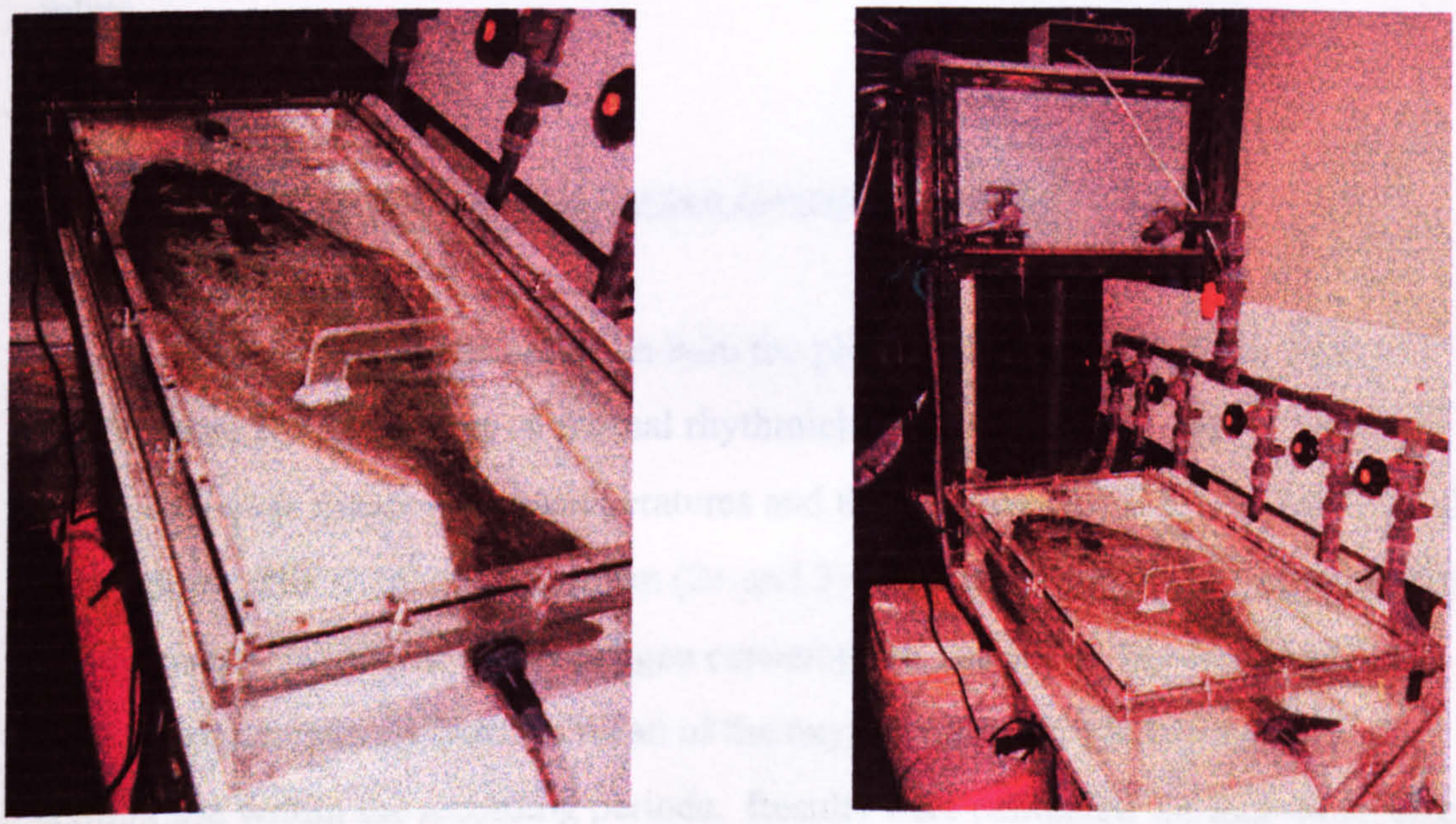


Fig. 3.3. An Atlantic halibut of 5861g in a large respirometer during resting oxygen consumption rate trials.

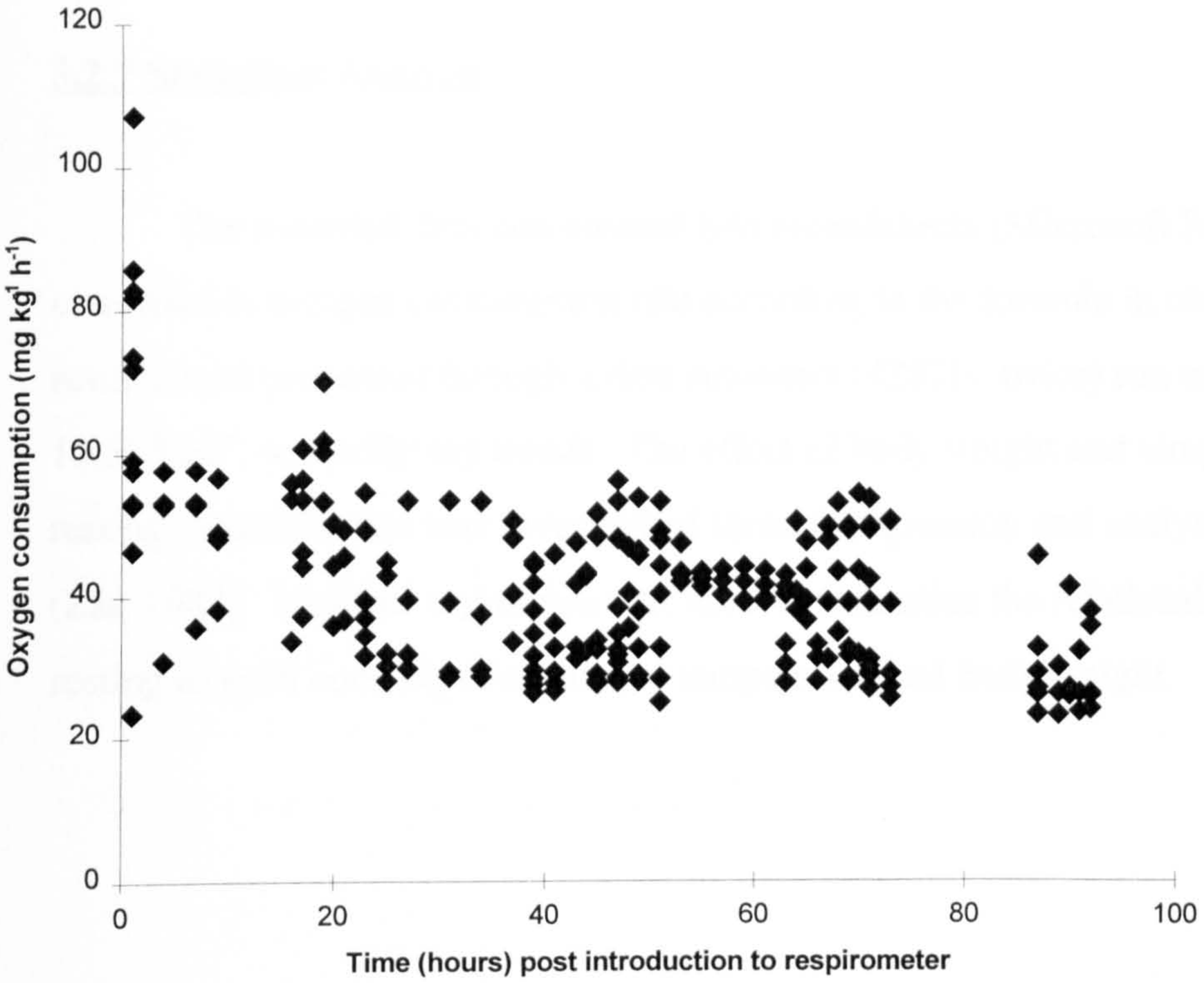


Fig. 3.4. Time series variation in oxygen consumption rate for *Hippoglossus hippoglossus* after introduction to respirometers. Compilation of data from 14 individual fish trials at 6°C. Fish weight range 92g -305g. Following the results of these initial trials, a settlement period of 30 hours was applied to halibut in respirometers prior to the determination of resting oxygen consumption rate values.

3.2.6. Effect of photoperiod on oxygen consumption rate.

Oxygen consumption data in both the photophase and scotophase was investigated for any degree of diurnal rhythmicity in a total of 133 trials. This was conducted over the three test temperatures and the weight range 53g - 5861g. The fish were grouped as small (1+), medium (2+ and 3+) and large (4+) according to size of respirometer. The mean of the oxygen consumption rate in the two photophase periods was compared with the mean of the oxygen consumption rate for the scotophase within the recording periods. Results were compared for individual size groups at the test temperatures of 6, 10 and 14°C to indicate differences in phase location of peak oxygen consumption rate. Where they occurred, peak oxygen consumption rates in the scotophase were expressed as a percentage increase over the photophase.

3.2.7 Statistical Analysis

The recorded data was entered into spreadsheets (Microsoft EXCEL 7.0), and converted to oxygen consumption rate according to the formula in chapter 2. The results were processed through a data smoother (4253H, twice) run on "Minitab 10.51Xtra", to clarify any trends. The effect of body weight and temperature on the resting metabolic rate was investigated through regression and analysis of variance (Zar, 1984). Multiple regression was used to determine the relationship between resting oxygen consumption rate and temperature and body weight.

3.3 Results

3.3.1 *The effect of body weight and temperature on oxygen consumption rate*

In common with many fish metabolism studies, there was an inverse relationship between $\log_{10}$ body weight and $\log_{10}$ oxygen consumption rate, with the rate of oxygen consumption increasing over the temperature range 6°C to 14°C. These results are represented in Fig. 3.5. Oxygen consumption conformed to the general allometric equation $Y = aW^b$, where Y is the resting oxygen consumption (mg h^{-1}), W is the body weight (g), a is the constant of the equation which is related to the test temperature, and b is the weight exponent. The regression equations, correlation coefficients and levels of significance are shown in Table 3.1.

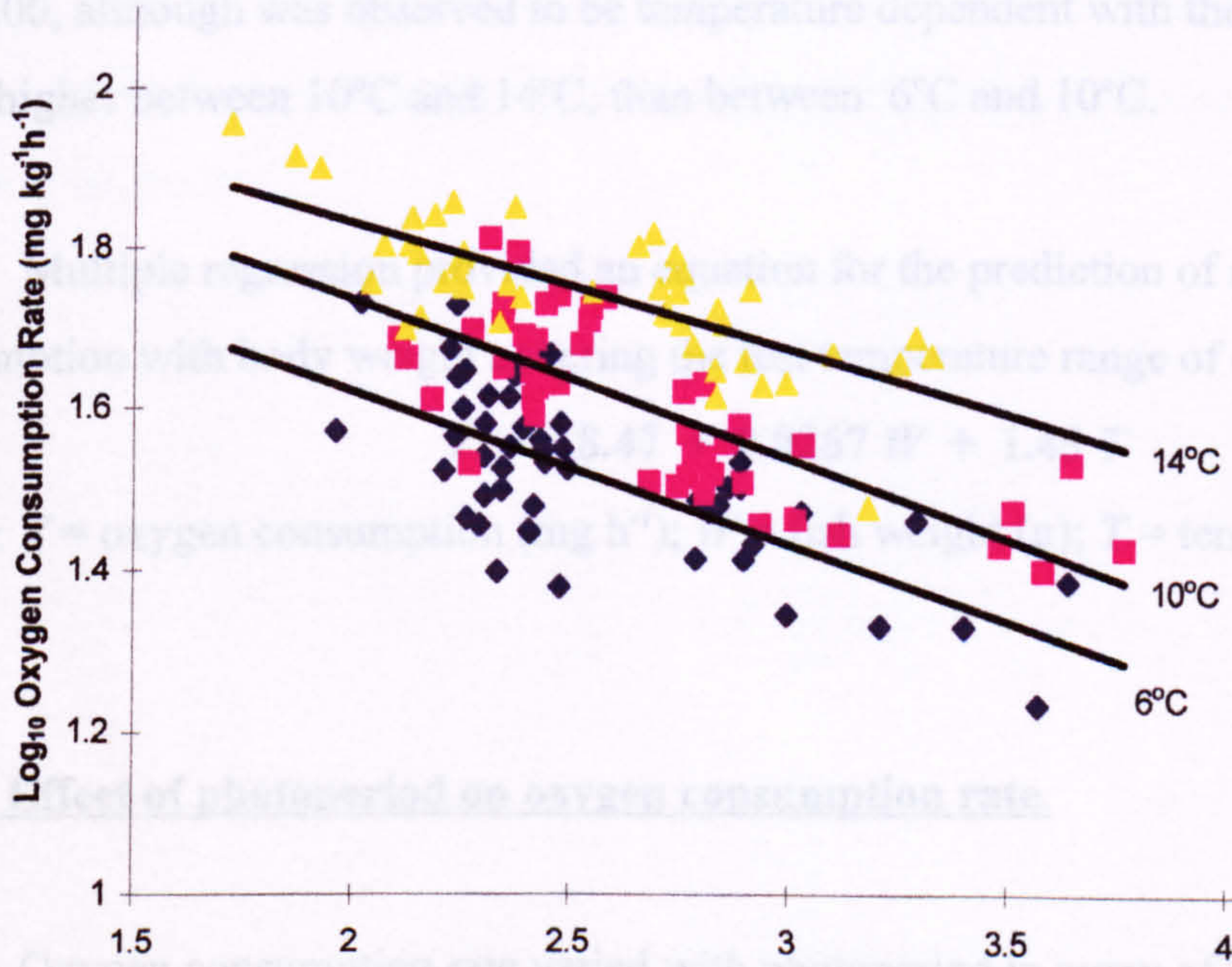


Fig. 3.5. Relationship between $\log_{10}$ oxygen consumption rate and $\log_{10}$ body weight at 6, 10 and 14°C in resting *Hippoglossus hippoglossus*.

Table 3.1. Regression statistics for the effect of temperature and body weight on resting oxygen consumption rate in *Hippoglossus hippoglossus* L. Equations are expressed in the form $Y = a + bX$, and $Y = a\,W^b$.

Temperature (°C)	Weight Range (g)	n	Regression equation and Allometric equation	r^2	Significance	Q_{10}
6	105 - 4372	50	$\text{Log}_{10}\,Y = 2.00 - 0.190\,\text{Log}_{10}\,W$	0.50	$p < 0.001$	
			$Y = 0.1\,W^{0.8101}$	0.94		
10	128 - 5861	50	$\text{Log}_{10}\,Y = 2.13 - 0.198\,\text{Log}_{10}\,W$	0.58	$p < 0.001$	1.84
			$Y = 0.1405\,W^{0.796}$	0.96		
14	53 - 2128	47	$\text{Log}_{10}\,Y = 2.15 - 0.160\,\text{Log}_{10}\,W$	0.56	$p < 0.001$	2.16
			$Y = 0.1363\,W^{0.8453}$	0.98		

Q_{10} values were calculated according to Van't Hoff's formula (Lucas, 1996), and were calculated for an individual of 1kg body weight. Q_{10} value over the entire test temperature range 6°C to 14°C was 2.00.

Regression analysis of variance confirmed the statistical significance of the plots for the three temperatures against a global plot of all temperature data ($F = 23.61$; d.f. = 4, 141; $p<0.001$). A mean weight exponent (b) value of 0.82 was recorded overall. The Q_{10} value calculated over the test temperature range 6°C to 14°C was 2.00, although was observed to be temperature dependent with the calculated Q_{10} value higher between 10°C and 14°C, than between 6°C and 10°C.

Multiple regression provided an equation for the prediction of resting oxygen consumption with body weight covering the test temperature range of 6 - 14°C:

$$Y = -8.47 + 0.0267\,W + 1.45\,T$$

where: Y = oxygen consumption (mg h^{-1}); W = fish weight (g); T = temperature (°C).

3.3.2. Effect of photoperiod on oxygen consumption rate.

Oxygen consumption rate varied with photoperiod in many of the halibut examined. Although not conclusive, the trend was for an elevated rate of oxygen consumption in the scotophase, with a total of 82 out of 133 investigated individuals exhibiting peak oxygen consumption in this phase. Figs. 3.6., 3.7. and 3.8. show examples of the respirometry trials in which peak oxygen consumption rates were observed in the scotophase at 6°C, 10°C and 14°C. Fig. 3.9. shows examples of trials

at the three test temperatures, where peak oxygen consumption rates did not correspond to this pattern. The phase location of peak oxygen consumption rate with fish size and temperature is shown in Fig. 3.10., and the percentage increases for each phase and size/temperature class are shown in Table 3.2. Generally, as fish weight increased peak oxygen consumption rate was more frequently observed in the scotophase, with smaller numbers of fish within the test groups showing peak oxygen consumption in the photophase. The exception was the small size class of fish examined at 14°C, in which there was a marked increase in number of fish showing peak oxygen consumption rate within the photophase over the scotophase. The percentage increase in oxygen consumption rate followed a similar pattern, with larger percentage increases observed in the scotophase than in the corresponding photophase, exceptions being small fish at 14°C and medium fish at 10°C.

Although the tendency is for an increase in oxygen consumption rate in the scotophase, the trend is not clearly defined and overall the pattern of rhythmicity is one of dualism. Peak oxygen consumption rates were observed in some individuals in the photophase and others in the scotophase, even within separate size and temperature groups.

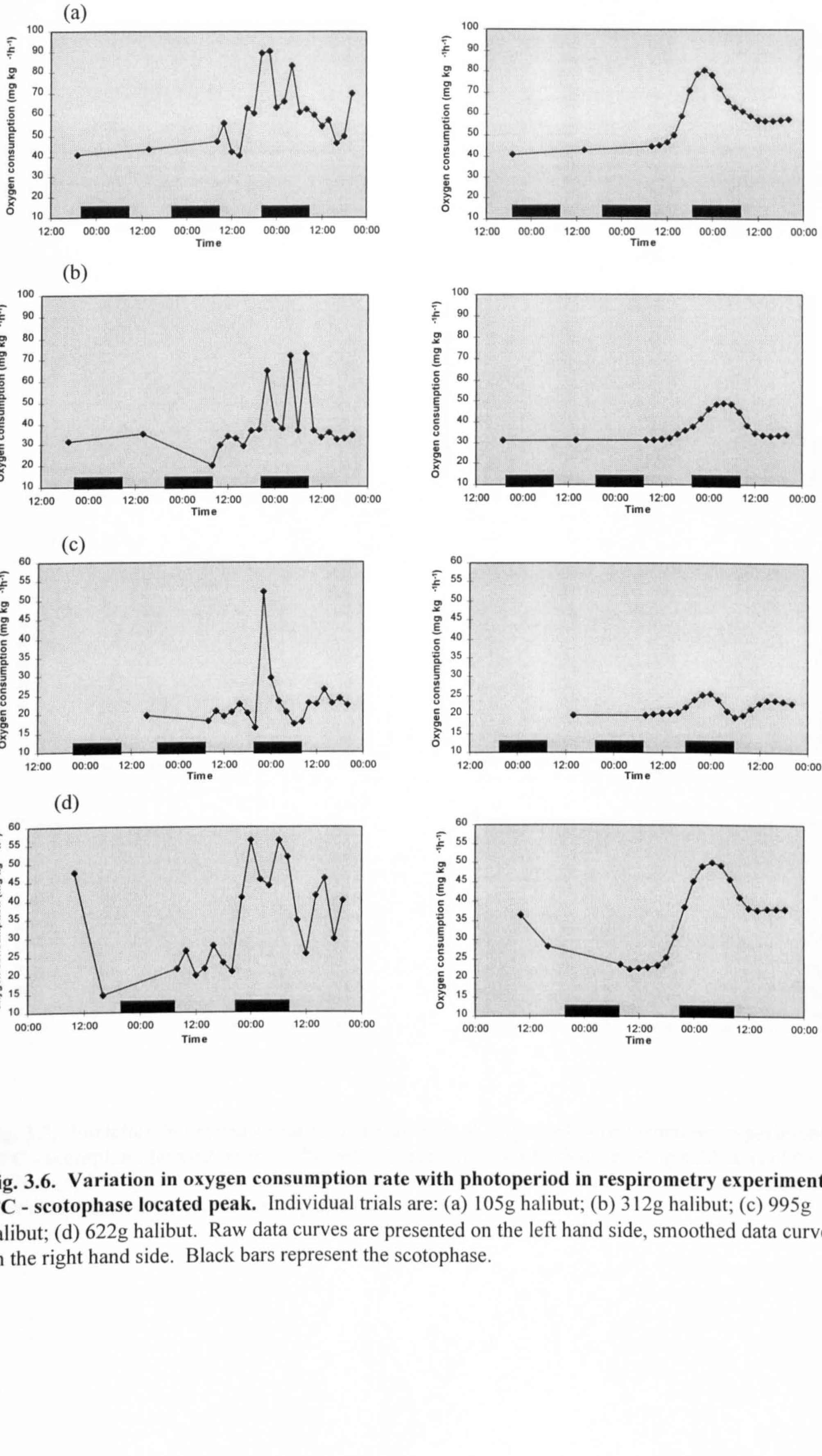


Fig. 3.6. Variation in oxygen consumption rate with photoperiod in respirometry experiments at 6°C - scotophase located peak. Individual trials are: (a) 105g halibut; (b) 312g halibut; (c) 995g halibut; (d) 622g halibut. Raw data curves are presented on the left hand side, smoothed data curves on the right hand side. Black bars represent the scotophase.

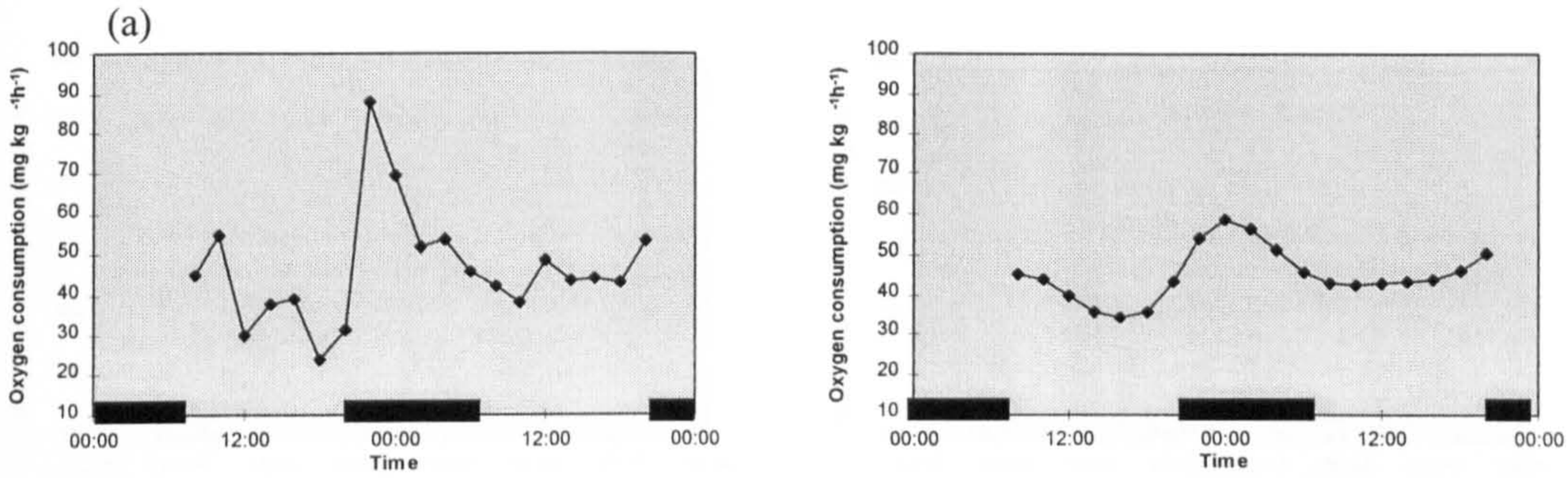


Fig. 3.7. Variation in oxygen consumption rate with photoperiod in respirometry experiments at 10°C - scotophase located peak. Individual trials are: (a) 260g halibut; (b) 555g halibut; (c) 589g halibut; (d) 3831g halibut. Raw data curves are presented on the left hand side, smoothed data curves on the right hand side. Black bars represent the scotophase.

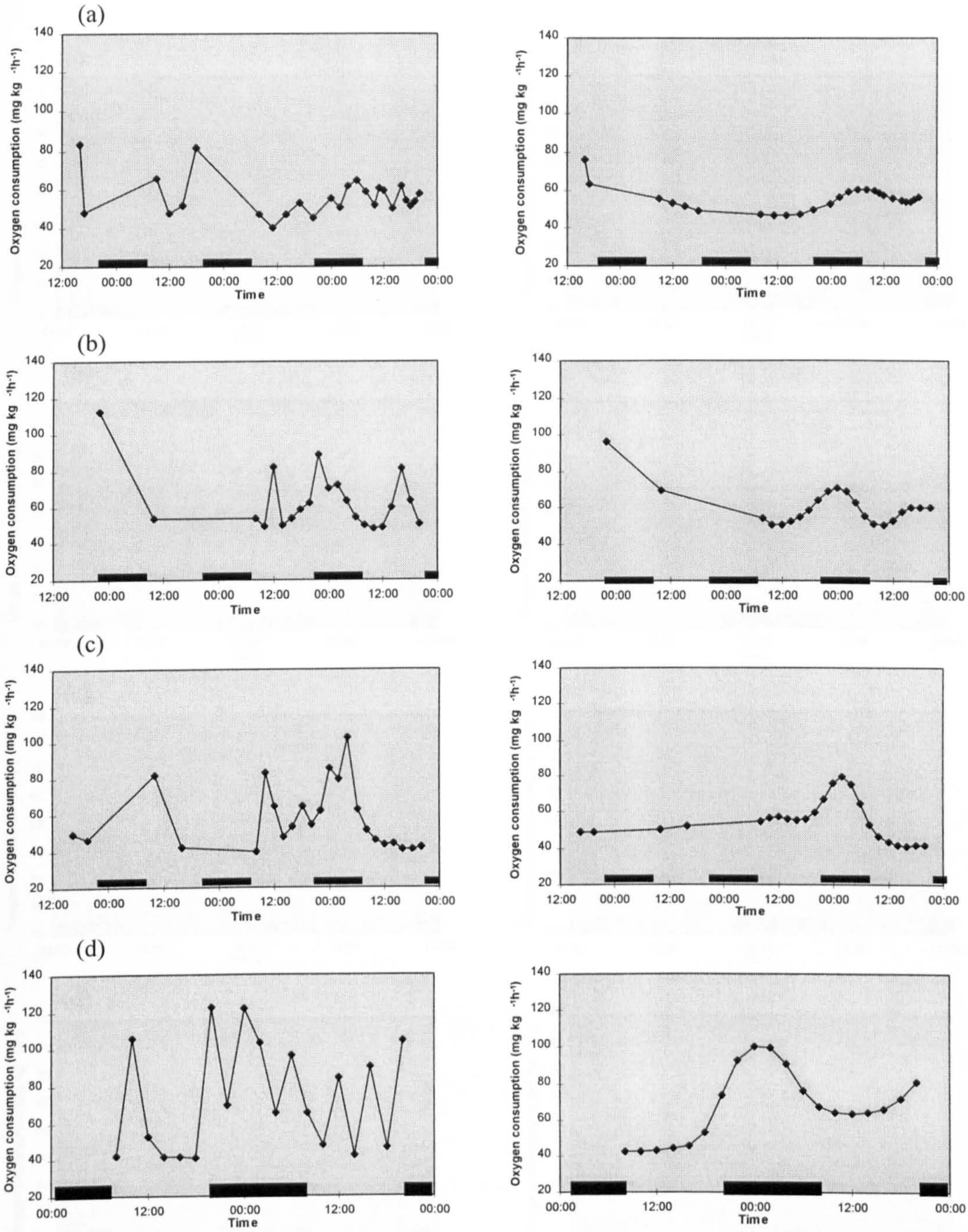


Fig. 3.8. Variation in oxygen consumption rate with photoperiod in respirometry experiments at 14°C - scotophase located peak. Individual trials are: (a) 520g halibut; (b) 530g halibut; (c) 678g halibut; (d) 1797g halibut. Raw data curves are presented on the left hand side, smoothed data curves on the right hand side. Black bars represent the scotophase.

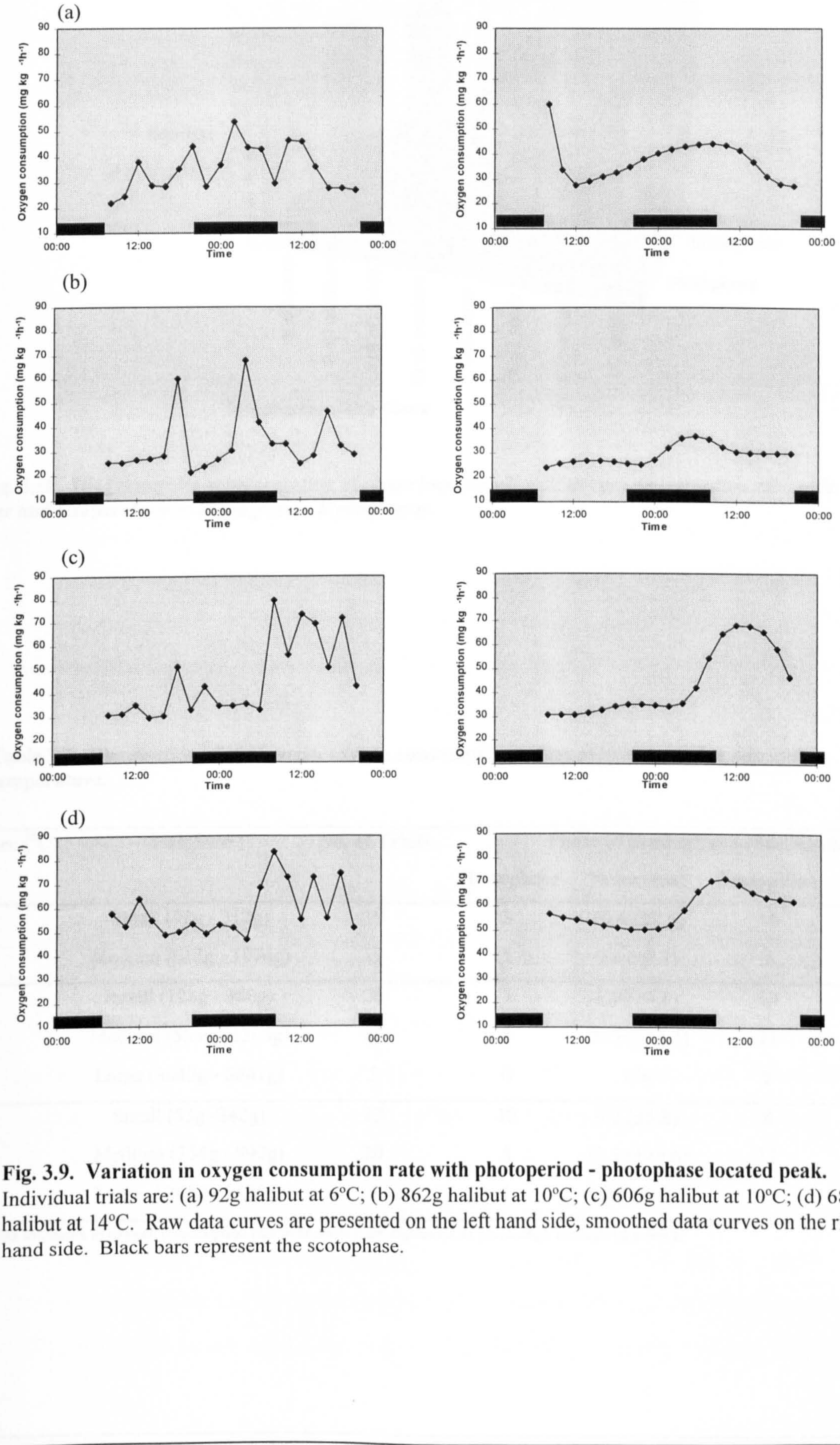


Fig. 3.9. Variation in oxygen consumption rate with photoperiod - photophase located peak. Individual trials are: (a) 92g halibut at 6°C; (b) 862g halibut at 10°C; (c) 606g halibut at 10°C; (d) 687g halibut at 14°C. Raw data curves are presented on the left hand side, smoothed data curves on the right hand side. Black bars represent the scotophase.

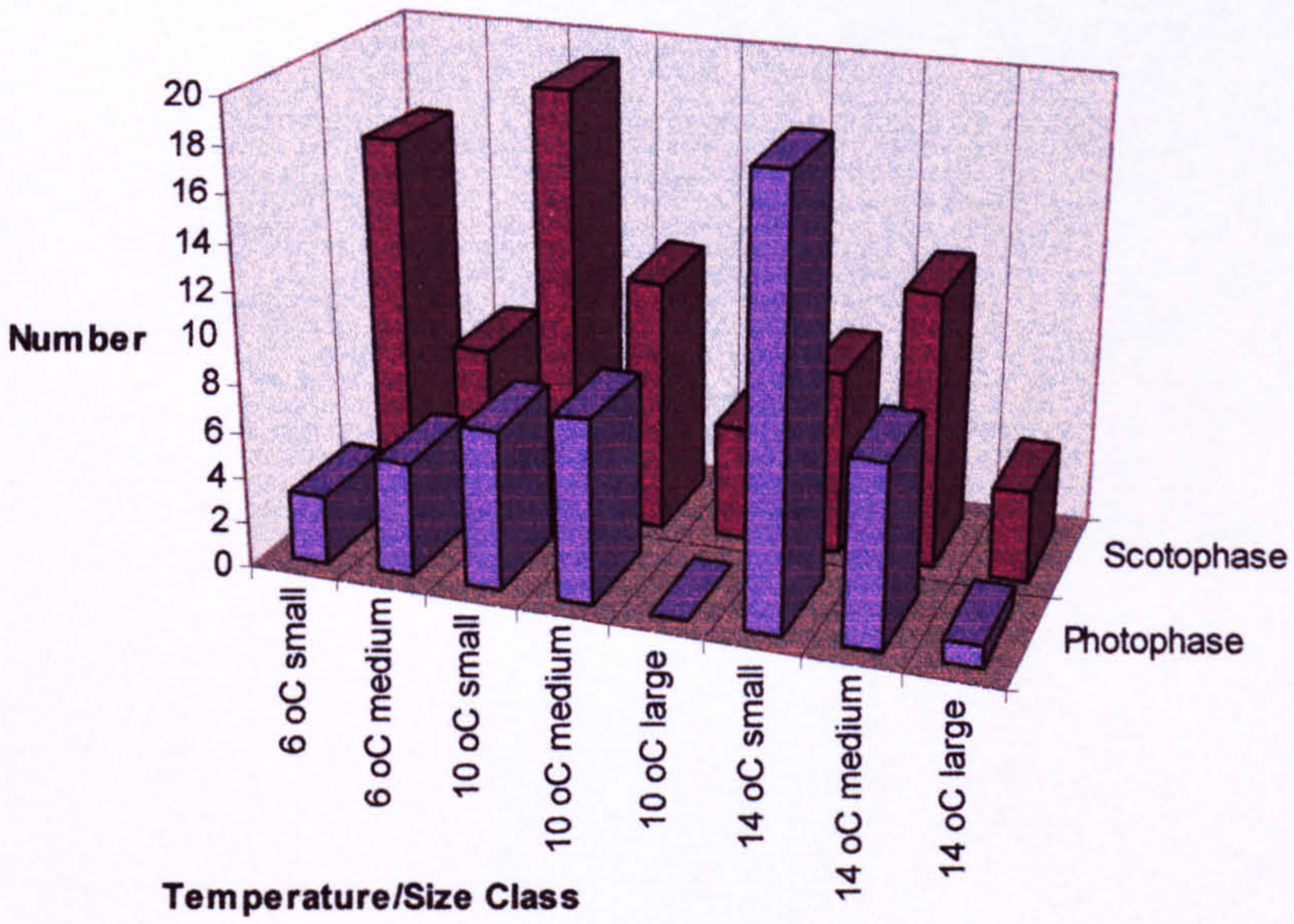


Fig. 3.10. Diagrammatic representation of phase location of peak oxygen consumption rate with size and temperature in *Hippoglossus hippoglossus*.

Table 3.2. Distribution of peak mean oxygen consumption values according to fish size and temperature.

Temperature (°C)	Fish Size	No. of Trials	Phase of peak oxygen consumption			
			Photophase	% increase	Scotophase	% increase
6	Small (92g - 312g)	19	3	10.4 (±9.3)	16	20.3 (±17.0)
	Medium (615g - 1074g)	12	5	9.6 (±7.1)	7	23.4 (±13.3)
10	Small (128g - 380g)	26	7	8.9 (±4.1)	19	22.0 (±23.1)
	Medium (555g - 1305g)	19	8	20.5 (±14.3)	11	18.7 (±13.4)
	Large (3042g - 5861g)	5	0	n/a	5	44.5 (±40.2)
14	Small (53g -242g)	27	19	9.2 (±5.8)	8	8.6 (±5.4)
	Medium (354g - 992g)	20	8	12.1 (±15.6)	12	24.9 (±21.8)
	Large (1265g - 2128g)	5	1	2.5 (n/a)	4	26.8 (±23.7)

Peak increases in oxygen consumption rate over resting are expressed as percentage increase (± s.dev.).

3.4 Discussion

This study provides information on the effects of temperature and body weight on the resting oxygen consumption rate of the Atlantic halibut, and is an important step towards producing an energy budget for this species.

The effect of body weight and temperature on oxygen consumption rate

The phenomenon of increased oxygen consumption rate per unit weight observed in smaller fish, and an increase in oxygen consumption rate with increasing temperature, is well documented for many fish species such as *Cyprinus carpio* (Beamish 1964a), *Carassius auratus* (Beamish and Mookherjii, 1964), *Cichlasoma urophthalmus* (Martinez-Palacios and Ross, 1986), *Anguilla anguilla* (Degani *et al.*, 1989), *Ptychocheilus oregonensis* (Cech *et al.*, 1994), and has also been identified in flatfish species such as *Pleuronectes platessa* (Edwards *et al.*, 1969; Jobling 1982), and *Platichthys flesus, Limanda limanda,* and *Microstomus kitt* (Duthie, 1982). From this study, it is clear that resting oxygen consumption rate in *H. hippoglossus* follows a similar pattern.

Resting oxygen consumption rate in some species of flatfish has previously been shown to be significantly lower than for roundfish (Wood *et al.*, 1979; Duthie, 1982), and is considered to reflect the relatively inactive behaviour patterns of these fish. A species comparison of resting oxygen consumption rate is presented in Table 3.3. and illustrated graphically in Fig. 3.11. The results of this study also confirm relatively low values for *H. hippoglossus* in comparison to cultured roundfish species. The data produced by Davenport *et al.*, (1995) for *H. hippoglossus* is significantly higher than that observed in this study, however these authors' data on resting oxygen consumption rates was gathered from a point 6 hours after entry to the respirometer, and completed after 24 hours. Stress induced oxygen consumption rate elevation was observed in Atlantic halibut in this study, and a period of 38 hours was allowed for the fish to acclimate to test conditions prior to data recording. Clearly this is an important

Table 3.3 A comparison of resting oxygen consumption rate by fish species, for temperate region species common in aquaculture, and flatfish.

Species	Temperature (°C)	Fish Size (g)	Oxygen cons. Rate (mg kg^{-1} h^{-1})	Source
Cultured species				
Oncorhynchus mykiss	15	273	72.5	Webb (1971)
Oncorhynchus mykiss	5	100	57.0	Rao (1968)
Oncorhynchus mykiss	15	100	112.0	Rao (1968)
Salmo trutta	10	216	80.8	Beamish (1964a)
Anguilla anguilla	18	150	35.8	Degani *et al.*, (1989)
Gadus morhua	5	124	35.5	Schurmann and Steffensen (1997)
Gadus morhua	10	158	57.0	Schurmann and Steffensen (1997)
Gadus morhua	15	167	78.2	Schurmann and Steffensen (1997)
Flatfish species				
Pleuronectes platessa	5	289	19.5	Priede and Holliday (1980)
Pleuronectes platessa	10	383	31.8	Priede and Holliday (1980)
Pleuronectes platessa	15	281	50.9	Priede and Holliday (1980)
Pleuronectes platessa	6	100	39.6	Fonds *et al.*, (1992)
Pleuronectes platessa	10	100	53.5	Fonds *et al.*, (1992)
Pleuronectes platessa	14	100	70.36	Fonds *et al.*, (1992)
Pleuronectes platessa	10	100	59.2	Edwards (1971)
Platichthys flesus	6	100	41.5	Fonds *et al.*, (1992)
Platichthys flesus	10	100	72.9	Fonds *et al.*, (1992)
Platichthys flesus	14	100	93.0	Fonds *et al.*, (1992)
Microstomus kitt	10	594 - 974	145.8 - 51.4	Davenport *et al.*, (1990)
Hippoglossus hippoglossus	10	742 - 2097	185.8 - 60.0	Davenport *et al.*, (1990)
Hippoglossus hippoglossus	6	100	41.7	This study
Hippoglossus hippoglossus	10	100	54.2	This study
Hippoglossus hippoglossus	14	100	67.6	This study

The presented results for a 100g fish from the work in this study are for comparative purposes.

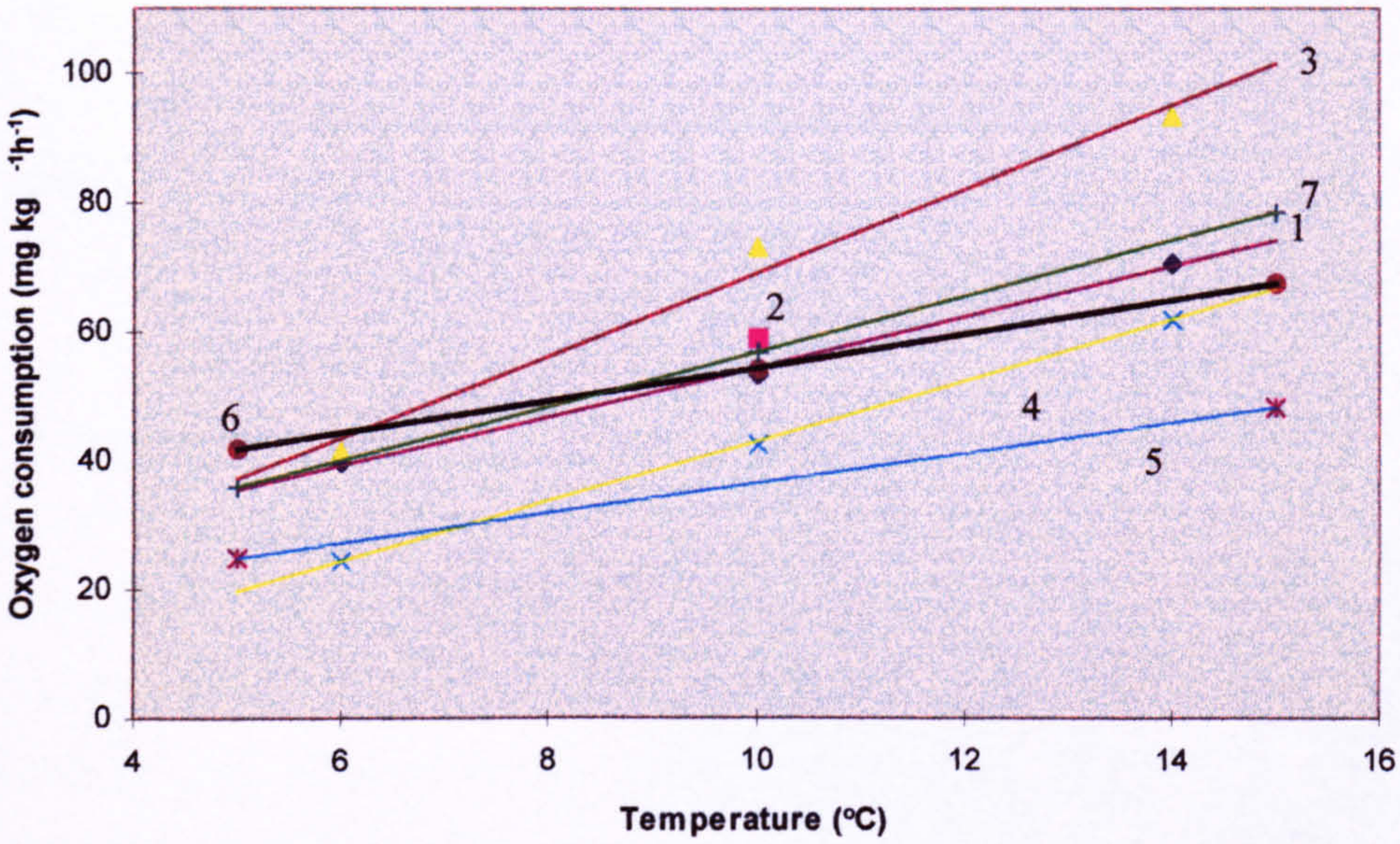


Fig. 3.11. Comparison of resting oxygen consumption rate in several marine fish species over the temperature range 5°C to 15°C. Data represented: 1, *Pleuronectes platessa* (Fonds, *et al*., 1992); 2, (single point), *Pleuronectes platessa* (Edwards, 1971); 3, *Platichthys flesus* (Fonds, *et al*., 1992); 4, *Microstomus kitt*, (Duthie, 1982); 5, *Platichthys flesus* (Duthie, 1982); 6, *Hippoglossus hippoglossus*, this study; 7, *Gadus morhua* (Schurmann and Steffensen, 1997). Resting oxygen consumption rates for a 100g fish are presented, with the exception of the data of Schurmann and Steffensen in which body weight is within the range 124 -167g.

factor which may account for the differences between these two studies, and further emphasises the importance of the adherence to a strict experimental protocol to facilitate comparisons. There is agreement with the data produced by Edwards (1971) and Fonds *et al*.,(1992) for *Pleuronectes platessa* and *Platichthys flesus*, although the values do appear somewhat elevated in comparison to those of Priede and Holliday (1980). Overall there appears to be a close correlation between resting oxygen consumption rates produced for Atlantic halibut in this study, and comparative values for other species of flatfish at similar temperatures and body weights, although there is little comparative data on the resting oxygen consumption rate of large flatfish.

A comparison of values obtained in this study with those for Atlantic cod (*Gadus morhua*) is particularly pertinent, since along with the Atlantic halibut this species was identified by Tilseth (1990) as a promising species in temperate marine

aquaculture. As an active piscivore, the cod occupies a similar niche to that of the halibut in temperate waters, with an overlapping temperature range and also possessing a similar sedentary lifestyle as a "sit and wait" predator. Further, the comparison of this roundfish species with the results for the Atlantic halibut is also of interest, with flatfish/roundfish species comparisons generally including data from the more active roundfish species. Although the weight range of fish in the study of Schurmann and Steffensen (1997) is relatively restricted, and the experiments were carried out at ambient photoperiod during September and October, the information provides a valuable comparison in resting metabolic rate between the two species. The resting oxygen consumption rate/temperature curve in Fig. 3.9. shows the cod in the study of Schurmann and Steffensen (1997) to possess a rate comparable to many of the flatfish studies. However, in a comparison with the results of this study, the two lines converge and cross, with the cod resting oxygen consumption rates elevated above the work of this study at temperatures greater than 8°C, although weight specific differences in metabolic rate suggest that a true comparison of data for 100g fish would elevate the data for the cod still further. The direct comparison of the data is unfortunately slightly restricted by this point, although a crude comparison suggests that resting oxygen consumption rates are higher in the cod at temperatures greater than 8°C. Below 8°C, the Atlantic halibut resting oxygen consumption rate is elevated above that of the cod, which may reflect an adaptive response of the halibut to low temperatures, given the natural temperature range of this species in the wild. In comparisons of polar, temperate and tropical fish species, the phenomenon of resting oxygen consumption rates in excess of those which would be predicted through extrapolation of oxygen consumption/temperature relationship data, has come to be defined by the term "Metabolic Cold Adaptation" (MCA). In essence the theory of MCA is associated with the relatively high metabolic rates which have been observed experimentally in some polar fish species (Wohlschlag, 1960) and is thought to be linked to the energy requirements of adaptive physiological processes seen at the organ level, e.g. the synthesis of antifreeze glycopeptides in the liver (MacDonald *et al.*, 1987). The use of the MCA term and the applicability of the concept remains contentious (Clarke, 1991; Eastman, 1993), and more recent work by Forster *et al.* (1987) raises some doubts over Wohlschlag's (1960) values for weight-specific

resting oxygen consumption in the Antarctic notothenoid *Pagothenia borchgrevinki*, further emphasising the importance of a strict protocol in such studies. Additional comparison of Antarctic and temperate gastropods (Houlihan and Allan, 1982) and polar and temperate region teleosts (Steffensen *et al.*, 1994) provides little evidence for MCA in the traditional sense of resting oxygen consumption rate at a value 2-4 times higher in polar species, although these organisms did show resting rate values significantly higher than would be predicted by extrapolation of the temperate species data suggesting an adaptive response to a low temperature environment in these species. Higher levels of metabolism may also be associated with increased protein synthesis and protein turnover rates which are thought to be related to growth rate (Randell Brown and Cameron, 1991a,b; Houlihan, 1991), and thus it may be hypothesised that this could confer a growth advantage on the Atlantic halibut in comparison to the cod at temperatures below 8°C for fish of about 100g weight. However, further work in this area is required to clarify weight specific resting oxygen consumption rate in the cod, and the exact nature of any differences between the two species.

Effect of Body Weight on Resting Oxygen Consumption Rate

The results of this study confirm that the Atlantic halibut conform to the general allometric equation $Y = a\,W^b$, with the value of the weight exponent b corresponding well with similar literature values, having a common value of 0.82 over the whole study. This is close to the value of 0.79 produced by Winberg (1956) for marine species. Brett and Groves (1979) quote a value of 0.86 for the weight exponent in fish, although these authors note that there is a large element of species specificity, and thus the value should be determined for individual species. Jobling (1985) gives a weight exponent range of 0.67 - 1.00 covering a variety of fish species. Literature values for the weight exponent in flatfish species are in the range 0.634 - 0.827 (Edwards *et al.*, 1969; Voyer and Morrison, 1971; Duthie, 1982), although all these studies were conducted over a comparatively small size range of fish. In contrast, the size range in this study was large at 50g - 5800g, in an effort to encompass a wide a range of animals of potential culture size.

Effect of Temperature on Resting Oxygen Consumption Rate

Increasing temperature is known to have the effect of elevating resting oxygen consumption rate in fish, within the organism's adaptive range (Winberg, 1956; Brett and Groves, 1979; Jobling 1994; Lucas 1996). This study shows that *H. hippoglossus* conforms to this paradigm, showing an increase in resting oxygen consumption rate with temperature over the range 6 - 14°C. However, the Q_{10} values show that the rate of change is not linear over the temperature range, with a lower value seen between 6°C and 10°C than that between 10°C and 14°C. Q_{10} values of 1.84 and 2.16 are reasonably close to Brett and Groves' (1979) multiplier of 2.3 which these authors provided as an approximation for temperature effects on metabolism.

The rate of change in physiological processes with temperature, and particularly oxygen consumption rates, has been found to decrease as temperature approaches the organism's thermal optimum (Winberg, 1956; Jobling 1994), suggesting in this instance that the thermal optimum for Atlantic halibut is in the region of 10°C or less, although it is difficult to draw any conclusions with a degree of precision since this work was carried out only at 6, 10 and 14°C. In addition, this study relates to a large size range of fish, and within the size range there are likely to be further size and age differences. Also, the probable sexual dimorphism in relation to the energetics of this species (Haug *et al.*, 1988) further complicates the overall picture. The higher growth rates seen in female Atlantic halibut in comparison to males are likely to be associated with higher rates of protein turnover, the physiological machinery of which may have a relatively higher energetic cost with the animals showing consequently increased resting respiratory rates. Current information on sex differences in growth rate of Atlantic halibut is limited to sexually mature wild fish, and possible differences between the sexes of immature fish warrants further research attention given the implications for culture of this organism. The sex ratio of the study fish in this work was not determined since all were immature and no reliable means of sexing Atlantic halibut at this stage of development currently exists. Although the significance of this factor is unknown, the

possibility exists for a deviation in sex ratio from 50:50 (male : female) to influence the results of such work. Only further respirometry on the resting oxygen consumption rate of individual fish of known sex will clarify this issue.

In work on Atlantic halibut, Björnsson and Tryggvadóttir (1996) produced values of thermal optima in relation to growth of 14°C for 10 - 60g fish, 11.4°C for 100 - 500g fish, and 9.7°C for 3 - 5kg fish, although this study was conducted at different times of the year and some of the effects may have been photoperiod and season induced. The results of this study suggest thermal optima for *H. hippoglossus* within this range, and corroborate these values. The selection of temperature preferenda of 2-8°C by wild fish (McCracken, 1958; Wheeler 1969), is a further point along this continuum, since the majority of fish landed at ports are large, mature adults of 50cm+ (Liewes, 1984), well in excess of the size of the study fish of Björnsson and Tryggvadóttir (1996). It appears that the preferred temperature range of *H. hippoglossus* coincides well with ambient annual sea water temperatures observed on the West coast of Scotland, and this reaffirms the potential of this organism for culture in these waters.

Effect of photophase on Resting Oxygen Consumption Rate

H. hippoglossus showed peak oxygen consumption rates in both the photophase and the scotophase in a 12L:12D photoperiod amongst all the groups examined in this study, although the results were not conclusive in identifying a particular rhythmicity in this species. This information is important, emphasising that the feeding of this species in the period of daylight as is currently the practice in Scotland, may not be appropriate. Without exception, Atlantic halibut farms are operated under identical conditions to the culture of salmonids in land-based seawater systems in Scotland, although the difference in the biology of the two organisms is great. The influence of a relatively easily controlled environmental parameter such as photoperiod on the physiology and behaviour of the Atlantic halibut is an extremely

important consideration in the potential culture of this organism, and should be a priority for future research into the ongrowing of this fish.

Photoperiod is described by Fry (1971) as a "Directive Factor" in fish metabolism, and an effect on resting metabolic rate has been documented in some species (De Silva *et al.*, 1986; Ross and McKinney, 1988a,b; Chakraborty *et al.*, 1992). Brett (1979) states that the effect of light on the metabolism of fish is a function of the quality and intensity as well as the photoperiod. In this study care was taken to conduct respirometry under identical conditions of light intensity, quality and photoperiod as had been applied in acclimation. In this way, the change in environmental parameters between acclimation and respirometry was minimised. Although every effort was made to contain the experimental apparatus in a noise restricted area of the laboratory, there were occasions when routine site husbandry elevated noise levels to such an extent that there may have been an effect on the oxygen consumption rate of individual halibut. Since any such interference always occurred between the hours of 09.00h and 18.00h, i.e. the photophase, there could have been an overall effect on the results for peak oxygen consumption rate related to light phase.

Anecdotal evidence suggests that Atlantic halibut may be active at night in the wild, drawing from increased mid-water activity of prey species at night (Leim and Scott, 1966). The results of research on rhythmicity in fish are generally indistinct, and Eriksson (1978) notes that this is characteristic of such studies, showing literature references providing evidence for diurnal, nocturnal and crepuscular rhythms in *Salmo trutta*. In addition to this, culture conditions may modify behaviour and any endogenous rhythm in this species may be overridden by husbandry activities such as feeding patterns. Goldfish (*Carassius auratus*) have shown increased activity in anticipation of meals, a "food-entrainable circadian oscillator" (Sánchez-Vázquez *et al.*, 1997). Dualism has been observed in cultured sea bass (*Dicentrarchus labrax*) in the laboratory (Sánchez-Vázquez *et al.*, 1995a,b), and such phasing of activity may be the evolutionary response of an opportunistic feeder. The high metabolic costs associated with locomotion in flatfish (Priede and Holliday, 1980) suggest that a large

flatfish such as a halibut is likely to become opportunistic in feeding pattern, and this species may exhibit a degree of dualism in the wild. Nocturnal activity is a successful strategy for predator evasion in many species, and this may have some bearing on the life history of the Atlantic halibut since the main predator is known to be the seal (Scott and Scott, 1988).

Clearly the existence of a diurnal, nocturnal or dualistic cycle has great implications for the culture of the Atlantic halibut. The results of this study indicate that endogenous physiological rhythms in this fish are not confined to the diurnal cycle, and this merits further attention with a view to maximising feeding efficiency in farmed populations.

Overall, this work produces values for the M_M component in the energy budget for *H. hippoglossus*, and the regression equations produced at the temperatures 6, 10 and 14°C allow the prediction of the energetic cost of this component across this temperature range for a range of fish sizes between 50g and 6 kg. A species comparison shows that the maintenance costs in the Atlantic halibut are lower than roundfish at comparable weights and temperatures, and similar to those of other flatfish species. This does suggest that *H. hippoglossus* may be a relatively energetically efficient organism, with the portion of ingested energy allocated to homeostasis proportionately small in comparison to other cultured species of fish.

Chapter 4.

Routine and post-prandial oxygen consumption and Ammonia excretion, in small populations of Atlantic halibut (*Hippoglossus hippoglossus* L.).

4.1. Introduction

There is a general lack of information on energy budgets of flatfish species in culture situations leading to the situation where units farming such species are likely to operate sub-optimally, with concomitant effects on both production costs and the environment. Cho *et al.* (1982) emphasise the importance of such studies in determining a nutritional and energetic balance, thereby facilitating a greater efficiency in the production of fish. Further research is therefore required in order to elucidate the energetics of cultured flatfish species, maximising the production of such systems. In particular, knowledge on *H. hippoglossus* is limited, and the unique characteristics of this organism together with the potential importance this species possesses for aquaculture in temperate marine systems, identify this species as an ideal candidate for such a study. Principally the energy transformations which occur following the ingestion of food, and the metabolic energy costs associated with the processing of food are important factors in the determination of an overall energy budget (see Chapter 1.). It is important to determine the energetics of this species when adapted to the tank environment to aid in the construction of models which allow the prediction of the performance of this species in such systems.

The routine metabolic rate is regarded as that level of metabolism influenced by random activity under experimental conditions in which movements are somewhat restricted, and the fish protected from external stimuli (Fry, 1971). Thus routine metabolic rate is a reflection of such random activity, the extent of which is dependent on Fry's "Directive" effects of environment, amongst other factors such as stock density, size of fish, and social interactions. Importantly, measurements are recorded on fish in the postabsorptive phase, as with measurements of resting metabolic rate, to ensure that there is no component of metabolism attributable to the processing of

food. Routine oxygen consumption rates generally range from standard metabolic rate to half the active metabolic rate depending on experimental conditions, and the peak routine rate for goldfish has been recorded at a level six times that of the standard rate (Fry, 1971). Consequently, recordings of routine rates have been considered to have limited relevance to fish energetics if the measurements are made without full knowledge of fish activity levels (Brett and Groves, 1979).

Some authors have developed methods of simultaneously measuring activity levels alongside routine oxygen consumption rates (Spoor, 1946; Beamish, 1964b; Beamish and Mookherjii, 1964; Sims *et al.*, 1993), although in many cases this was undertaken in order that a prediction of resting metabolic rate through extrapolation to zero activity could be established. If prior knowledge of the resting oxygen consumption rates has been obtained, routine oxygen consumption rate will provide information on the energy cost of spontaneous activity, a factor which is useful in the overall energy budget calculation, and has direct application in the culture environment. Inter-specific comparisons may be difficult since experimental conditions will vary, although such routine rate measurements also provide important information on diurnal activity cycles when recordings are taken over 24 hour periods (Cech, 1990). Notwithstanding the difficulties associated in drawing meaningful conclusions from such work, there are many literature studies on routine metabolism in fish, including those on the effects of food deprivation (Beamish, 1964; Glass, 1968), the effects of salinity (Moser and Miller, 1994), the effects of salinity and temperature (Marais, 1978), the effects of smoltification in salmonids (Wiggs *et al.*, 1989), nocturnal versus diurnal rhythms in fish (Du Preez *et al.*, 1986), as well as the routine oxygen consumption of different species within the Pleuronectidae (Waller, 1992; MacIsaac *et al.*, 1996).

Feeding has long been known to exert an influence on metabolic rate, with the experiments of Lavoisier and Laplace in 1780 on fasted and recently fed subjects the first documented evidence (see Kleiber, 1961). The post-prandial increase in metabolic rate was originally termed the Specific Dynamic Effect, following the work of Rubner (1902) who demonstrated the phenomenon in the dog, and described the

effect as "the chemical work of glands in the metabolism of absorbed nutrients". This term is regarded as something of a mistranslation from the original German and Specific Dynamic Action (S.D.A.) has been generally applied to physiological studies. The descriptive relevance of other terms such as "heat increment" and "calorigenic effect" has been argued (Kleiber, 1961; Beamish and Trippel, 1990), however S.D.A. has been used to denote the post-prandial increase in metabolic rate in the majority of fish studies.

An early study showed an increase in oxygen consumption and carbon dioxide production rates following injection of amino acids in hepatectomised dogs, similar to the increases seen in control animals following a meal (Wilhelmj *et al.*, 1928). Much further work has been achieved since this time, with some attention devoted to this phenomenon in fish. More recently, S.D.A. has been considered to comprise the energy costs of digestion, assimilation and storage of digestion products, and the deamination of amino acids in the synthesis of nitrogenous excretory products (Jobling, 1985). S.D.A. has also been regarded as an "entropy tax" paid during food conversion (Ware, 1975), and Winberg (1956) suggested that in fish the metabolic rate could double from resting levels following the ingestion of a meal, a figure also quoted by Steffens (1989). According to Brett and Groves (1979), this figure leads to an underestimate although the ratio may provide a reasonable prediction if routine metabolic rates are used as the base for calculation. Using an inert diet, Tandler and Beamish, (1979, 1981) showed that there is a mechanical component to the overall energy cost of S.D.A. (grasping, chewing, swallowing and intestinal muscular work - peristalsis), although in other work the peristaltic energetic proportion is not considered to be significant in terms of the overall cost (Jobling and Davies, 1980; Jobling, 1993). Such energy requirements are difficult to distinguish from those physiological costs associated with the metabolism of the food, and the prefix "apparent" has been applied to denote the inclusion of both components (Medland and Beamish, 1985).

S.D.A. is customarily measured through indirect calorimetry by calculation of oxygen consumption rate, although the technique of direct calorimetry has also

successfully been applied (Smith *et al.*, 1978). The magnitude of S.D.A. is determined by plotting the curve of post-prandial oxygen consumption with time (Chakraborty, 1992). When the area under the curve is calculated, and the resting metabolic rate subtracted from this value, the S.D.A may be quantified in terms of oxygen consumed (mg O_2), which may then be transformed into energy (J). Commonly, S.D.A. is then expressed in terms of the energy content of the food (Jobling, 1985), or as the percentage of ingested energy, also known as the S.D.A. coefficient (Jobling and Davies, 1980).

In fish studies, three components of S.D.A. have been identified as important: the maximum or peak metabolic rate following feeding, the magnitude, and the duration of effect (Jobling, 1980). The phenomenon of S.D.A. is complex, and a range of biotic and abiotic factors influence the response (Beamish and Trippel, 1990). In general S.D.A. is dependent on the quantity and quality of the foodstuff (Muir and Niimi, 1972; Hamada and Ida, 1973; Beamish, 1974; Brett, 1976; Scott *et al.*, 1978; Vahl and Davenport, 1979; Jobling, 1985; Guinea and Fernandez, 1997), with increasing size of meal lengthening the duration of the elevated metabolic rate. Tandler and Beamish (1979, 1981) observed a positive relationship between S.D.A. and the quantity of ingested energy, and in another study the same authors showed that S.D.A. was positively related to the proportion of protein in the diet (Tandler and Beamish, 1980). An increased level of energy cost attributable to S.D.A. was also recorded by Cho *et al.* (1976), with increasing dietary protein levels of 40-60% in the rainbow trout. A review of "heat increment" in fish bioenergetic models by Beamish and Trippel (1990) notes that the energetic cost increases with meal size, fish weight and increasing temperature, but decreases with body weight for a given feed rate. In work on juvenile cod, Soofiani and Hawkins (1982) observed that the elevation in oxygen consumption rate following a meal increased with ration size, and the rate of oxygen consumption increased with temperature, reaching a maximum at 15°C. Cho and Slinger (1980) observed a linear relationship between increasing digestible energy intake and heat increment (S.D.A.) in rainbow trout, and this was correlated with increasing levels of feed intake to a maximum at 15°C, which was assumed to be the optimum temperature in this species. As with many aspects of fish respirometry,

experimental error resulting from a high inter-individual variability or increased stress levels during sampling may result in a wide range of results for S.D.A. (Knights, 1985). Excitability and increased activity during feeding are not unusual phenomena, and the associated energy costs must be discounted from any study of S.D.A. (Brett and Groves, 1979).

At this juncture, the biochemistry associated with the S.D.A. processes remains incompletely understood, however, Jobling (1983) hypothesised that S.D.A. may "represent a short-term increase in rates of protein synthesis and turnover following feeding" with the process regulated by plasma thyroid hormone concentration. More recent studies on S.D.A. in channel catfish following infusion of essential amino acids, confirmed an increase in plasma amino acid concentration and protein synthesis rate in white muscle tissue associated with S.D.A. (Randell Brown and Cameron, 1991a,b). The administration of a protein synthesis inhibitor (cycloheximide) negated the S.D.A. and the effect on plasma amino acid concentration and protein synthesis rate, suggesting that S.D.A. may be directly attributable to the energetic cost of protein synthesis, and therefore to the metabolic cost of growth. These results add strength to the hypotheses of various authors for a link between S.D.A. and growth rate (Jobling, 1981a, 1985; Knights, 1985; Hepher, 1988). In a review of protein dynamics in ectotherms, Houlihan (1991) draws several correlations between oxygen consumption rate and levels of protein synthesis in fish tissues, in particular noting the parallel increase in oxygen consumption rate and protein synthesis rate in tissues and the whole organism following a meal.

As noted by Pandian (1987) the process of nitrogen excretion in fish may be separated into two categories, namely the endogenous component associated with catabolism of proteins, and the exogenous component associated with the energy loss from ingested food. In this respect, nitrogen excretion may be regarded as somewhat analogous to the situation with oxygen consumption, with a base level energy expenditure observed at lower metabolic levels, and increasing over a defined metabolic range. Primarily energy losses as nitrogen in fish are in the form of ammonia excreted across the gill surface, although other compounds such as urea and

trimethylamine oxide contribute a small proportion of energy loss (Brett and Groves, 1979). Ammonia comprises 75-85% of nitrogenous excretory losses in marine fish, and the estimated daily loss is 9-11% of the food intake (Pandian, 1987). The excretion of ammonia does confer an energetic advantage in that the ammonia molecule is simple in structure, and comparatively there is a greater energy cost associated with the formation of urea and trimethylamine oxide as excretory products (Brett and Groves, 1979). The disadvantage to the formation of ammonia is its relative toxicity, not only in terms of mortality but also with sublethal levels impacting growth rates (Jobling, 1994).

In a study of juvenile plaice, Jobling (1981b) observed that ammonia represented 75-85% of the nitrogenous excretion in starved plaice, and that the endogenous rate of ammonia excretion increased with temperature. The weight specific endogenous ammonia excretion rate was also observed to be higher in small fish within the weight range 5-90g, with the rate of ammonia excretion related to body weight to the power 0.67. Jobling (1993) quotes values of 30-300 mg N kg^{-1} d^{-1} for endogenous nitrogen excretion in fish, with the value dependent on species, body weight and water temperature.

The ingestion of a meal leads to an increase in the rate of ammonia excretion, which peaks at a period some hours after the consumption of food (Jobling, 1993). Experimentally this has been observed in species of fish such as sockeye salmon (Brett and Zala, 1975), plaice (Jobling, 1981b), Atlantic cod (Ramnarine *et al.*, 1987) and the common carp (Chakraborty *et al.*, 1992b). A post feeding ammonia excretion rate elevated between two and eleven times that of the endogenous excretion rate was observed by Jobling (1981b) in the plaice, and both the peak rate and the duration increased with the amount of protein absorbed from the diet. Ramnarine *et al.* (1987) also derived a significant relationship between peak ammonia excretion rate and ration size in the Atlantic cod.

The aim of this study was to further explore the bioenergetics of the Atlantic halibut by quantifying routine oxygen consumption rate and the energetic costs

attributable to S.D.A. In this way, the partitioning of energy within the metabolic component of the energy budget (M_M, M_A and M_F) was elucidated. Additionally, endogenous ammonia excretion rate and ammonia excretion following a single meal was examined to aid in the determination of energy losses due to excretion - the "U" component of the energy budget. This work was carried out in small populations of Atlantic halibut held in tank systems at stocking densities likely to be achieved in culture, with the results of such a study providing information directly relevant to the husbandry of this species under intensive farming conditions.

4.2 Materials and Methods

4.2.1 Fish

Test animals were Atlantic halibut of the 1994 and 1995 year classes.

4.2.2 Facilities

The $1m^2$ holding tanks were operated as flow through respirometers, with central sumps and external standpipes facilitating arrangement of dissolved oxygen probes in tank effluent water, allowing measurement of the dissolved oxygen content of the effluent before the water was exposed to the atmosphere. Each tank possessed an opaque glass fibre lid which minimised disturbance effects of any external influences. The arrangement of this system is shown schematically in Fig. 4.1. a & b. Inflowing seawater was gravity fed from the site header tank through a system of 0.75 inch ABS pipework, finally entering the trial tanks through a 90° bend and falling approximately 5cm to the tank water level. The tanks were assumed to be well mixed and to operate efficiently as flow through respirometers, with the diffusion of atmospheric oxygen over the water-air interface also assumed to have a negligible effect on the subsequently determined oxygen consumption rates. Stock populations of halibut were maintained in these tanks, and a minimum period of 4 weeks was allowed between any new introductions and the commencement of a trial.

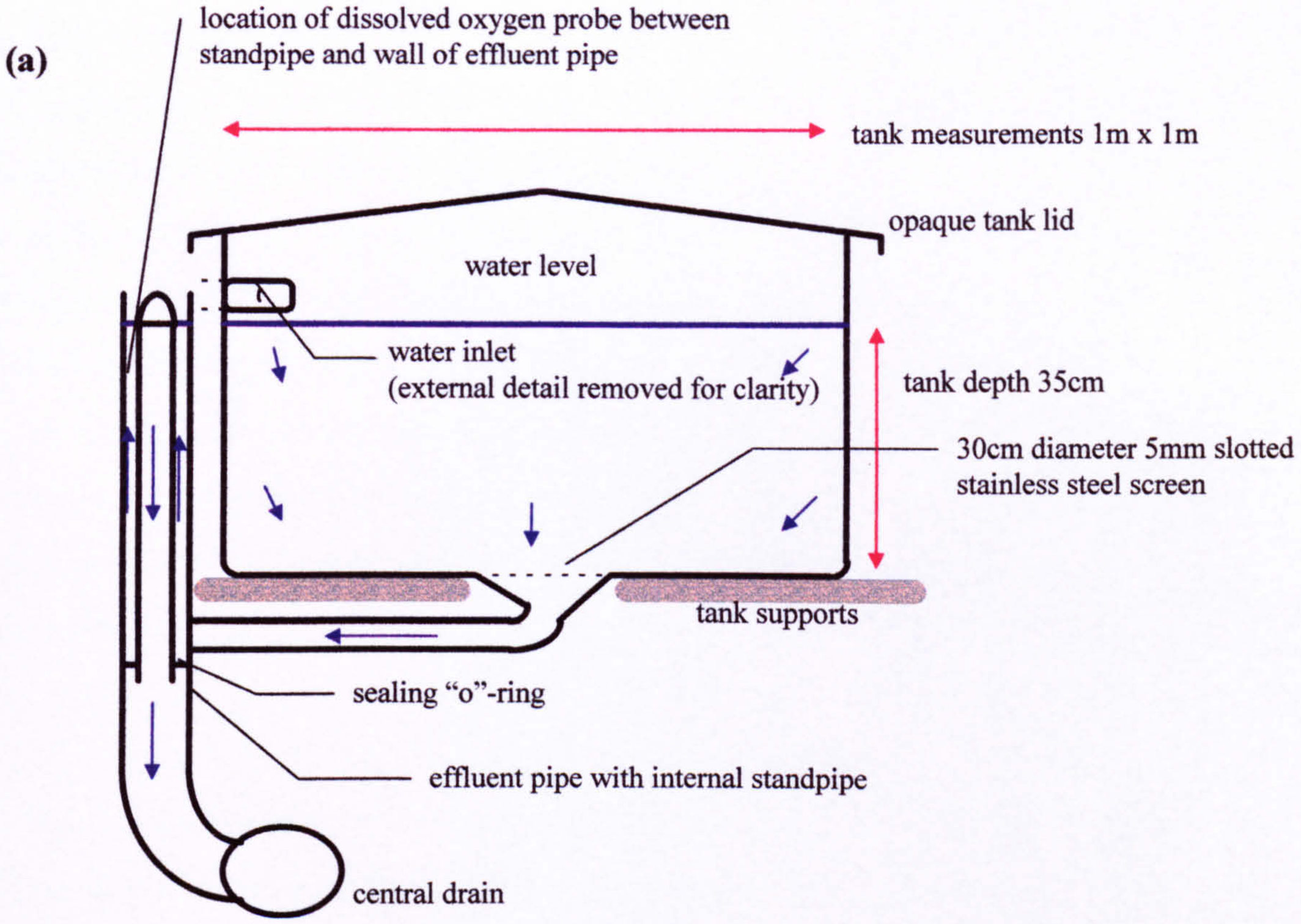


Fig. 4.1. Side (a) and Plan (b) views of the 1m^2 tanks employed as flow through respirometers in this study. Seawater flow direction is indicated by blue arrows.

4.2.3. Experimental Protocol

Tank populations numbered in the range 5-14 individuals, the number dependent on average weight, and thus tank biomass. Halibut were maintained within distinct size classes. Water flow rate measurements are critical to the precise determination of oxygen consumption and ammonia excretion rates, and were measured periodically throughout each trial. A stopwatch was used to quantify the length of time taken for the inflowing water to fill an exact volume in a graduated beaker, and these values used to calculate flow rate. Water flow rates were measured approximately every six hours during individual trials, and measurements were taken immediately after the recording of dissolved oxygen data to reduce the possible influence of this action on fish activity levels, and hence oxygen consumption rate values.

The original intention of this work was to monitor the effect of the ingestion of a single meal on the oxygen consumption rate of individual Atlantic halibut held within respirometers. Preliminary attempts to feed halibut in respirometers were undertaken at the end of some of the resting oxygen consumption rate trials conducted as experimental work for Chapter 3. In total, 17 individual halibut ranging in size from 300g to 1200g, were offered feed when held in respirometers at temperatures of 10°C and 14°C. Pellets were allowed to remain within the respirometers up to a maximum of 3 hours after introduction, and at no point were individuals observed to ingest pellets. This necessitated a modified approach to the determination of post-prandial oxygen consumption rate, consequently small populations of halibut maintained routinely in small flow-through tanks were chosen as a substitute to individual fish analysis. These small populations were maintained as stock tanks for the respirometry acclimation unit, and were held continuously in the $1m^2$ tanks. Thus the halibut were not introduced into a novel environment prior to this experimental work, and conditions for the fish were maintained similar prior to, and throughout, the period of this study.

Airstones were removed, and the water flow measured in trial tanks one hour prior to the commencement of each trial. Flow rates had previously been adjusted to experimental velocities 24 hours before each trial. Flow rates ranged between 390 l h^{-1} and 710 l h^{-1} depending on water temperature and fish biomass. Trials were categorised as either routine rate (measurements on starved halibut according to the periods defined in chapter 2.) or "fed" rate (measurements recorded on halibut following a single meal). Trials were conducted at ambient temperatures over the period 16 June 1996 to 9 November 1997 (temperature range 7.7°C - 14.8°C). Photoperiod was ambient. Empty tanks were operated as controls for each experimental run in order that background oxygen consumption rates could be measured. Dissolved oxygen readings were taken hourly from 08.00h to 18.00h, and 2-hourly between 18.00h and 08.00h.

Nitrogenous excretion rates in the form of total ammonia were examined in both starved and fed populations through the collection of water samples from effluent pipes. This was carried out for a total of 7 tanks at temperatures 8, 10 and 12°C. Water samples of approximately 300ml of seawater were collected by siphon from the effluent pipe into a glass beaker, at a position adjacent to that of the dissolved oxygen probe. These samples were immediately filtered (Whatman GF/C papers), and stored at -20°C, for subsequent analysis of ammonia levels (see Chapter 2 for method of ammonia analysis and calculation of oxygen consumption and ammonia excretion rates). Background ammonia levels were measured through analysis of water samples taken from the control tank at four hour intervals. These values were measured in the range 0.8 - 4.7μg NH_3-N l^{-1}. Ammonia excretion rates were calculated according to the following equation:

$$\textbf{Ammonia excretion (mg kg}^{-1}\textbf{ h}^{-1}\textbf{)} = \textbf{(A - B) x F x (1000/W)}$$

where A = ammonia in the test tank effluent, B = ammonia in the control tank effluent, F = flow rate (l h^{-1}) and W = fish biomass (g).

4.2.4. Effect of body weight and temperature on routine oxygen consumption rate

Routine oxygen consumption rate data was compiled for a total of 73 trials. Water temperature was recorded for each dissolved oxygen reading, and the average calculated for each trial. All results were grouped within the temperature ranges 7 - 9°C; 9 - 11°C; 11 - 13°C; 13 - 15°C. Fish weight varied from 287g to 1462g.

Trials commenced at 09.00h on day 1, and oxygen consumption rate was monitored with readings recorded hourly between 09.00h and 20.00h, 2-hourly to 08.00h the following day, and then hourly, finishing at 10.00h on day 2. Mean 24-hour oxygen consumption rate values in the routine rate trials were calculated, and investigated for any relationship between body weight and temperature on routine metabolic rate.

4.2.5. Effect of photoperiod on routine oxygen consumption rate.

Routine rate oxygen consumption data was measured throughout the light and dark cycles under ambient photoperiod, and this was investigated for any degree of diurnal rhythmicity. Photoperiod over the duration of the trials varied between 18L:6D and 9L:15D. Extreme care was taken to ensure that there was no disturbance to the test fish during the procurement of dissolved oxygen readings. When readings were taken in the dark phase, no artificial illumination was applied, and a low intensity hand held torch carrying a red filter was employed for personal orientation. Six individual routine rate trials were conducted in conjunction with the activity work (see Chapter 5.), and these were discounted from the analysis.

4.2.6. Effect of body weight on meal size

In all fed trials the halibut were offered a single meal of 1% body weight at 10.00h on day one, following the initial two recordings of oxygen data. Feed composition and pellet size were are presented in Table 4.1. After a period of 2 hours,

uneaten pellets were removed, counted, and the actual amount of feed ingested by each test population was calculated.

Table 4.1 Composition of test diets.

	Pellet Size (length)		
	8.0mm	**13.0mm**	**18.0mm**
Fish weight	**40-500g**	**500-1000g**	**>1000g**
Analysis %			
Protein	**56.0**	**52.0**	**52.0**
Oil	**16.0**	**20.0**	**20.0**
Carbohydrate	**9.0**	**9.0**	**9.0**
Ash	**11.0**	**11.0**	**11.0**
Phosphorus	**1.5**	**1.5**	**1.5**
Nitrogen	**9.0**	**8.3**	**8.3**
Gross Energy kJ g^{-1}	**20.5**	**21.1**	**21.1**

Dietary composition is presented according to that defined by the manufacturer (B.O.C.M. Pauls). Subsequent analysis of feed samples produced values corresponding closely to those presented.

4.2.7. Effect of a single meal on post-prandial oxygen consumption rate and Specific Dynamic Action (S.D.A.)

Oxygen consumption rate following a single meal was monitored in a total of 38 trials. Fed trials commenced at 09.00h on day 1, and oxygen consumption rate was measured hourly until 20.00h, 2-hourly until 08.00h, then hourly until the termination of the trial at 18.00h on day 2. Minimum starvation periods according to those defined in Chapter 2. were applied prior to the commencement of each trial. Diet and pellet size was as described in Table 4.1. Halibut were offered a single meal of 1.0% body weight at 10.00h. At intervals of 1-2 days prior to these trials, routine rate measurements had been undertaken on the same fish tank groups. The routine rate data was then compared with data from the fed trials, and this comparison used to investigate the quantity and duration of the S.D.A. effect. Fed trials within temperature groups were all performed within the same experimental period.

4.2.8. Effect of body weight and temperature on endogenous Ammonia excretion rate.

Water samples were collected from effluent pipes at 4-hourly intervals over the 24 hour trial duration in endogenous ammonia excretion rate trials, with this work carried out in conjunction with some of the routine metabolic rate trials. A total of 21 trials were conducted. This experimental work was timed to coincide with ambient sea water temperatures reaching 8, 10 and 12°C in the spring and early summer of 1997, and seven individual tanks were monitored over this period. Fish weight was 402g - 1420g.

4.2.9. Effect of a single meal on Ammonia excretion rate.

In conjunction with some of the fed oxygen consumption rate trials, water samples were collected at 2-hourly intervals over the 33 hour trial duration. The work was conducted 24 to 48 hours after the starved trials in the same tanks and fish populations as studied in 4.2.6., allowing comparison between endogenous ammonia excretion rate and ammonia excretion rate following a single meal in discrete populations of Atlantic halibut. A total of 21 trials were conducted at test temperatures of 8°C to 12°C, with fish populations as for 4.2.6.

4.2.10. Statistical Analysis.

Dissolved oxygen and ammonia data was entered onto spreadsheets (Microsoft EXCEL 7.0) for analysis. Oxygen consumption and ammonia excretion rates were subsequently calculated from flow rates and fish weights according to the formulae presented above and in chapter 2. The results for both oxygen consumption and ammonia excretion rates were processed through a data smoother (4253H, twice) run on "Minitab 10.51Xtra", to clarify any trends. Trials were grouped according to temperature, and the effect of body weight and temperature on the routine metabolic rate was investigated through linear regression and analysis of variance (Zar, 1984). Regression lines produced for individual temperatures were compared against a global

regression for all data to test the relationships of oxygen consumption and ammonia excretion rates with temperature. Multiple regression was used to develop models for the relationship between both oxygen consumption and ammonia excretion with temperature and fish weight. The data from the fed trials was investigated for S.D.A. effect of a single meal, including time to peak oxygen consumption and ammonia excretion following a meal, magnitude and duration of effect. The magnitude of the effect was calculated as the difference between pre-prandial and post-prandial rate, from analysis of routine and fed rate trials on individual populations at the same water temperature. The effect of fish size on size of meal ingested (% body weight) was explored through regression analysis.

4.3 Results

4.3.1. *Effect of body weight and temperature on routine oxygen consumption rate*

There was an inverse relationship between Log_{10} body weight and routine oxygen consumption rate, oxygen consumption rate increasing with temperature over the experimental range 8 - 14°C. These results are represented in Fig.4.2. The regression equations, correlation coefficients and levels of significance are shown in Table 4.2.

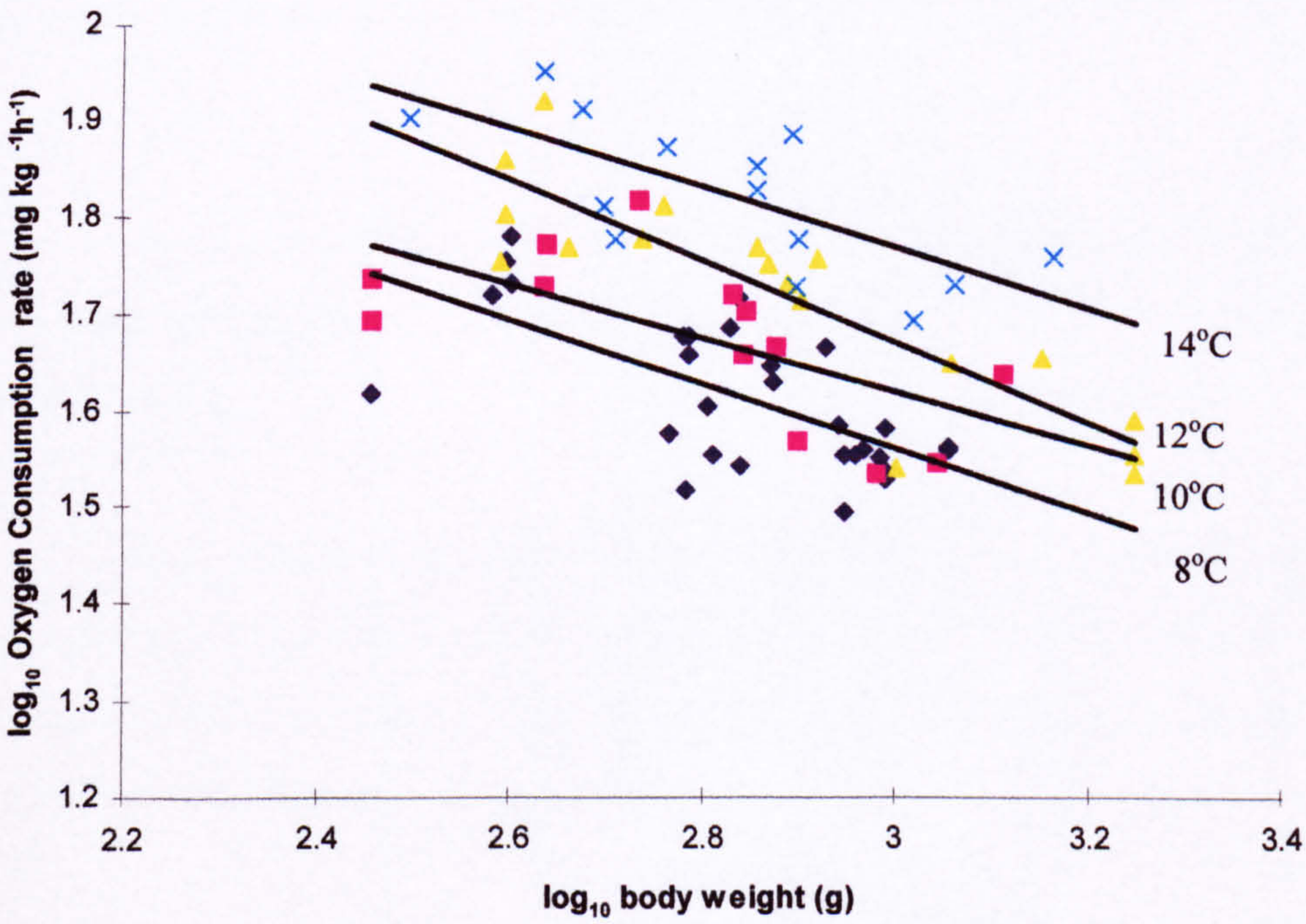


Fig. 4.2. Relationship between log_{10} oxygen consumption rate and log_{10} body weight at routine levels of activity from 8°C to 14°C in tank populations of *Hippoglossus hippoglossus*.

Table 4.2. Regression statistics for the effect of temperature and body weight on routine oxygen consumption rate in small populations of *Hippoglossus hippoglossus* L.

Temperature (°C)	n	Weight range (g)	Logarithmic Regression equation	Allometric equation ($Y = a\ W^b$)	Q_{10}
8	29	287 - 1137	$\text{Log}_{10}\ Y = 2.57 - 0.336\ \text{Log}_{10}\ W$ ($r^2 = 0.44$, $p < 0.001$)	$Y = 0.3687\ W^{0.66}$ ($r^2 = 0.76$, $p < 0.01$)	
10	13	287 - 1297	$\text{Log}_{10}\ Y = 2.45 - 0.278\ \text{Log}_{10}\ W$ ($r^2 = 0.50$, $p < 0.025$)	$Y = 0.2817\ W^{0.72}$ ($r^2 = 0.85$, $p < 0.01$)	1.86
12	17	390 - 1774	$\text{Log}_{10}\ Y = 2.92 - 0.416\ \text{Log}_{10}\ W$ ($r^2 = 0.79$, $p < 0.001$)	$Y = 0.8247\ W^{0.58}$ ($r^2 = 0.86$, $p < 0.01$)	1.91
14	14	315 - 1462	$\text{Log}_{10}\ Y = 2.70 - 0.312\ \text{Log}_{10}\ W$ ($r^2 = 0.52$, $p < 0.005$)	$Y = 0.5008\ W^{0.69}$ ($r^2 = 0.83$, $p < 0.01$)	2.88

Q_{10} values were calculated according to Van't Hoff's formula (Lucas, 1996), and were calculated for an individual of 1kg body weight. Q_{10} value over the entire test temperature range 8°C to 14°C was 2.17.

Regression analysis confirmed the statistical significance of the plots for the four temperatures against a global plot for all temperature results ($F = 20.86$; d.f. = 6, 66; $p < 0.001$).

The Q_{10} value calculated over the full test temperature range 8°C to 14°C was 2.22, although Q_{10} was observed to be temperature dependent with the highest calculated value between 12°C and 14°C, decreasing over the temperature range with the lowest value between 8°C and 10°C.

As with the data on resting oxygen consumption, further exploration of the data was achieved through an investigation into the relationship between body weight and oxygen consumption at the four test temperatures, the results of which are also shown in Table 4.2. The relationship is described by the allometric equation $Y = aW^b$, where Y is the resting oxygen consumption (mg h^{-1}), W is the body weight (g), a is the constant of the equation which is related to the test temperature, and b is the weight exponent.

Multiple regression provided an equation for the prediction of routine oxygen consumption with body weight covering the test temperature range of 6 - 14°C:

$$Y = -14.8 + 0.0296\,W + 2.70\,T$$

where: Y = oxygen consumption (mg h^{-1}); W = fish weight (g); T = temperature (°C).

4.3.2. *Effect of photoperiod on routine oxygen consumption rate*

Out of a total of 67 routine oxygen consumption rate trials carried out under ambient photoperiod, 58 trials exhibited increased oxygen consumption rates nocturnally. Examples of the raw data and smoothed data produced from trials within the temperature groups 8°C, 10°C, 12°C and 14°C are illustrated in Fig. 4.3., 4.4., 4.5. and 4.6. Temperature did not appear to influence the pattern, however trials in which nocturnal routine oxygen consumption rates were diminished generally had fish weights in the lower end of the size range (396g - 964g). The phase location of peak oxygen consumption rate with temperature for all the routine rate trials is shown in Fig. 4.7., and the percentage increase for each phase over all test temperatures is shown in Table 4.3. The percentage increase in routine oxygen consumption was higher when the peak was observed nocturnally for all test temperature groups.

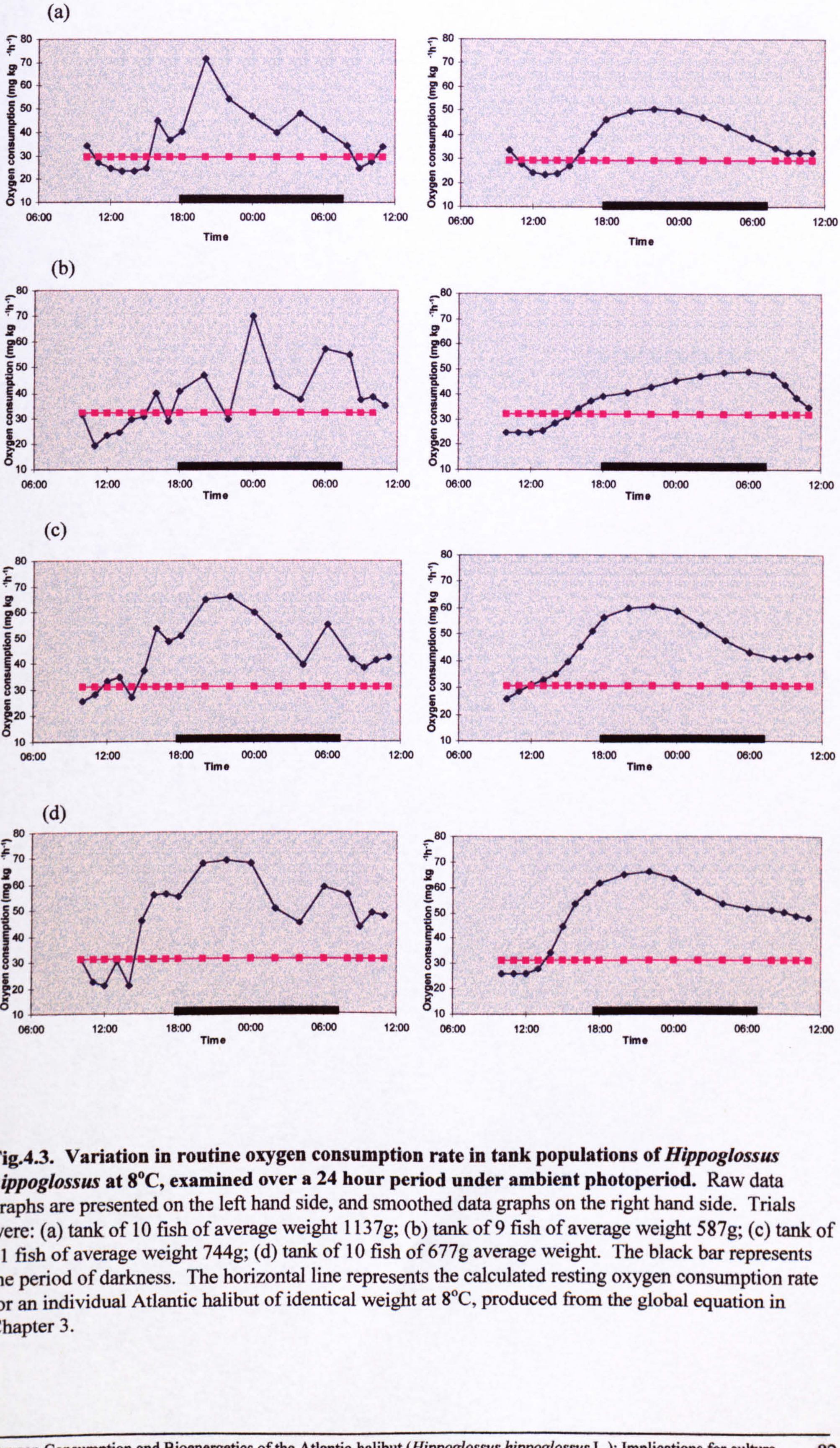
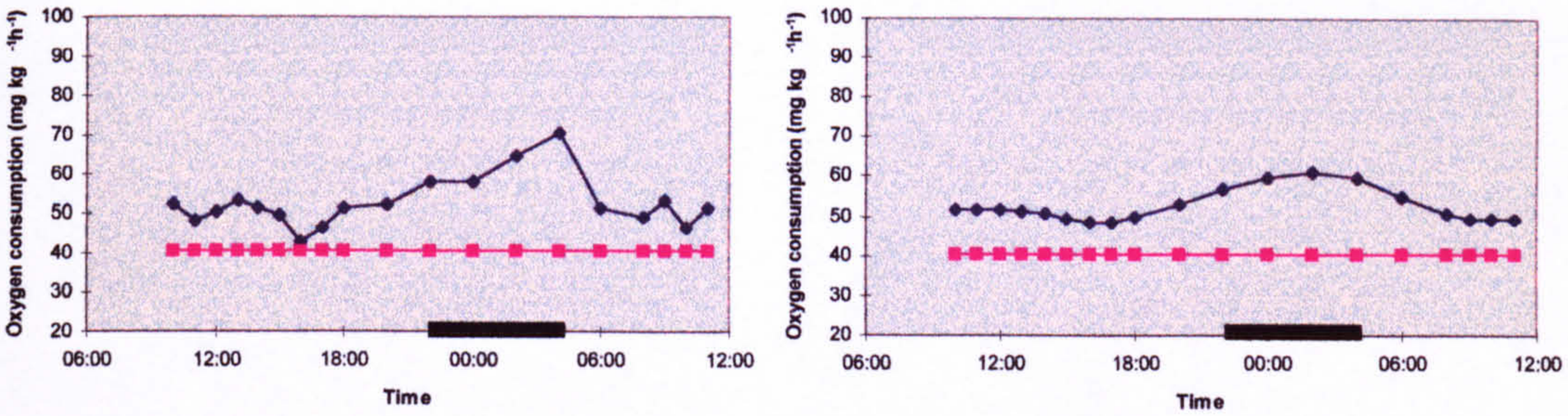
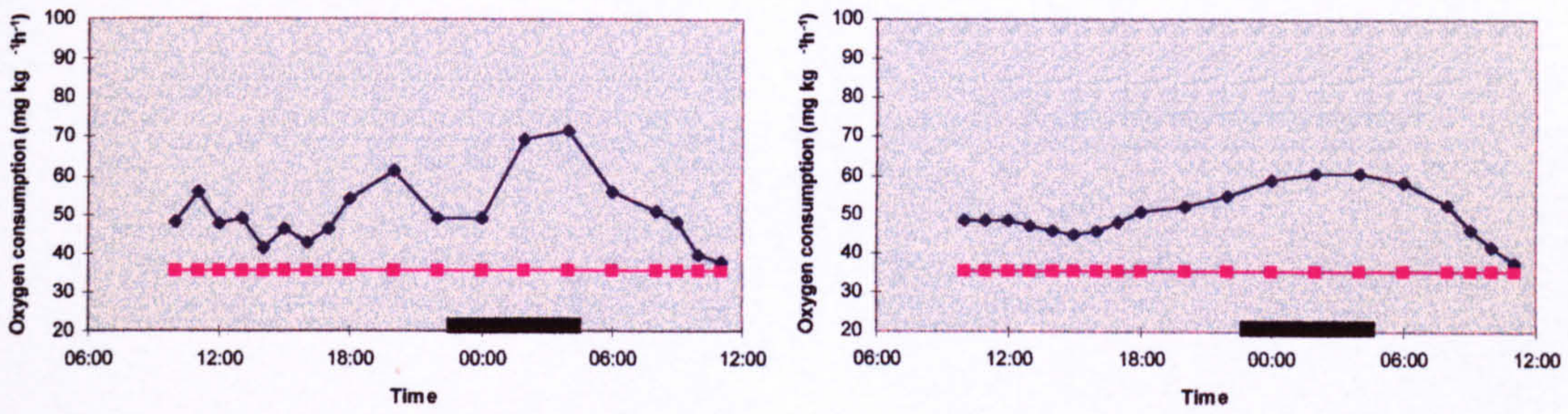


Fig.4.3. Variation in routine oxygen consumption rate in tank populations of *Hippoglossus hippoglossus* at 8°C, examined over a 24 hour period under ambient photoperiod. Raw data graphs are presented on the left hand side, and smoothed data graphs on the right hand side. Trials were: (a) tank of 10 fish of average weight 1137g; (b) tank of 9 fish of average weight 587g; (c) tank of 11 fish of average weight 744g; (d) tank of 10 fish of 677g average weight. The black bar represents the period of darkness. The horizontal line represents the calculated resting oxygen consumption rate for an individual Atlantic halibut of identical weight at 8°C, produced from the global equation in Chapter 3.

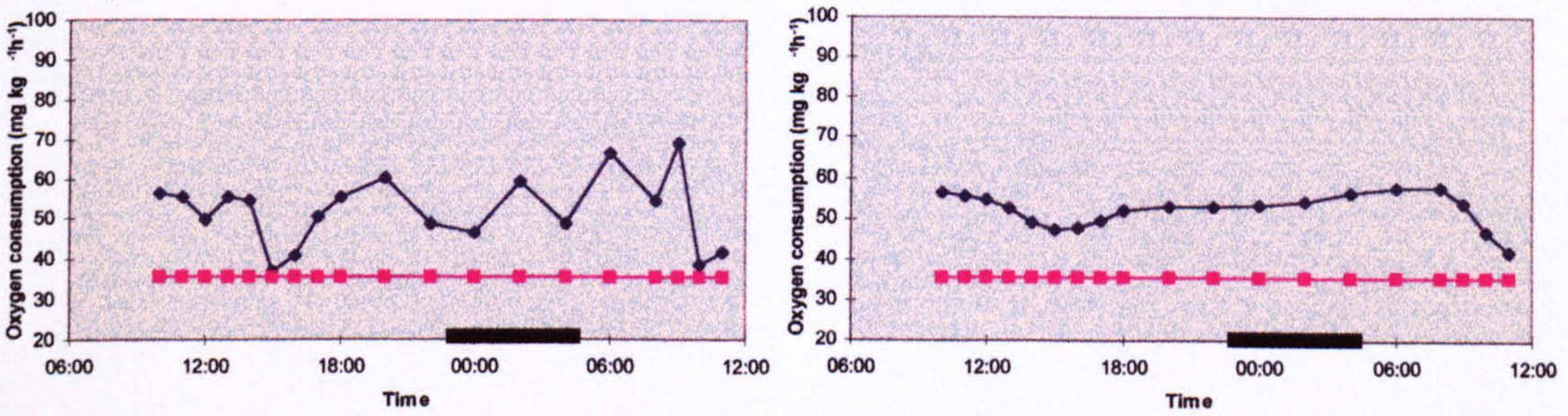
(a)



(b)



(c)



(d)

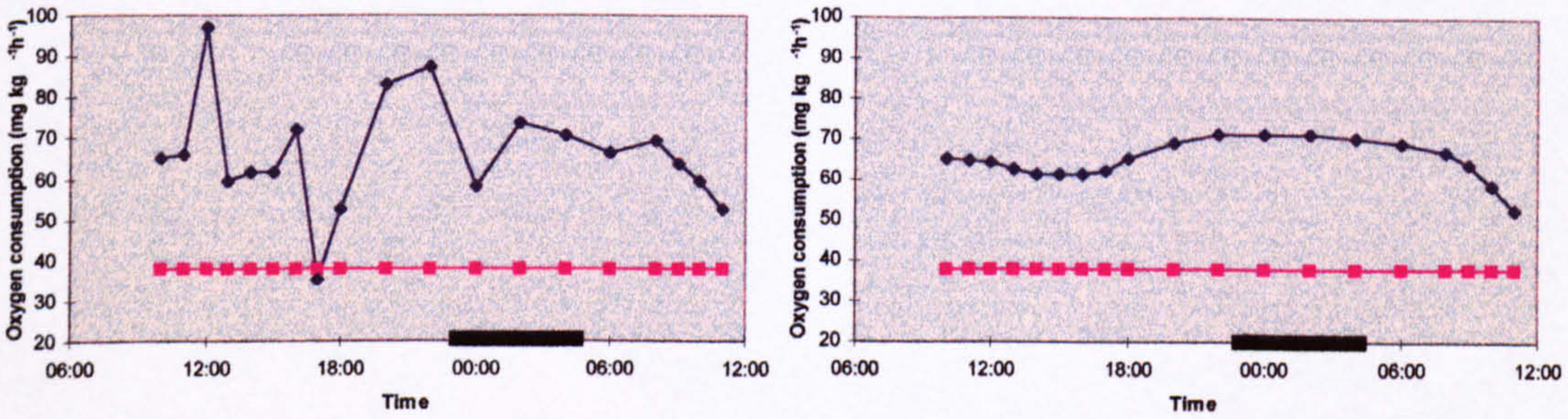


Fig.4.4. Variation in routine oxygen consumption rate in tank populations of *Hippoglossus hippoglossus* at 10°C, examined over a 24 hour period under ambient photoperiod. Raw data graphs are presented on the left hand side, and smoothed data graphs on the right hand side. Trials were: (a) tank of 11 fish of average weight 433g; (b) tank of 9 fish of average weight 703g; (c) tank of 6 fish of average weight 680g; (d) tank of 6 fish of 546g average weight. The black bar represents the period of darkness. The horizontal line represents the calculated resting oxygen consumption rate for an individual Atlantic halibut of identical body weight at 10°C, produced from the global equation in Chapter 3.

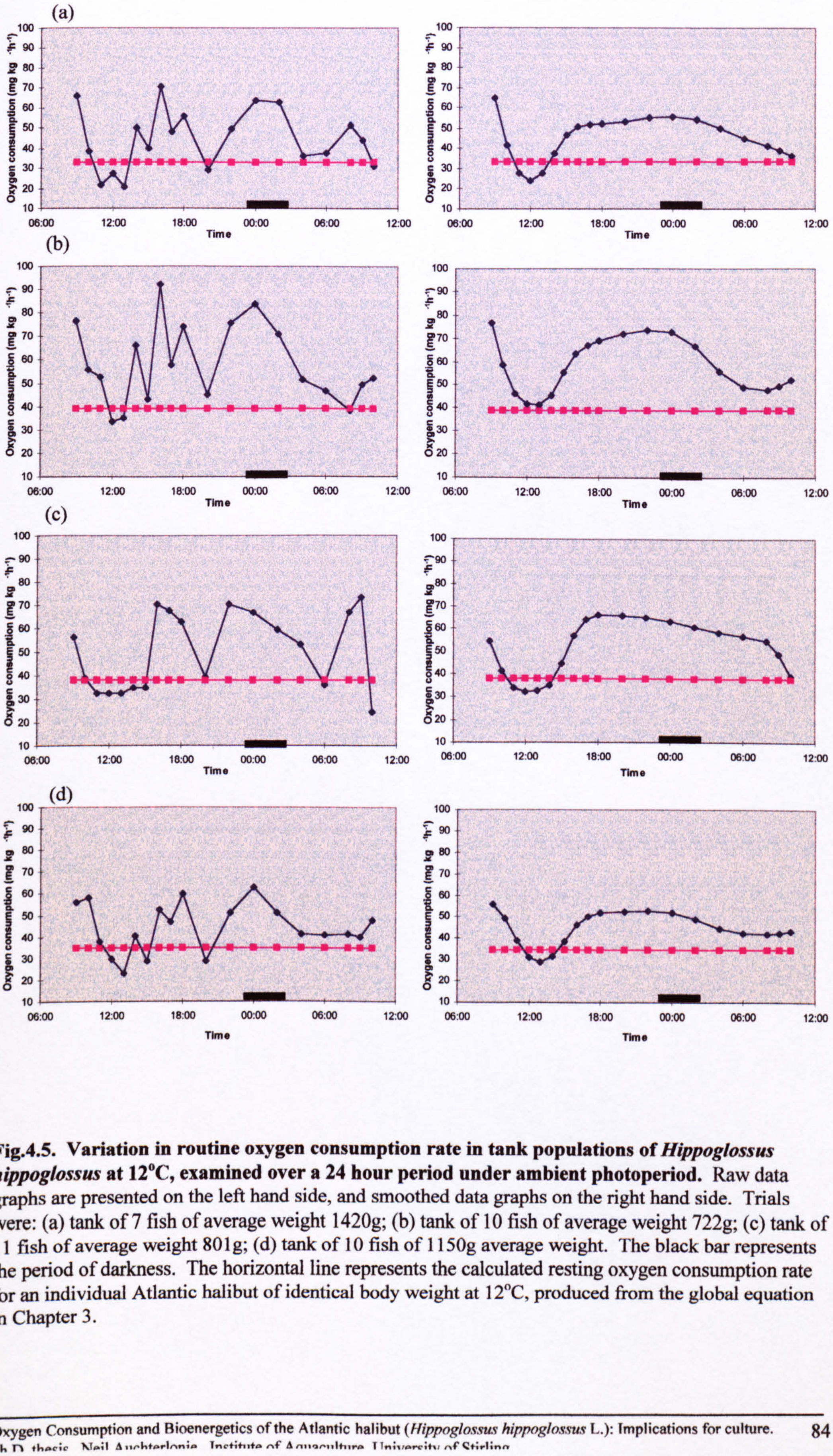


Fig.4.5. Variation in routine oxygen consumption rate in tank populations of *Hippoglossus hippoglossus* at 12°C, examined over a 24 hour period under ambient photoperiod. Raw data graphs are presented on the left hand side, and smoothed data graphs on the right hand side. Trials were: (a) tank of 7 fish of average weight 1420g; (b) tank of 10 fish of average weight 722g; (c) tank of 11 fish of average weight 801g; (d) tank of 10 fish of 1150g average weight. The black bar represents the period of darkness. The horizontal line represents the calculated resting oxygen consumption rate for an individual Atlantic halibut of identical body weight at 12°C, produced from the global equation in Chapter 3.

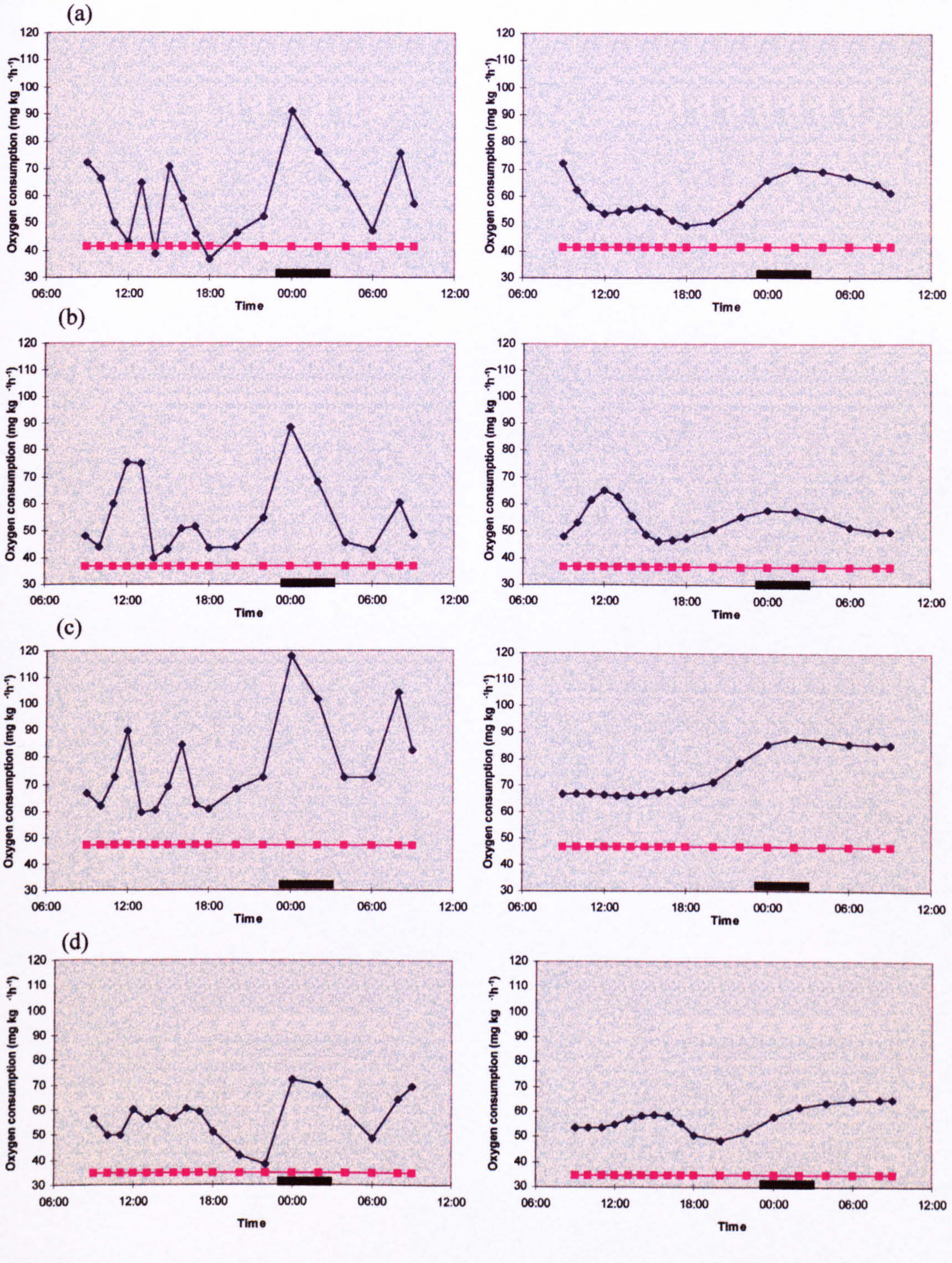


Fig.4.6. Variation in routine oxygen consumption rate in tank populations of *Hippoglossus hippoglossus* at 14°C, examined over a 24 hour period under ambient photoperiod. Raw data graphs are presented on the left hand side, and smoothed data graphs on the right hand side. Trials were: (a) tank of 11 fish of average weight 797g; (b) tank of 10 fish of average weight 1156g; (c) tank of 9 fish of average weight 581g; (d) tank of 7 fish of 1462g average weight. The black bar represents the period of darkness. The horizontal line represents the calculated resting oxygen consumption rate for an individual Atlantic halibut of identical body weight at 14°C, produced from the global equation in Chapter 3.

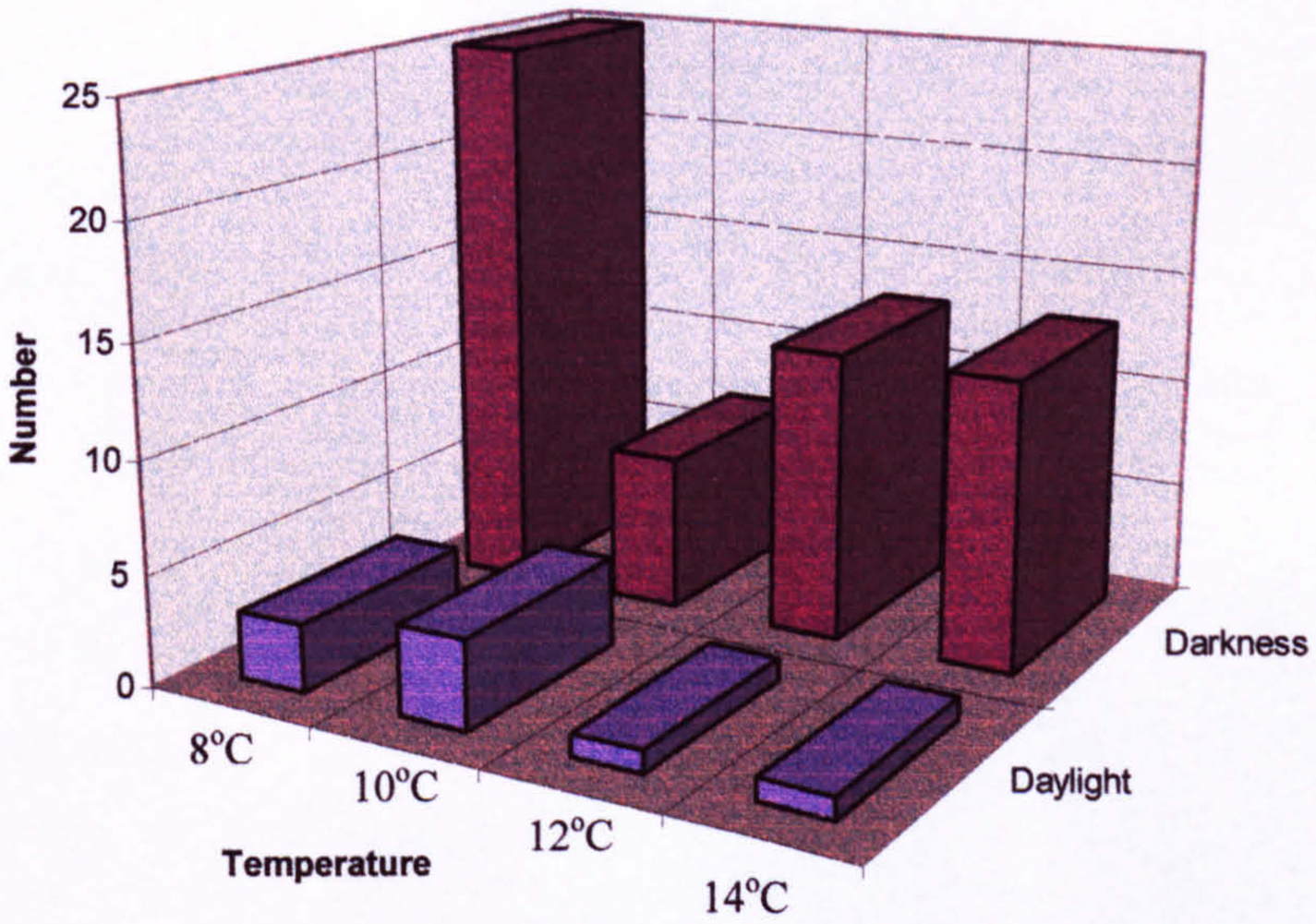


Fig. 4.7. Location of peak routine oxygen consumption rate within the period of daylight or darkness in the Atlantic halibut (*Hippoglossus hippoglossus* L.). Results are from a total of 67 separate 24 hour trials conducted under conditions of ambient photoperiod and temperature. Trial period was 16 June 1996 to 9 November 1997. Temperature varied from 7.7°C to 14.8°C, and results for individual trials were grouped within 2°C bands.

Table 4.3. Distribution of peak routine oxygen consumption rates in daylight and darkness in Atlantic halibut under ambient conditions of temperature and photoperiod.

Temperature (°C)	Fish Size (g)	No. of Trials	Phase of peak oxygen consumption			
			Daylight	% increase	Darkness	% increase
8	382 - 1137	28	3	1.8 (2.3)	25	28.9 (17.8)
10	433 - 1297	11	4	6.6 (7.6)	7	11.8 (6.0)
12	390 - 1420	14	1	2.0 (n/a)	13	18.8 (11.2)
14	315 - 1462	14	1	4.5 (n/a)	13	16.5 (9.3)

Values for percentage increase in oxygen consumption rate are (± standard deviation).

4.3.3. *Effect of body weight on meal size and energy intake.*

Over the entire test temperature range, there was an inverse relationship between meal size (percentage of body weight) and fish wet weight following a standard starvation period which was found to be statistically significant ($r^2 = 0.32$; d.f. = 36; $p < 0.01$), (Fig. 4.8a.). Energy intake was determined for each individual fish biomass in kJ g^{-1} (wet weight of fish) from the values for meal size and feed gross energy. Calculations of both meal size and energy intake assume that the feed intake rate is similar for all individuals within the single tank population, i.e. that no hierarchical behaviour resulted in the uneven distribution of feed amongst individuals of any test group. Further assumptions that there was no later regurgitation of food, and that all uneaten pellets were collected, are also made. A significant relationship ($r^2 = 0.42$; d.f. = 36; $p < 0.01$) existed between energy intake (kJ g^{-1} wet weight) and fish weight and this is presented graphically in (Fig. 4.8b.).

Multiple regression confirmed the significance of the relationships between meal size and fish weight with temperature over the test range 8°C to 14°C ($F = 13.40$, d.f. = 35, $p < 0.01$), and energy intake and fish weight with temperature over the test range 8°C to 14°C ($F = 14.43$, d.f. = 35, $p < 0.01$). These relationships are presented graphically in Fig. 4.9.(a, b), and may be represented by the following global equations:

$$Y = 1.12 - 0.0276\,T - 0.000401\,X$$ where Y is the meal size as a percentage of body weight, X is the fish weight (g), and T is the temperature (°C),

and

$$Y = 2.41 - 0.0375\,T - 0.00116\,X$$ where Y is the energy intake (J g^{-1} wet weight), X is the fish weight (g), and T is the temperature (°C).

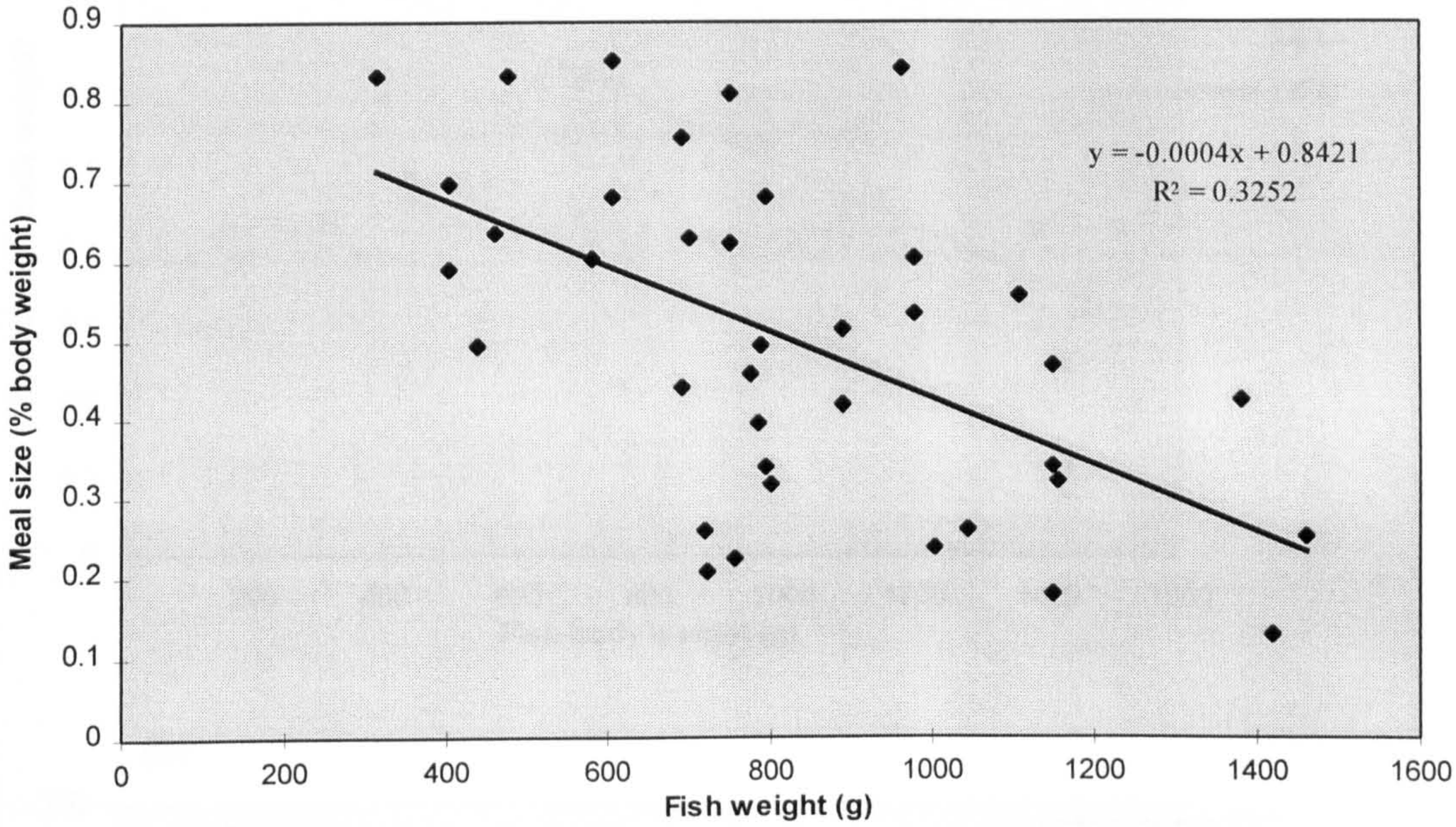
The equations describing the relationship between meal size and energy intake with body weight at the individual test temperatures 8°C, 10°C, 12°C and 14°C are presented in Table 4.4. The results show a high degree of variability in the data

produced at 10°C, and a correspondingly poor regression fit. The results for the other temperatures indicate maximum feed and energy intakes at 8°C, followed by 14°C and 12°C. Generally, there was no clear pattern of feed and energy intake with changing temperature over the test range, however there appeared to be a greater feed and energy intake at the lower temperatures of 8°C and 10°C.

Table 4.4. Regression equations describing the relationship between meal size and energy intake with temperature, for the test temperatures 8°C, 10°C, 12°C and 14°C.

Temperature (°C)	Meal size (% body weight)	Energy intake (J g^{-1} wet weight)
8	*Y = 0.894 - 0.000384 W* (r^2 = 0.36, d.f. = 13, p = 0.023)	*Y = 2.11 - 0.00118 W* (r^2 = 0.50, d.f. = 13, p = 0.005)
10	*Y = 0.545 + 0.000002 W* (r^2 = 0, d.f. = 6, p = 0.995)	*Y = 1.49 - 0.000333 W* (r^2 = 0.004, d.f. = 6, p = 0.669)
12	*Y = 0.730 - 0.000466 W* (r^2 =0.67, d.f. = 6, p = 0.023)	*Y = 1.72 - 0.00115 W* (r^2 = 0.71, d.f. = 6, p = 0.019)
14	*Y = 0.896 - 0.000541 W* (r^2 = 0.66, d.f. = 9, p = 0.05)	*Y = 2.27 - 0.00155 W* (r^2 = 0.64, d.f. = 9, p = 0.006)

(a)



(b)

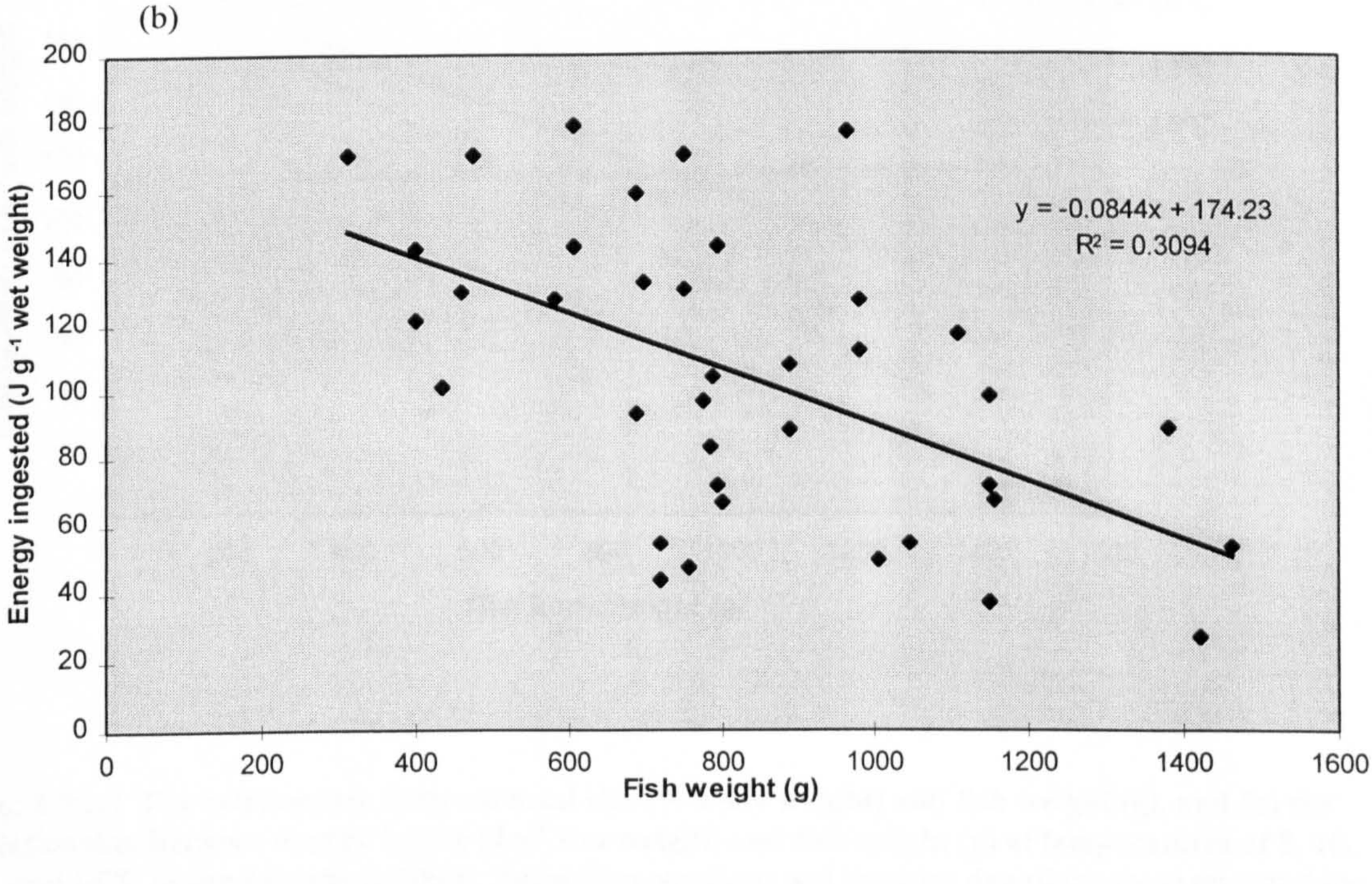
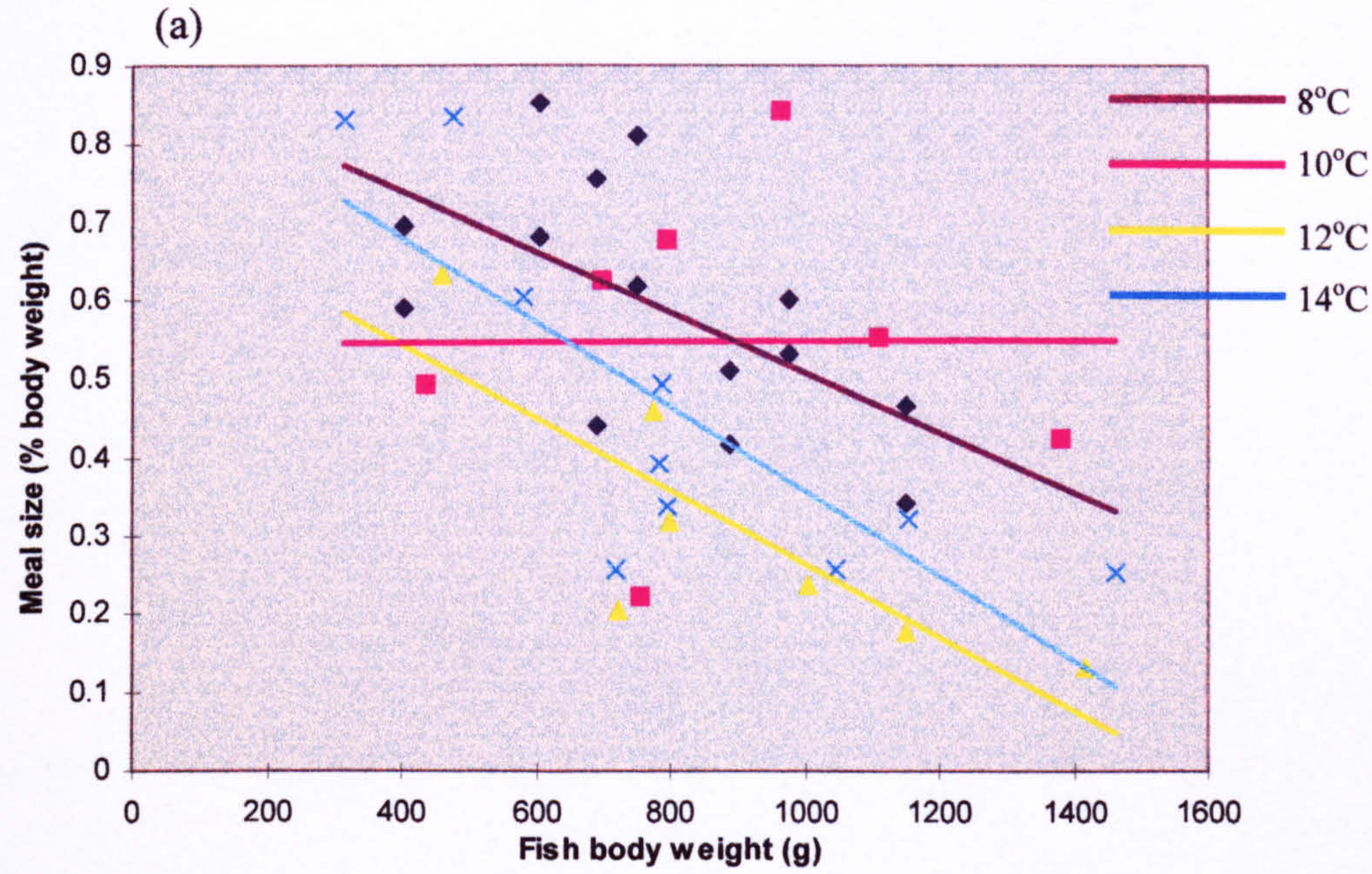


Fig. 4.8. Relationship between meal size and body weight (a), and Energy ingested (kJ g^{-1} wet weight) and body weight (b) in the Atlantic halibut, *Hippoglossus hippoglossus* (L.) for test temperatures of 8°C to 14°C.



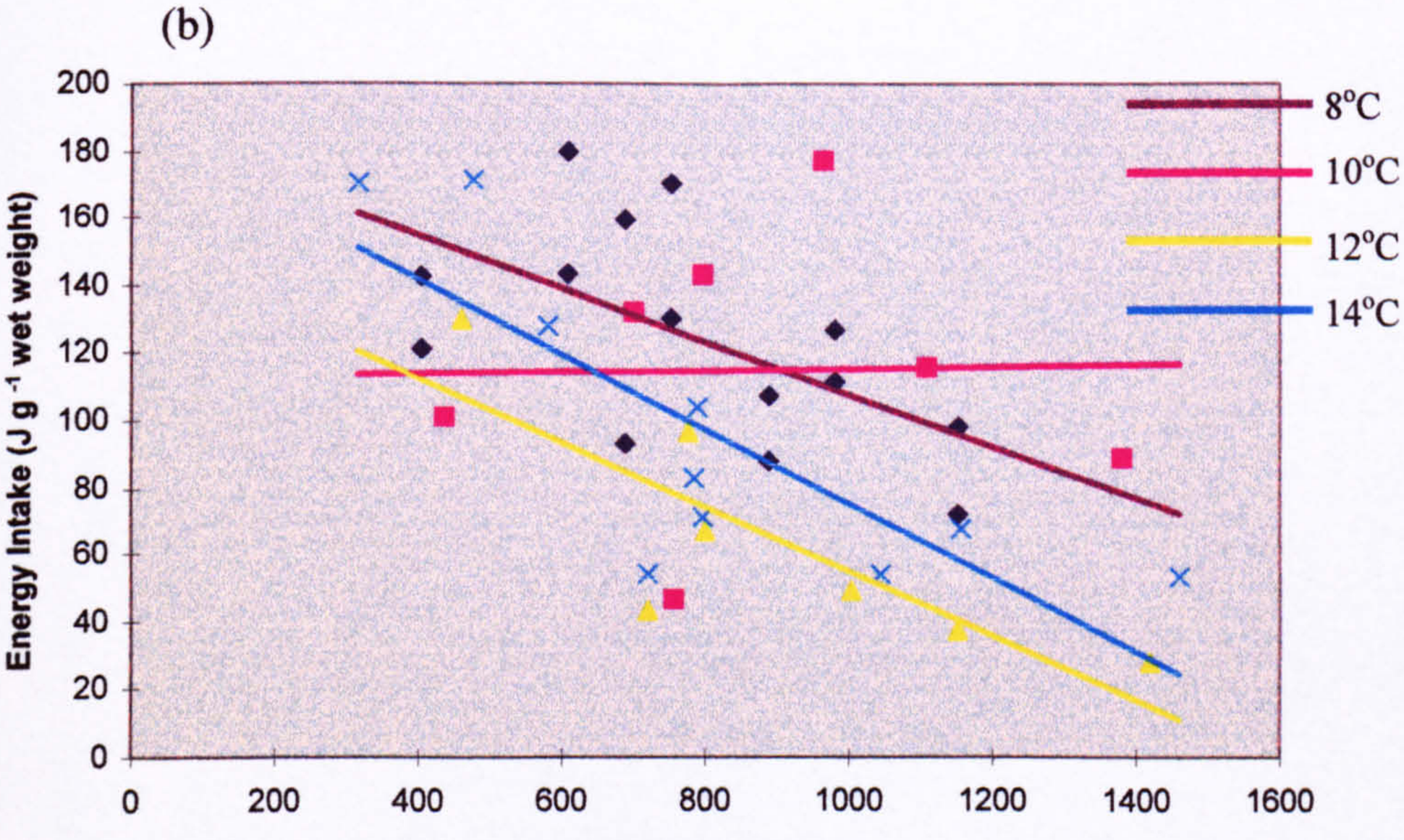


Fig. 4.9.(a) The relationship between meal size (% body weight) and fish weight (g), and (b) the relationship between energy intake (J g^{-1} wet weight) and fish weight (g) at temperatures of 8, 10, 12 and 14°C in the Atlantic halibut. Regression equations and statistics describing these relationships are presented in Table 4.5.

Table 4.5. Regression statistics describing the relationship between meal size (% body weight) and energy ingested (J g^{-1} wet weight) with fish weight (g) at temperatures of 8, 10, 12 and 14°C.

Relationship	Temperature (°C)	Equation	r^2	d.f.	p value
Meal size and fish weight	8	Y = 0.894 -0.000384 X	0.36	12	0.023
	10	Y = 0.545 +0.000002 X	0	5	0.995
	12	Y = 0.730 -0.000466 X	0.67	5	0.023
	14	Y = 0.890 -0.000536 X	0.64	7	0.010
nergy ingested and fish weight	8	Z = 185 - 0.0772 X	0.34	12	0.031
	10	Z = 112 + 0.0031 X	0.10	5	0.961
	12	Z = 151 - 0.0955 X	0.67	5	0.024
	14	Z = 183 - 0.108 X	0.66	8	0.005

here, X is fish weight (g), Y is meal size (% body weight) and Z is energy ingested (J g^{-1} wet weight).

A plot of ration size (as percentage of body weight) with temperature is presented in Fig.4.10. Although the results presented in this figure encompass body weights of 315g to 1462g, throughout the studies involving fed fish, there is a clear variation in ration size with temperature. Maximum ration size was observed at the temperatures of 8°C and 10°C, followed by 14°C. Feed intake at 12°C was reduced in comparison to the other test temperatures.

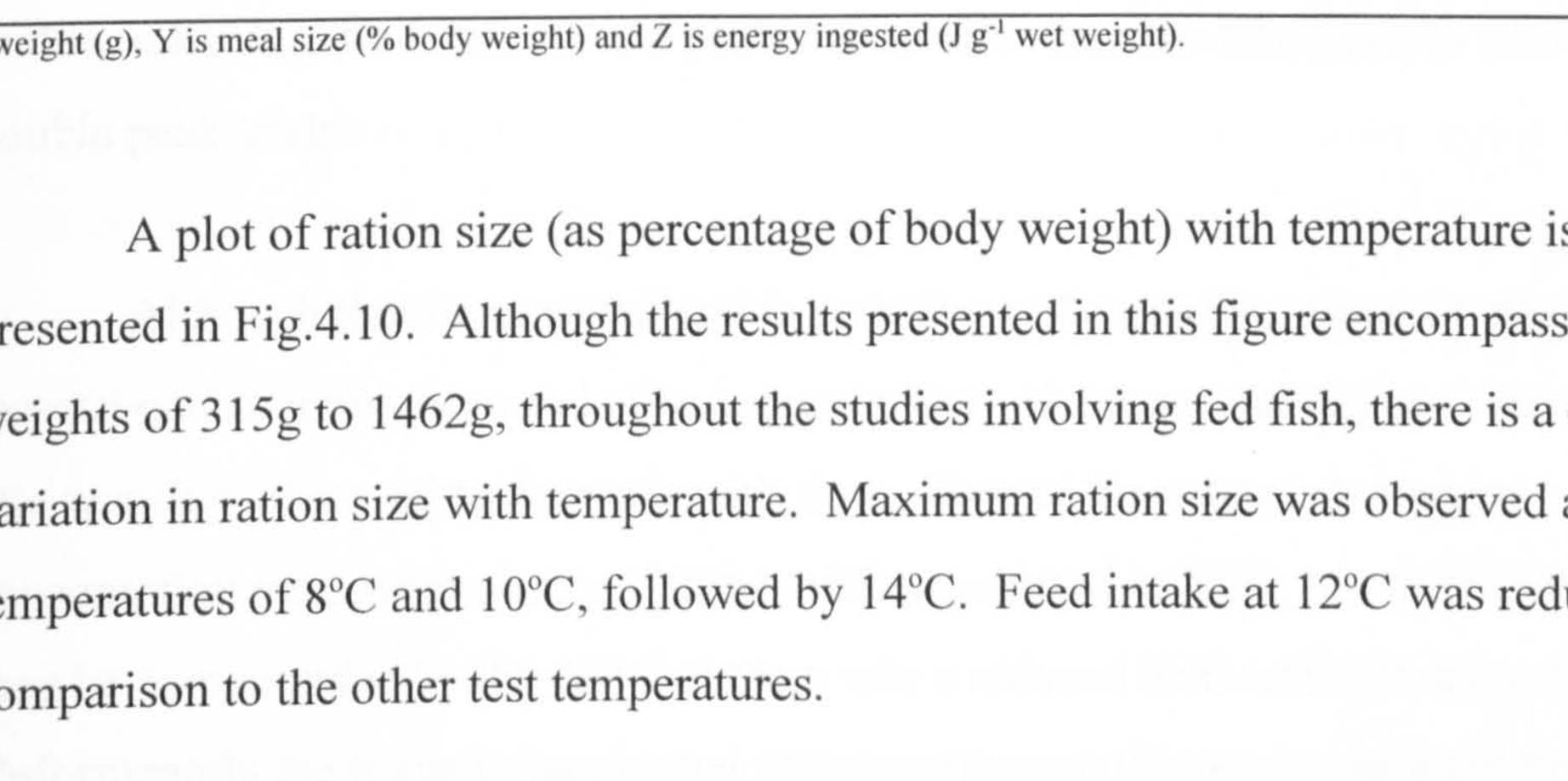


Fig.4.10. Effect of temperature on ration size (percentage body weight) in the Atlantic halibut, *Hippoglossus hippoglossus*.

4.3.4. *Effect of a single meal on post-prandial oxygen consumption rate and Specific Dynamic Action (S.D.A.)*

Fig. 4.11., 4.12., 4.13. and 4.14. show examples of the post-prandial increase in oxygen consumption rate in individual tank trials at 8°C, 10°C, 12°C and 14°C, and the results for all trials are presented in Table 4.6. Oxygen consumption rates exhibited an increase above the routine level following the ingestion of a single meal, steadily increasing to a single or a double peak over the measurement period, then declining in all trials. Double peaks were observed in a total of 19 out of 38 trials, comprising one trial at 8°C, 7 trials at 10°C, 7 trials at 12°C and 4 trials at 14°C, and within individual trials the second peak was smaller than the first peak in 12 of the 19 double peak trials.

Although there was considerable variation in the results, generally peak oxygen consumption occurred after approximately 18 hours, and oxygen consumption rate was then observed to decrease with time. Shorter times to peak oxygen consumption rates were observed in the trials conducted at 12°C, however the effect may be compounded by the fact that there was a reduced feed intake in these trials. Unfortunately the logistics associated with carrying out this work restricted the time period of data recording, and many trials did not reach peak oxygen consumption within the experimental period. Only in a total of 16 of the 38 trials was oxygen consumption rate observed to peak, and then to decline to routine levels. Extrapolation of the post-prandial oxygen consumption curves in a further 18 trials was used to provide values for the length of time taken to decrease to routine rate. Such extrapolation is based on the assumption that there is no change in the rate of decrease of oxygen consumption over the predictive time period. In two trials (459g fish at 12°C and 581g fish at 14°C) post-prandial oxygen consumption rate was still increasing at the end of the trial period, and extrapolation of these results was not possible. The minimum time to regain routine oxygen consumption rate was found to be 24 hours. Mean peak oxygen consumption rate expressed as a percentage of the routine rate was 67.6 (± 24.5)%. The magnitude of the S.D.A. effect was quantified in 36 of the 38 trials in which there was an observed or a predicted reduction of oxygen

consumption rate to routine levels following feeding. S.D.A. coefficients (energy cost of S.D.A. (J)/energy intake (J), expressed as a percentage) were determined following recalculation of the values for oxygen consumption as energy equivalents in Joules (J). The Q_{ox} values applied for conversion of the oxygen consumption data were: the value of 13.56 J mg^{-1} O_2 was applied to starved fish, as the value of Brett and Groves (1979) for fish metabolising there own tissues; for fed fish Q_{ox} values were determined from the composition of feed administered from figures of 13.6 for protein, 13.73 for lipid and 14.78 for carbohydrate respectively (Elliott and Davison, 1975). The results are summarised in Table 4.7. The relationships between meal size (and energy ingested) with S.D.A. magnitude, S.D.A. duration, S.D.A. coefficient, peak oxygen consumption value, time to peak oxygen consumption, and the relationships between fish weight and peak oxygen consumption and S.D.A. coefficient were explored for each test temperature. Figs. 4.15., 4.16., 4.17., 4.18., 4.19., 4.20. and 4.21. represent these results graphically, and the regression statistics for these relationships are presented in Table 4.8.

S.D.A. magnitude varied between 193.2 and 1831.6 mg O_2 kg^{-1} fish, with the higher values observed at the highest test temperature of 14°C. No significant correlation between meal size and energy ingested with S.D.A. magnitude was determined at the test temperatures, although there was a trend for slightly increasing S.D.A. magnitude with meal size and energy ingested at the test temperatures of 8°C, 10°C and 12°C (Fig.4.15.). The results for 14°C show a reversal of this trend, with a decrease in S.D.A. magnitude with increasing meal size and quantity of energy ingested, although there is a high degree of variability in the results at this temperature. Generally the lowest values for S.D.A. magnitude were obtained at 8°C and the highest values at 14°C.

The results for S.D.A. duration (Fig. 4.16.) closely followed the pattern of results obtained with magnitude of S.D.A. effect, and again there was no clear correlation between this factor and meal size and quantity of energy ingested. Temperature did not appear to influence the duration of the S.D.A. effect over the test range. The minimum length of time to regain routine oxygen consumption rate was

observed to be 24 hours, with the maximum duration of the S.D.A. effect predicted at 57 hours through extrapolation of the data.

S.D.A. coefficient decreased with increasing meal size and quantity of energy ingested (Fig. 4.17.), although this relationship was only observed to be significant at the $p < 0.05$ level at 14°C (see Table 4.8.). S.D.A. coefficient values tended to increase with increasing temperature. There was a significant variation in the value of the S.D.A. coefficient between 2.1 and 30.0, although the mean value for all temperatures was 9.8.

The results produced at 8°C and 10°C provided no evidence for a relationship between meal size and energy ingested with peak post-prandial oxygen consumption rate. At 12°C and 14°C peak post-prandial oxygen consumption rate was found to increase with increasing meal size and quantity of energy ingested (Fig.4.18.).

The time to peak oxygen consumption rate following ingestion of a single meal showed a high degree of variability in the data points at all temperatures in Fig.4.19. The lowest values were obtained at 12°C, although the significance of these results is unclear, since meal size and energy intake in these trials was lower than at the other temperatures.

The value of peak post-prandial oxygen consumption rate was observed to decline with increasing fish weight, as shown in Fig.4.20. This relationship proved statistically significant at the $p < 0.05$ level at 12°C, and at the $p < 0.01$ level at 8°C and 14°C, and follows the pattern of routine oxygen consumption rate, with values at individual fish weights increasing over the temperature range.

No significant relationship between S.D.A. coefficient and fish weight was determined at any of the test temperatures (Fig.4.21.).

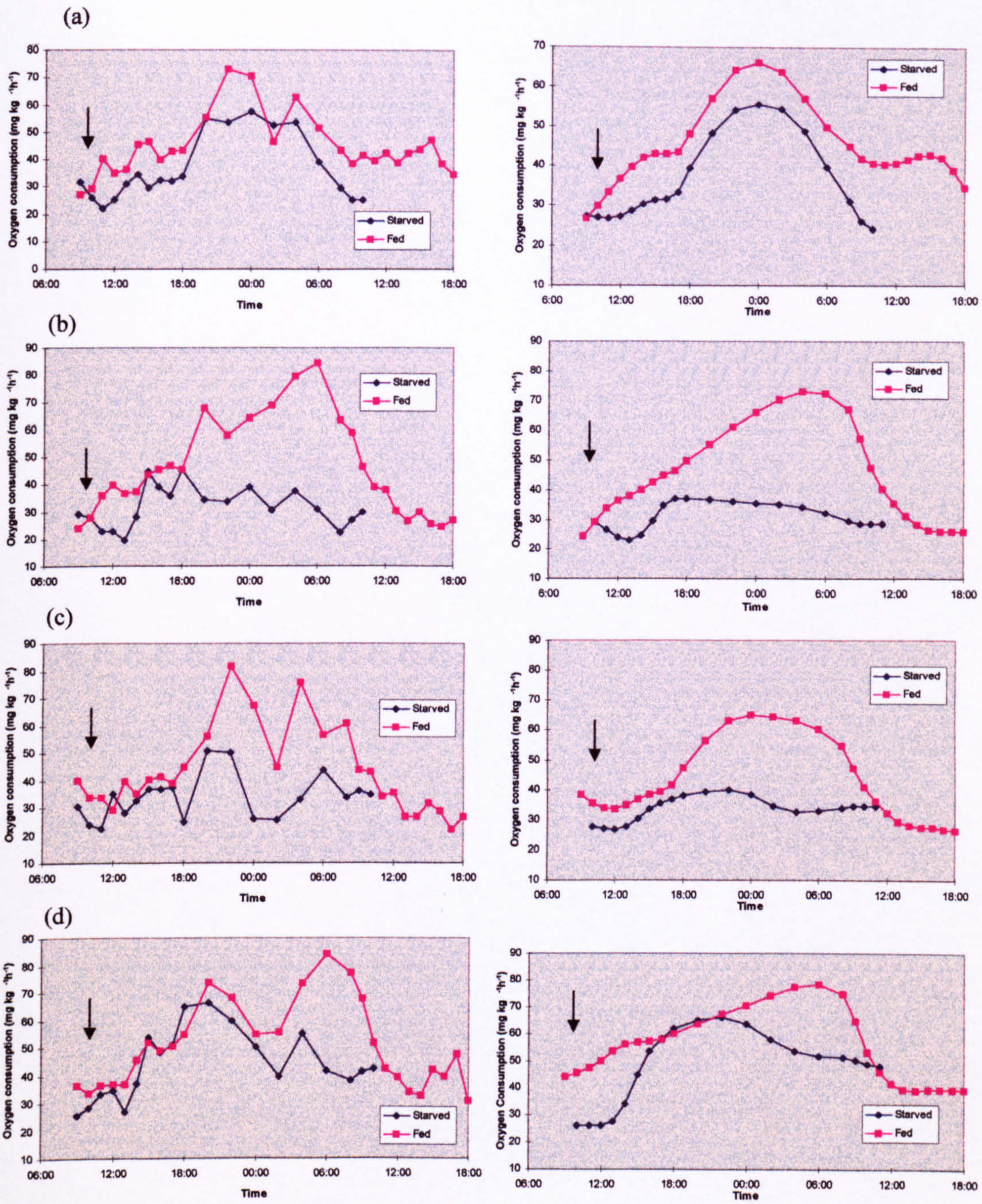


Fig. 4.11. Examples of routine oxygen consumption and post-prandial oxygen consumption in tank populations of *H. hippoglossus* at 8°C. Raw data curves are presented on the left hand side, smoothed data on the right hand side. In the fed trials, the fish were fed at 10.00h, immediately following the first reading. Individual trials are: (a) tank population of 9 fish, average weight 1150g, meal size 0.47% body weight; (b) tank population of 10 fish, average weight 889g, meal size 0.51% body weight; (c) tank population of 6 fish, average weight 979g, meal size 0.53% body weight; (d) tank population of 10 fish, average weight 677g, meal size 0.44% body weight. Vertical black arrows mark the point of feeding.

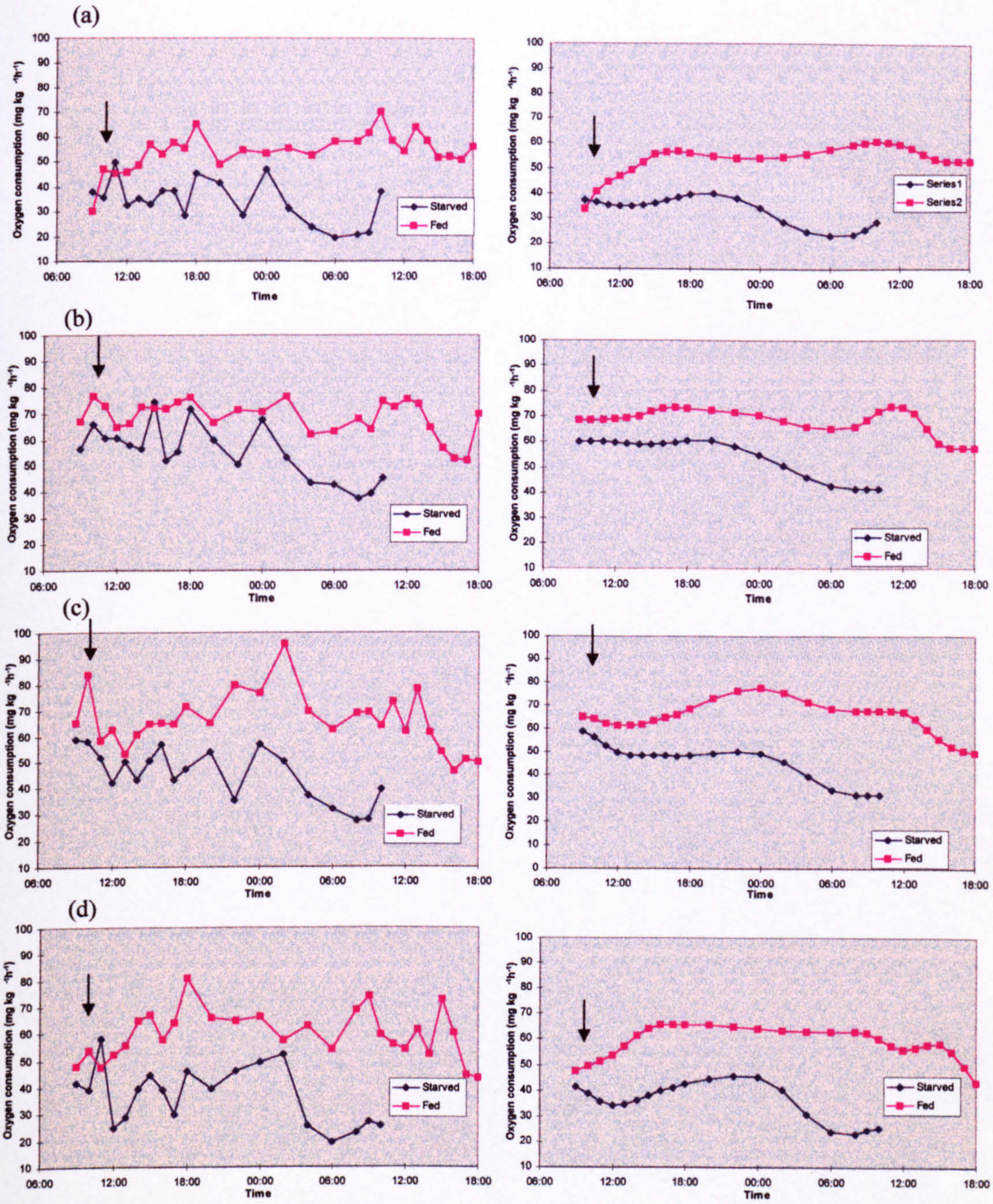


Fig. 4.12. Examples of routine oxygen consumption and post-prandial oxygen consumption in tank populations of *H. hippoglossus* at 10°C. Raw data curves are presented on the left hand side, smoothed data on the right hand side. In the fed trials, the fish were fed at 10.00h, immediately following the first reading. Individual trials are: (a) tank population of 11 fish, average weight 964g, meal size 0.84% body weight; (b) tank population of 11 fish, average weight 436g, meal size 0.49% body weight; (c) tank population of 11 fish, average weight 700g, meal size 0.63% body weight; (d) tank population of 11 fish, average weight 795g, meal size 0.68% body weight. Vertical black arrows mark the point of feeding.

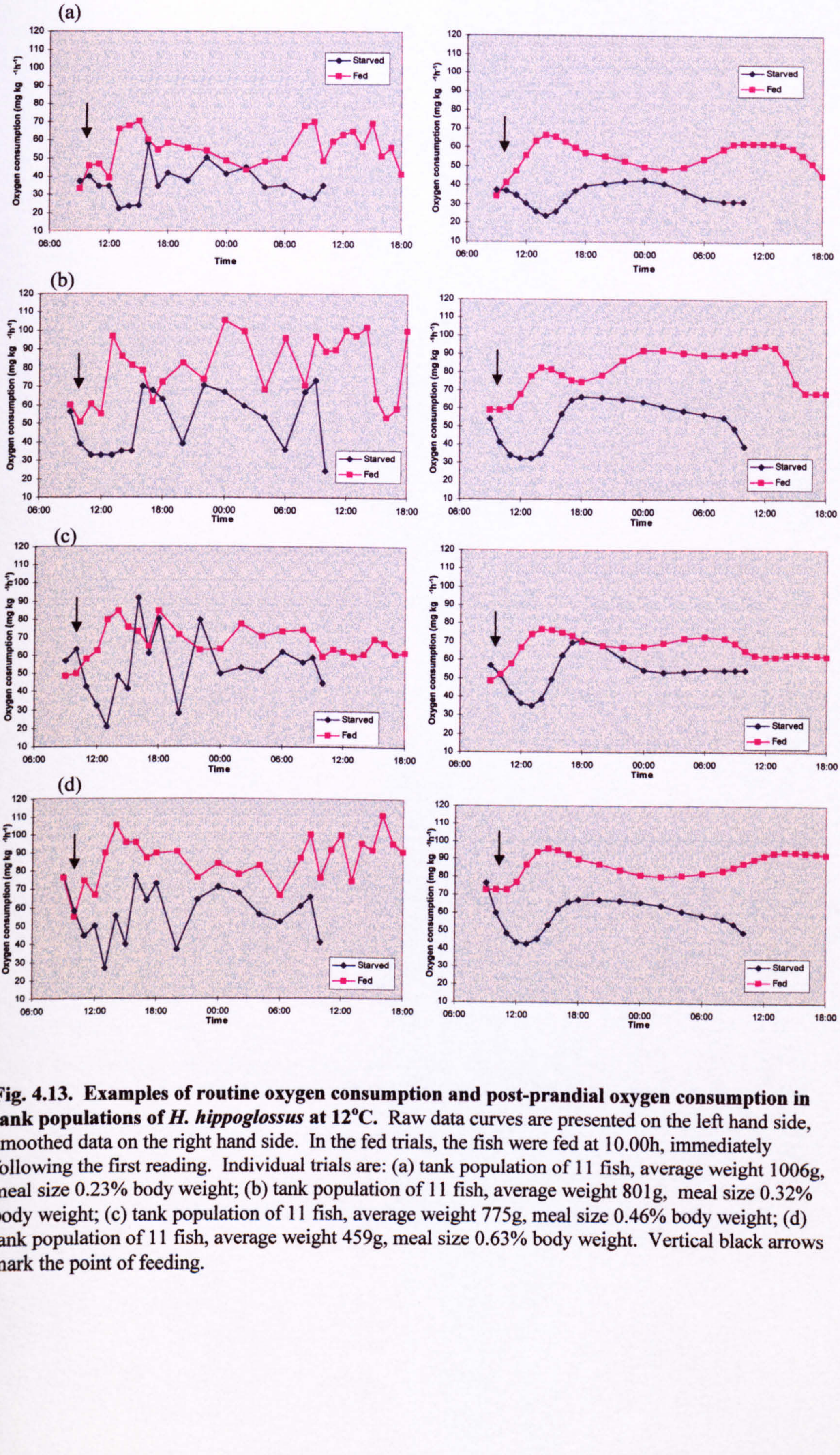


Fig. 4.13. Examples of routine oxygen consumption and post-prandial oxygen consumption in tank populations of *H. hippoglossus* at 12°C. Raw data curves are presented on the left hand side, smoothed data on the right hand side. In the fed trials, the fish were fed at 10.00h, immediately following the first reading. Individual trials are: (a) tank population of 11 fish, average weight 1006g, meal size 0.23% body weight; (b) tank population of 11 fish, average weight 801g, meal size 0.32% body weight; (c) tank population of 11 fish, average weight 775g, meal size 0.46% body weight; (d) tank population of 11 fish, average weight 459g, meal size 0.63% body weight. Vertical black arrows mark the point of feeding.

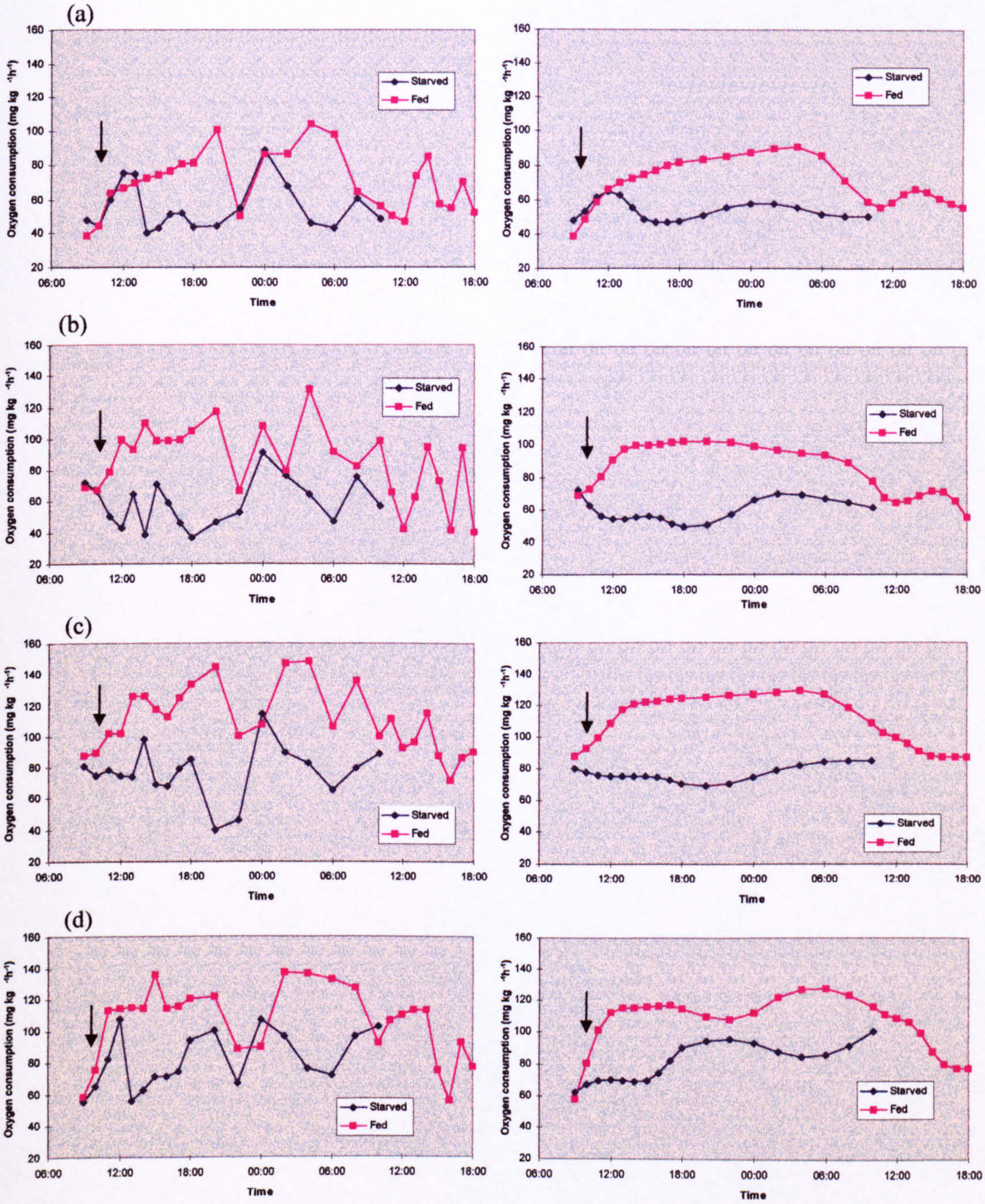


Fig. 4.14. Examples of routine oxygen consumption and post-prandial oxygen consumption in tank populations of *H. hippoglossus* at 14°C. Raw data curves are presented on the left hand side, smoothed data on the right hand side. In the fed trials, the fish were fed at 10.00h, immediately following the first reading. Individual trials are: (a) tank population of 10 fish, average weight 1156g, meal size 0.32% body weight; (b) tank population of 11 fish, average weight 797g, meal size 0.34% body weight; (c) tank population of 11 fish, average weight 786g, meal size 0.39% body weight; (d) tank population of 6 fish, average weight 476g, meal size 0.83% body weight. Vertical black arrows mark the point of feeding.

Table 4.6. Analysis of post-prandial oxygen consumption in tank populations of the Atlantic halibut, *Hippoglossus hippoglossus*.

Temperature (°C)	Weight (g)	n	Meal (% body weight)	Routine Oxygen consumption (calculated)	Post-prandial peak oxygen consumption	Peak oxygen cons. (% of routine rate)	Time of peak (hours from meal)	Time to diminish to Routine Rate (h)
8	1150	9	0.34	35.5	59.4	67.3	18	24
8	979	6	0.53	36.5	64.8	77.4	14	26
8	606	9	0.68	40.8	84.7	107.6	20	36*
8	889	10	0.51	37.3	72.7	95.3	18	28
8	752	11	0.62	38.6	72.8	88.5	20	25
8	690	10	0.44	39.5	78.2	98.1	20	25
8	402	11	0.59	46.5	91.5	96.8	16	24
8	1150	9	0.47	35.5	66.2	86.5	14	31
8	979	6	0.60	36.5	46.5	27.2	28	32
8	606	9	0.85	40.8	72.6	77.8	14	33*
8	889	10	0.42	37.3	62.8	68.6	12	36*
8	752	11	0.81	38.6	69.0	78.6	12	40*
8	690	10	0.75	39.5	68.8	74.5	18	39*
8	402	11	0.70	46.5	73.4	57.8	16	47*
10	1380	7	0.42	38.4	58.5	52.3	29	39*
10	1109	10	0.55	40.6	86.8	113.9	8	57*
10	700	11	0.63	47.0	77.3	64.4	12	33*
10	964	11	0.84	42.3	60.7	43.6	24	41*
10	795	11	0.68	44.9	65.7	46.2	7	32
10	757	11	0.22	45.7	75.5	65.1	30	34*
10	436	11	0.49	57.6	73.8	28.1	25	29
12	1420	7	0.13	42.0	62.8	49.4	4	42*
12	1150	10	0.18	44.9	60.4	34.6	4	25
12	722	10	0.21	54.0	71.9	33.2	4	27
12	1006	11	0.23	47.1	66.2	40.5	4	32
12	801	11	0.32	51.6	94.6	83.5	26	47*
12	775	11	0.46	52.3	76.5	46.3	4	39*
12	459	11	0.63	68.0	95.6	40.7	5	‡
14	1462	7	0.25	45.3	84.3	47.3	29	46*
14	1156	10	0.32	49.5	90.4	69.1	18	47*
14	720	11	0.26	61.5	104.1	46.6	18	28
14	1045	11	0.26	51.6	78.7	60.0	18	31
14	788	11	0.49	58.8	94.0	83.1	29	44*
14	797	11	0.34	58.5	102.2	71.8	10	32
14	476	11	0.83	77.9	127.4	57.3	20	30
14	315	14	0.83	102.7	133.0	69.0	4	25
14	786	6	0.39	58.9	129.7	69.6	18	40*
14	581	9	0.60	69.2	128.5	75.9	16	‡

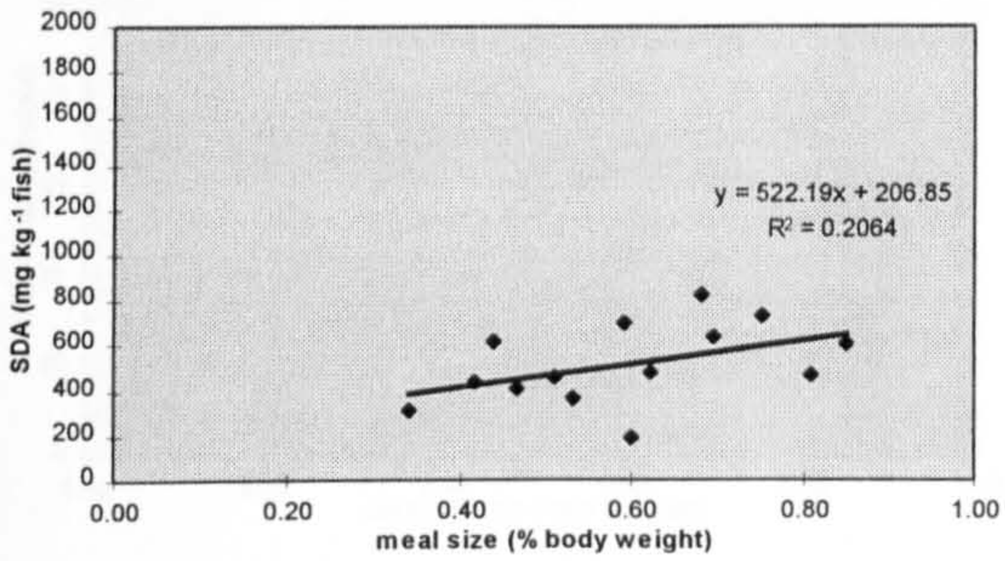
Oxygen consumption rates are expressed as mg kg^{-1} h^{-1}. Routine oxygen consumption rate values are calculated from the equation in 4.3.1. * denotes trials where the time to diminish to routine oxygen consumption rate was predicted from individual post-prandial oxygen consumption curves. ‡ denotes trials where the point at which post-prandial oxygen consumption rate would diminish to routine levels could not be predicted.

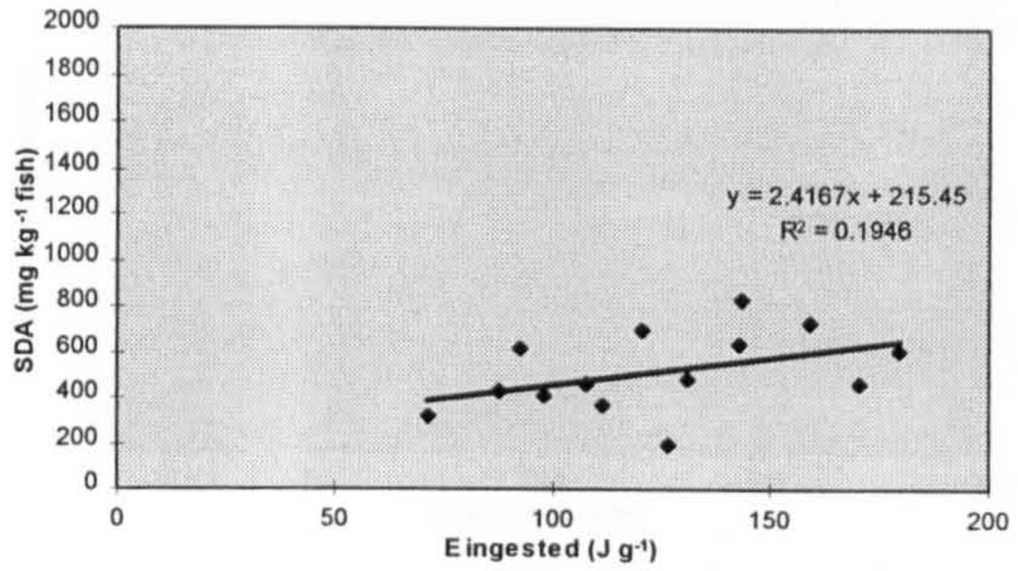
Table 4.7. Components of S.D.A. in the Atlantic halibut, *Hippoglossus hippoglossus*, fed a single meal of commercial dry pelleted diet.

Temperature (°C)	wt. (g)	n	E ingested (kJ)	E ingested (J g^{-1} body wt.)	S.D.A. magnitude (mg O_2 kg^{-1} fish)	S.D.A. magnitude (mg O_2)	S.D.A. magnitude (kJ)	SDA coefficient
8	1150	9	742.5	71.74	315.0	3260.6	44.3	6.0
8	979	6	656.6	111.83	368.4	2162.8	29.4	4.5
8	606	9	782.8	143.48	827.8	4516.4	61.4	7.8
8	889	10	956.4	107.61	461.1	4097.9	55.7	5.8
8	752	11	1081.5	130.82	481.4	3980.0	54.1	5.0
8	690	10	640.4	92.84	612.9	4227.8	57.5	9.0
8	402	11	535.2	120.95	700.9	3101.4	42.2	7.9
8	1150	9	1018.2	98.38	408.0	4222.5	57.4	5.6
8	979	6	744.1	126.74	191.9	1126.7	15.3	2.1
8	606	9	979.0	179.44	601.9	3283.9	44.7	4.6
8	889	10	783.2	88.12	435.6	3871.7	52.7	6.7
8	752	11	1409.8	170.54	463.6	3832.2	52.1	3.7
8	690	10	1096.5	158.96	727.9	5021.0	68.3	6.2
8	402	11	631.4	142.69	635.1	2810.4	38.2	6.1
10	1380	7	861.6	89.17	337.6	3261.7	44.4	5.1
10	1109	10	1292.3	116.49	1540.3	17088.2	232.4	18.0
10	700	11	1018.2	132.29	623.6	4800.1	65.3	6.4
10	964	11	1879.8	177.27	446.6	4735.8	64.4	3.4
10	795	11	1253.2	143.32	498.9	4362.1	59.3	4.7
10	757	11	391.6	47.03	445.5	3709.4	50.4	12.9
10	436	11	483.8	100.94	348.7	1671.3	22.7	4.7
12	1420	7	274.1	27.58	503.9	5008.5	68.1	24.8
12	1150	10	430.8	37.46	202.7	2331.5	31.7	7.4
12	722	10	313.3	43.41	193.2	1394.3	19.0	6.1
12	1006	11	548.3	49.56	292.6	3236.4	44.0	8.0
12	801	11	587.4	66.68	1007.2	8873.4	120.7	20.5
12	775	11	822.4	96.42	523.0	4460.9	60.7	7.4
12	459	11	656.0	129.95	n.d.	n.d.	n.d.	n.d.
14	1462	7	548.3	53.58	999.2	10224.7	139.1	25.4
14	1156	10	783.2	67.73	781.7	9039.6	122.9	15.7
14	720	11	430.8	54.36	826.0	6544.9	89.0	20.7
14	1045	11	626.6	54.50	508.8	5850.2	79.6	12.7
14	788	11	900.7	103.96	744.6	6451.0	87.7	9.7
14	797	11	626.6	71.49	932.6	8174.2	111.2	17.7
14	476	11	893.8	170.77	1008.3	5277.5	71.8	8.0
14	315	14	750.3	170.41	330.5	1455.0	19.8	2.6
14	786	6	391.6	83.08	1831.6	8634.1	117.4	30.0
14	581	9	665.7	127.27	n.d.	n.d.	n.d.	n.d.

n.d. = not determined

(a)





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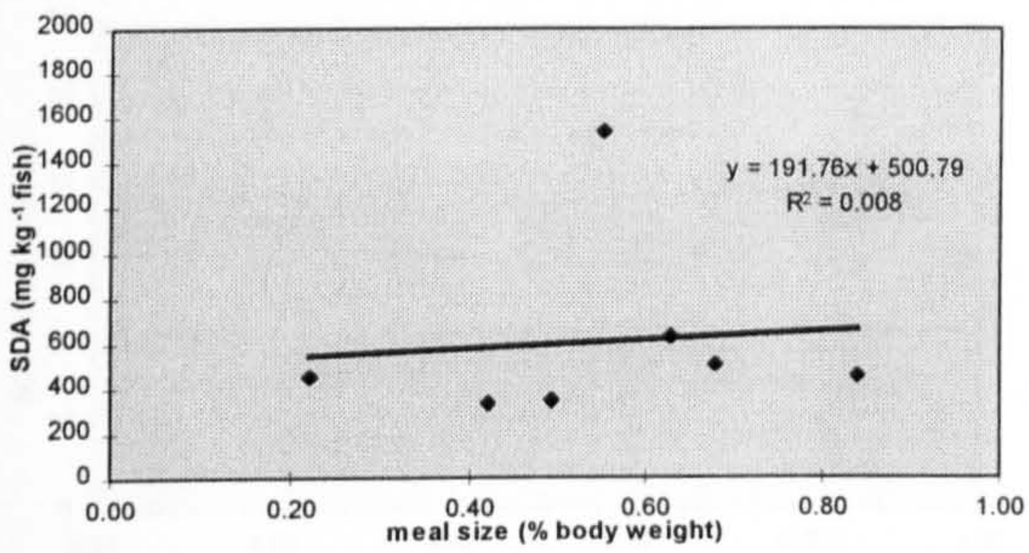

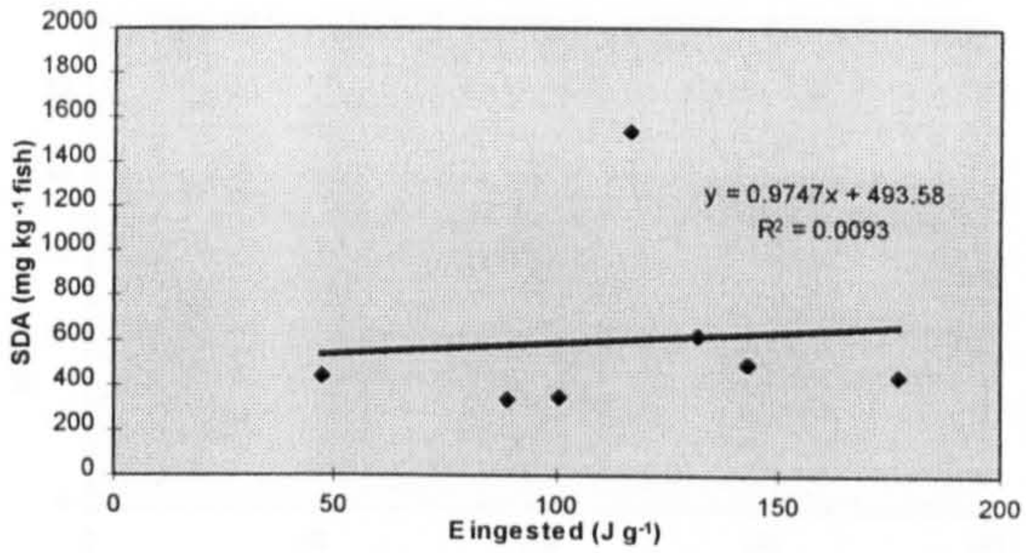

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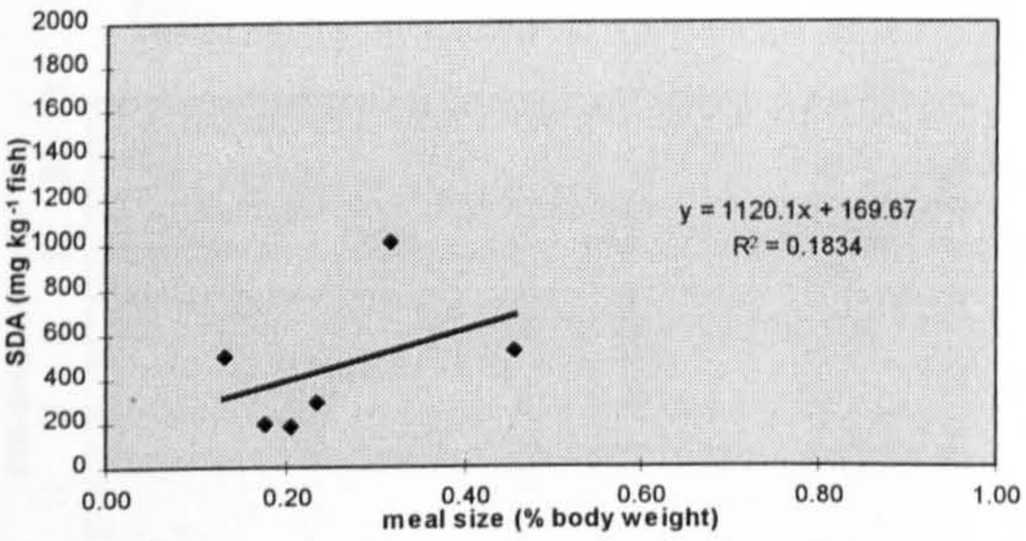

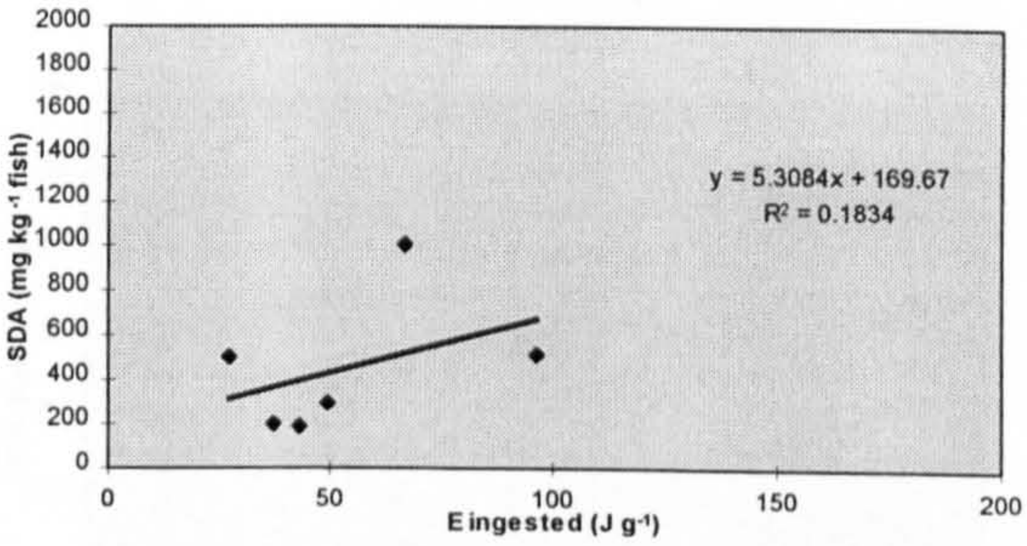

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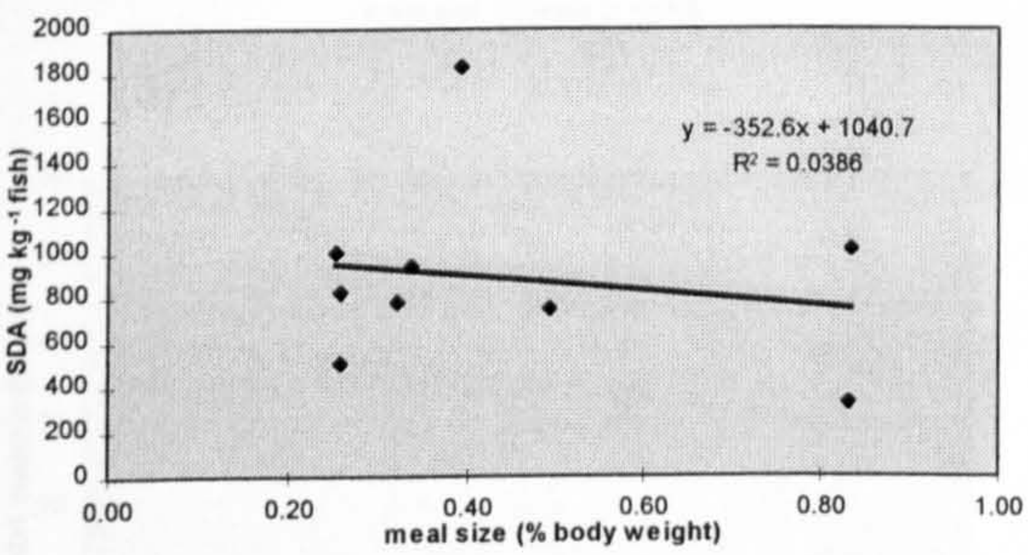


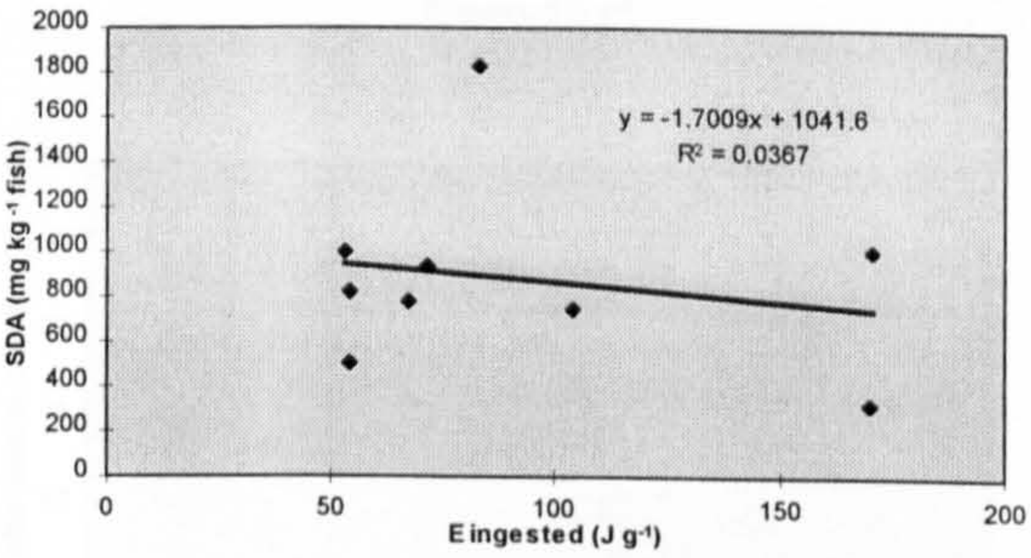
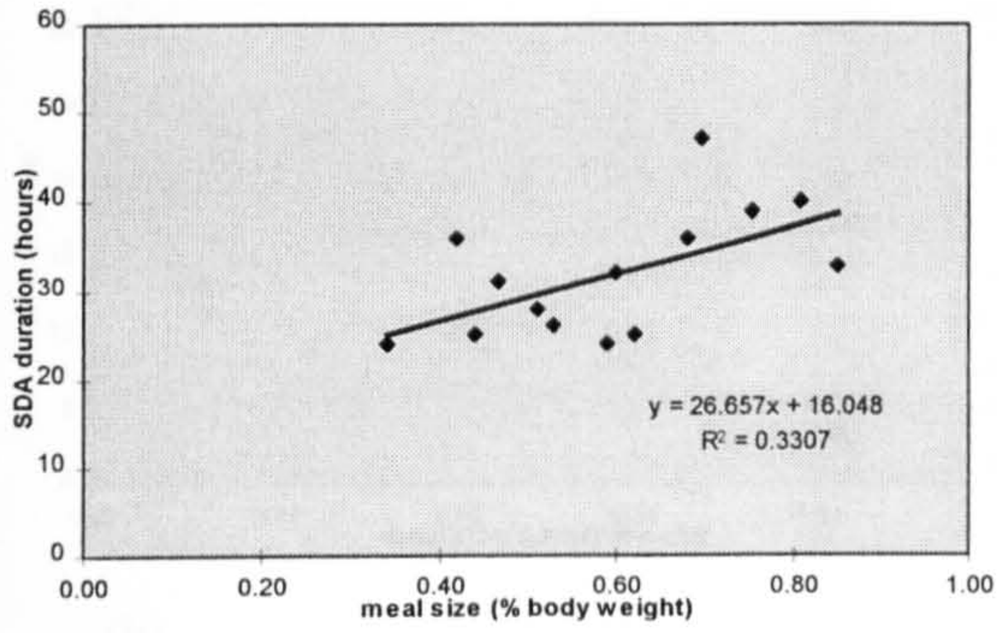
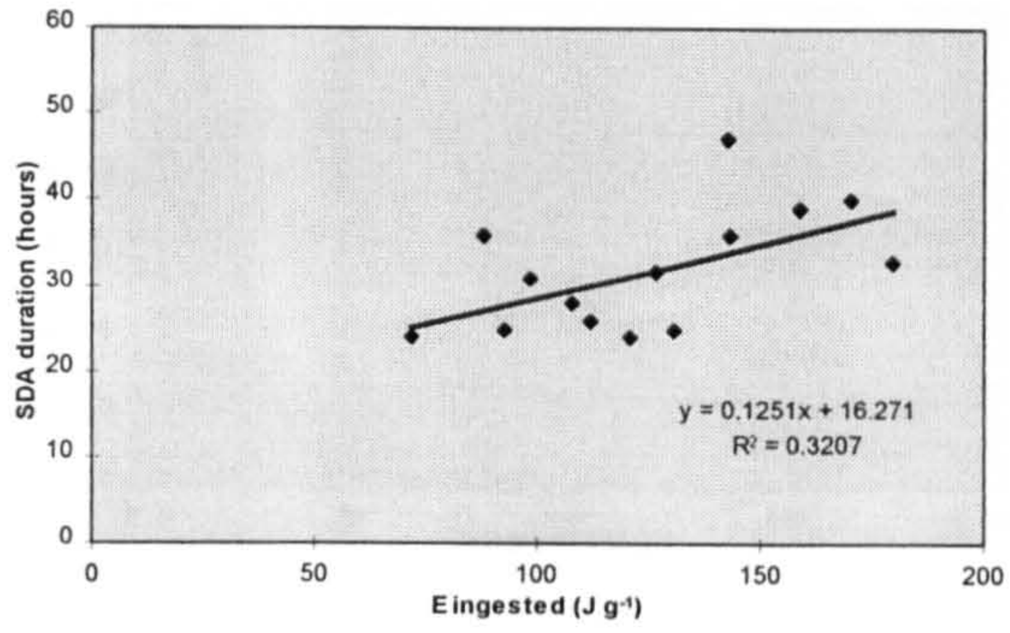


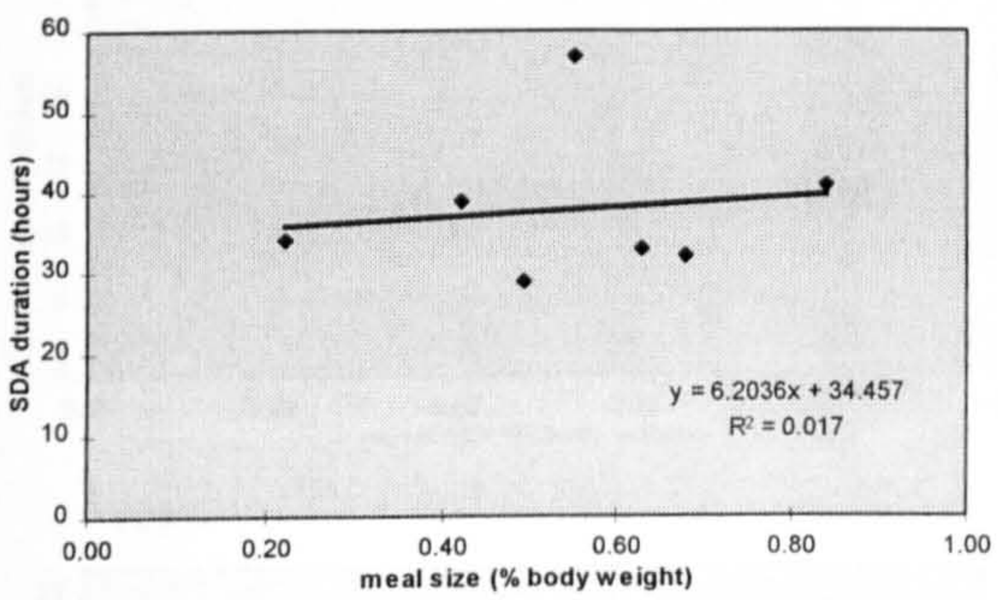
Fig. 4.15. Relationship between meal size and Energy ingested with magnitude of S.D.A. effect in the Atlantic halibut, *Hippoglossus hippoglossus* following the ingestion of a single meal. Diet was a commercial dry pelleted feed. Temperatures are (a) 8°C, (b) 10°C, (c) 12°C and (d) 14°C. Regression equations and r^2 values are printed on individual graphs in the form $Y = a + bX$.

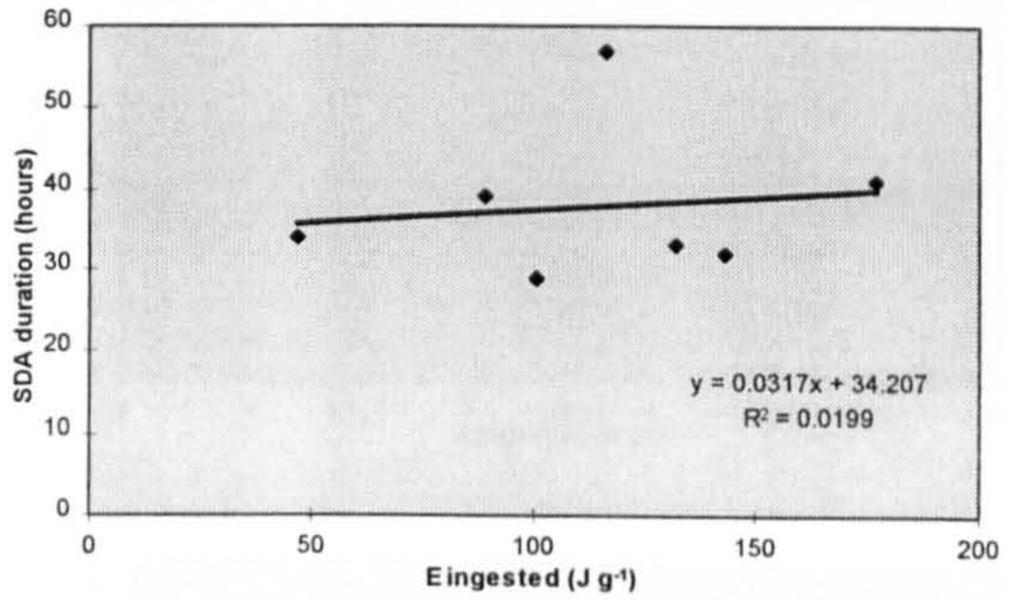
(a)





(b)





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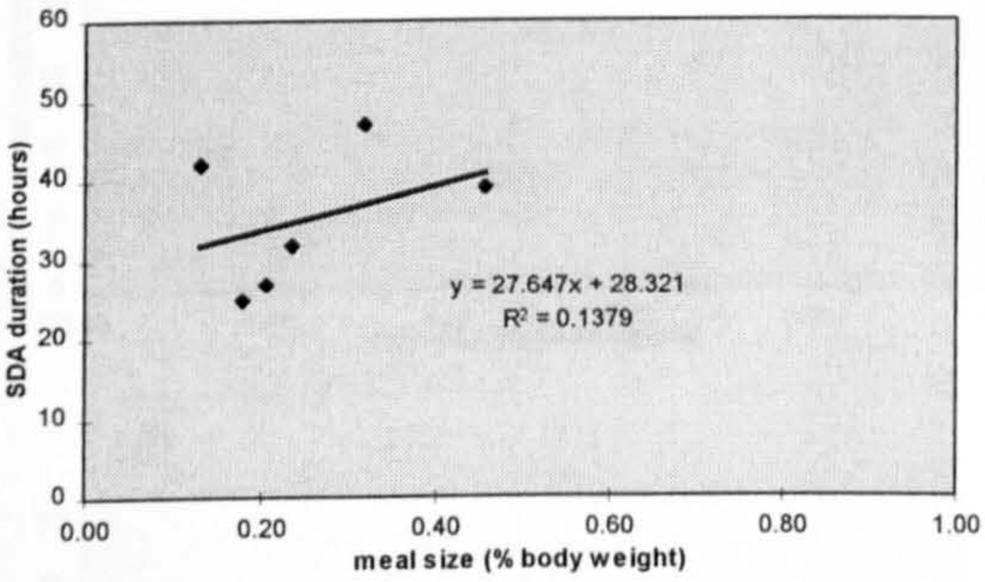
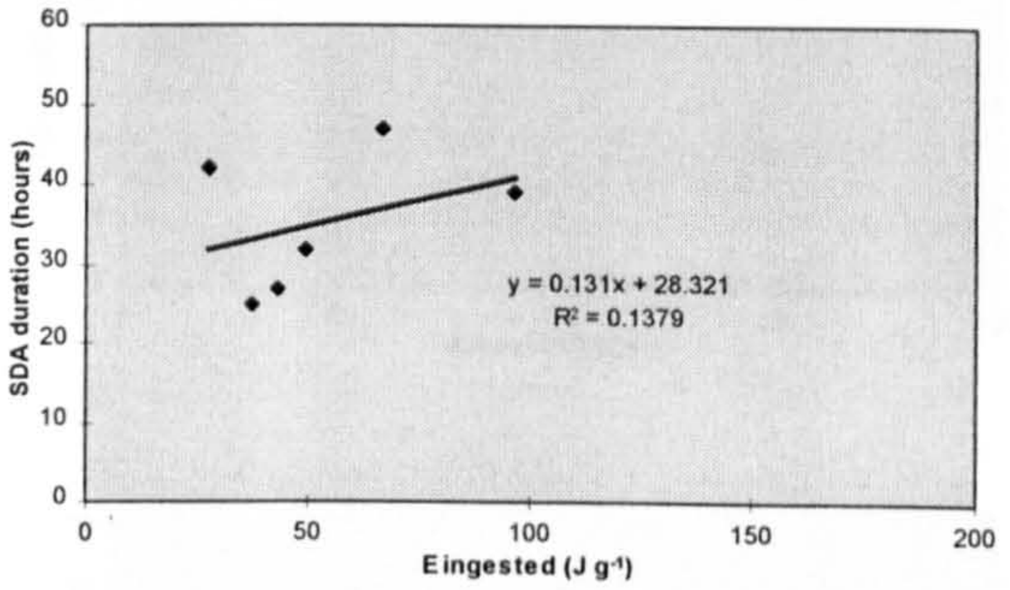
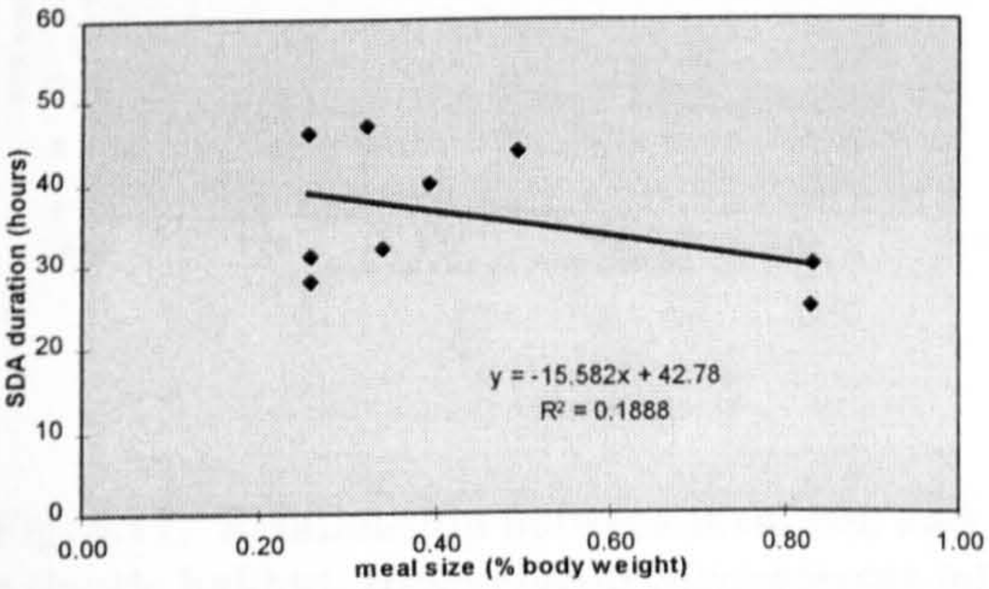




(d)



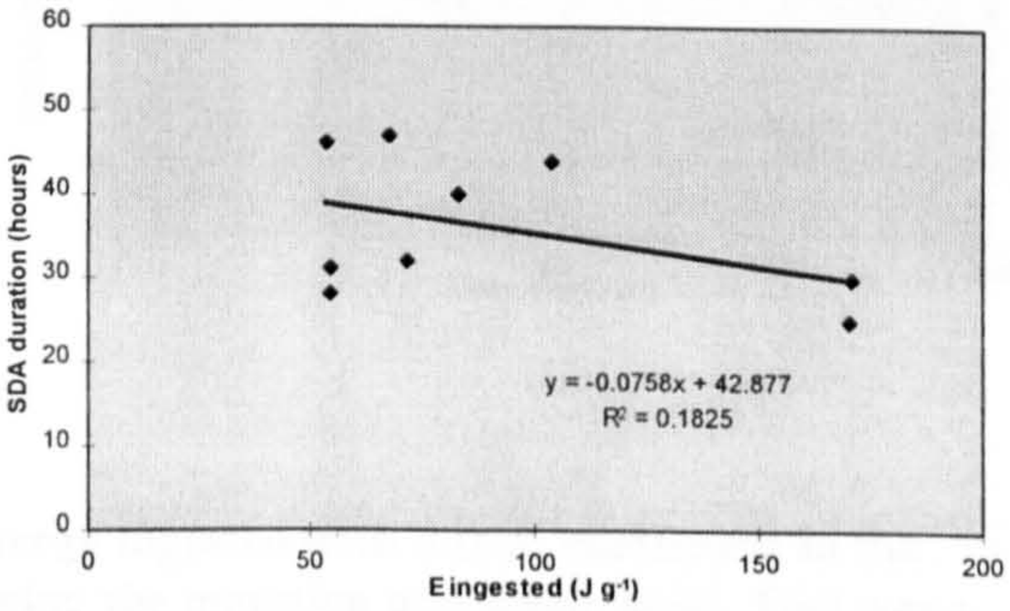
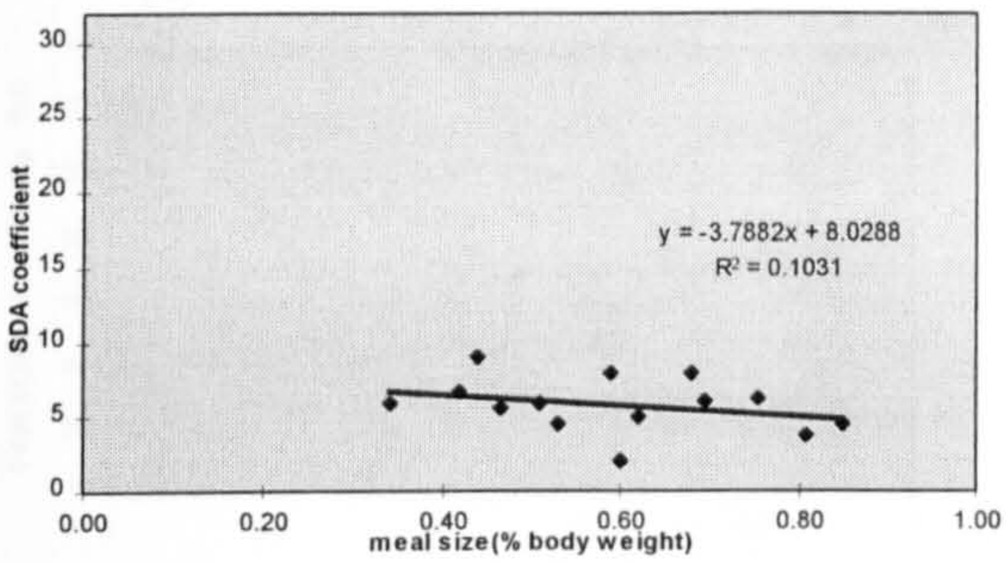
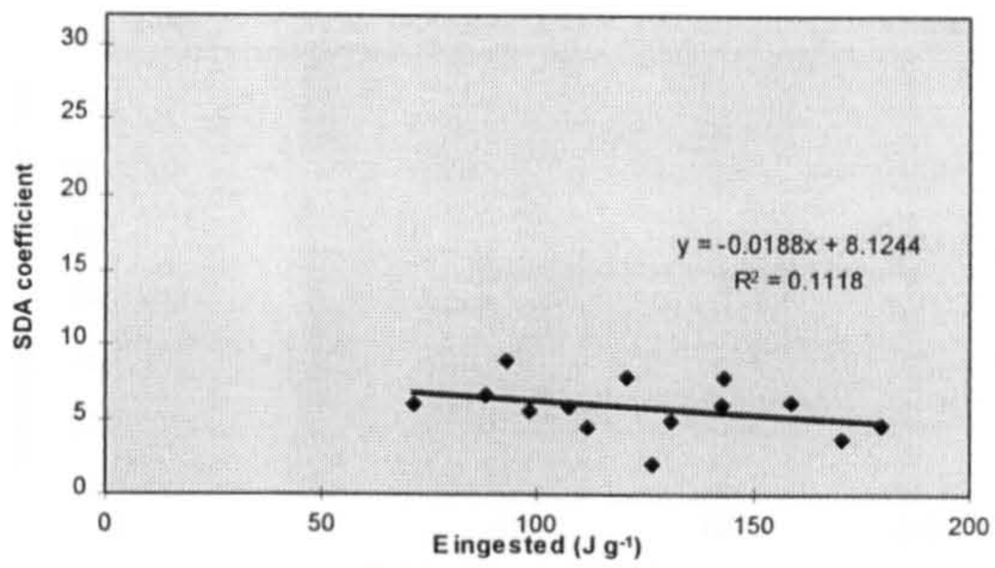


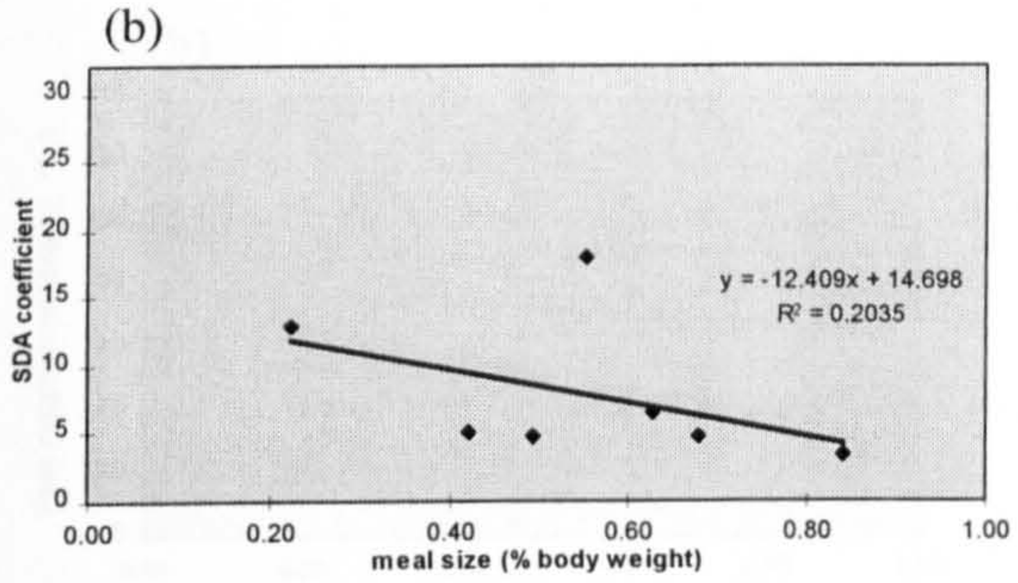
Fig. 4.16. Relationship between meal size and Energy ingested with duration of S.D.A. effect in the Atlantic halibut, *Hippoglossus hippoglossus* following the ingestion of a single meal. Diet was a commercial dry pelleted feed. Temperatures are (a) 8°C, (b) 10°C, (c) 12°C and (d) 14°C. Regression equations and r^2 values are printed on individual graphs in the form $Y = a + bX$.

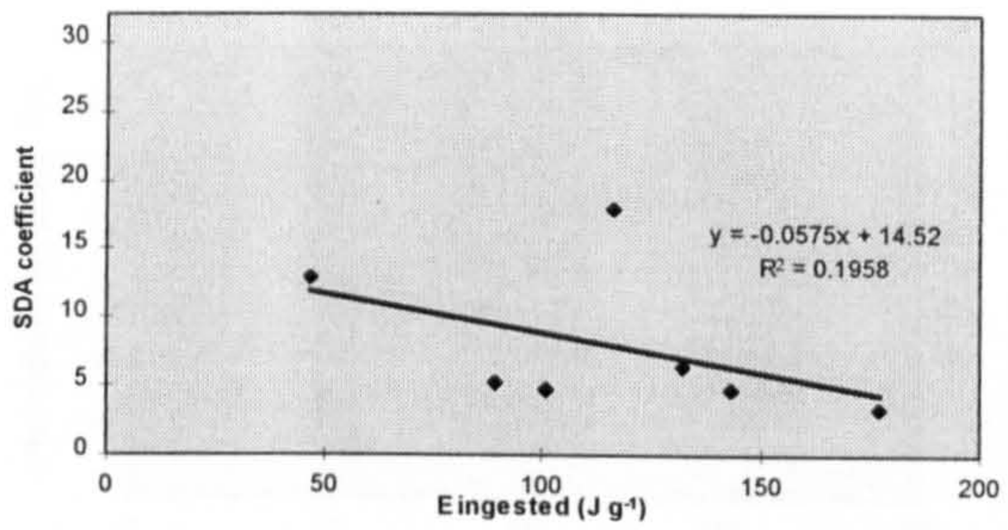
(a)





(b)





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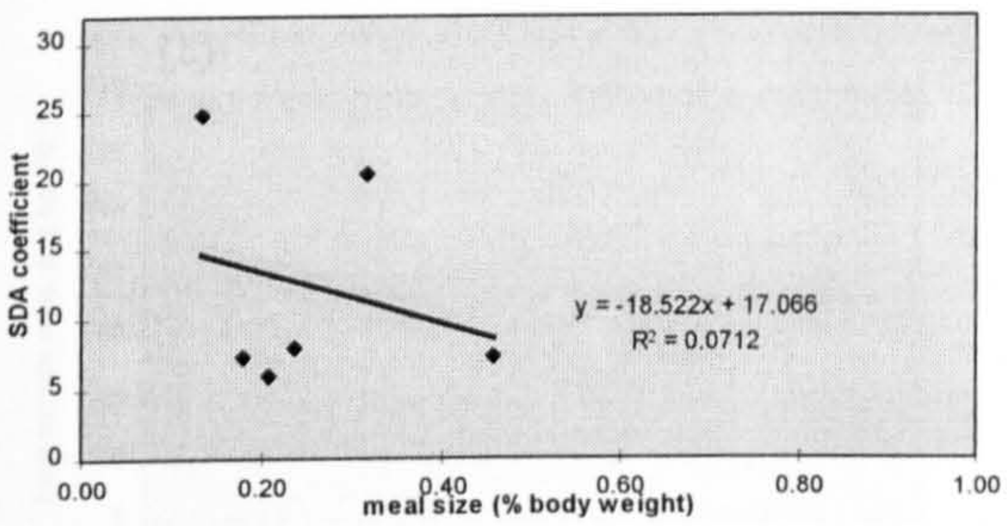
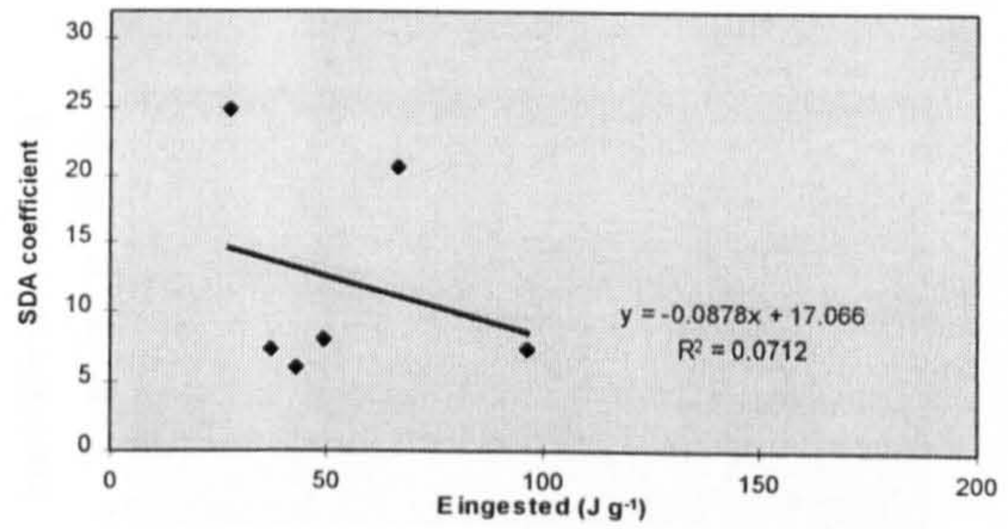
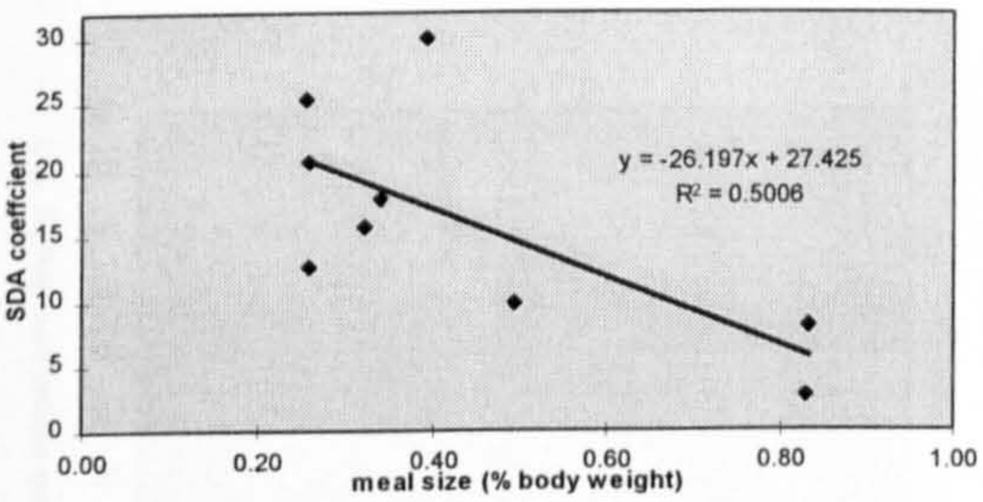




(d)



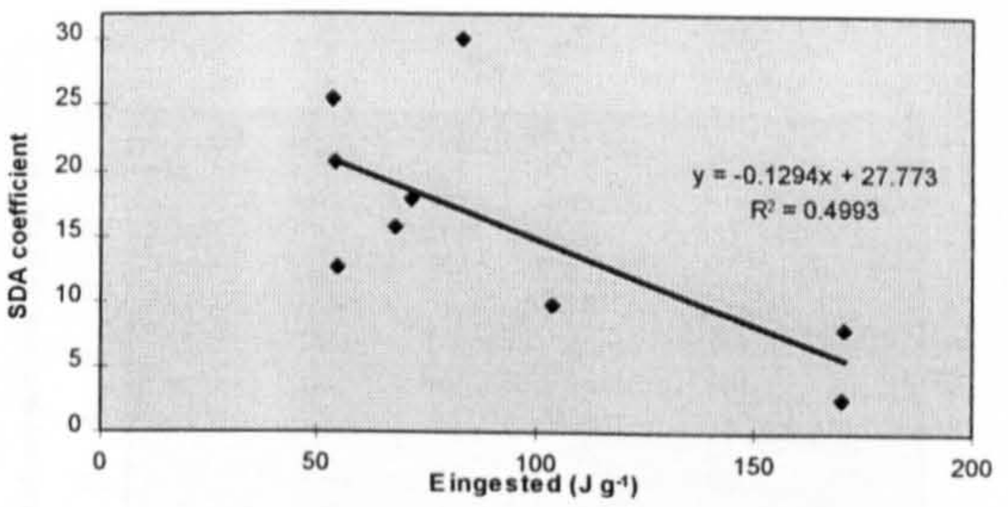
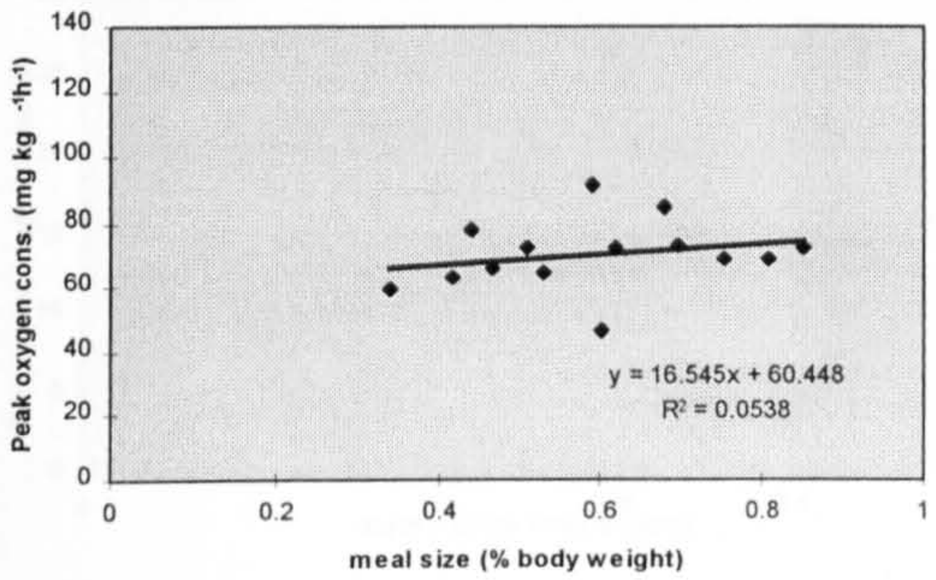
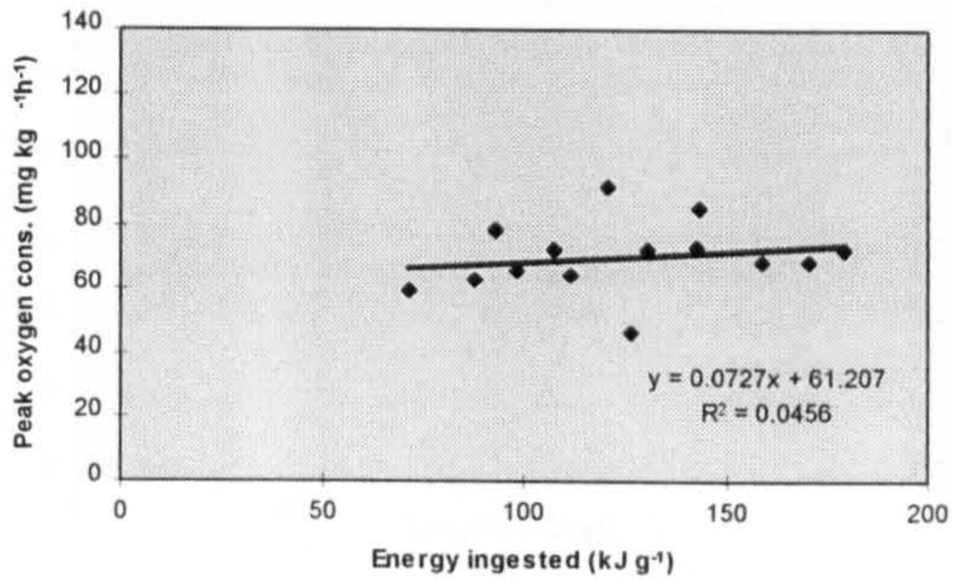


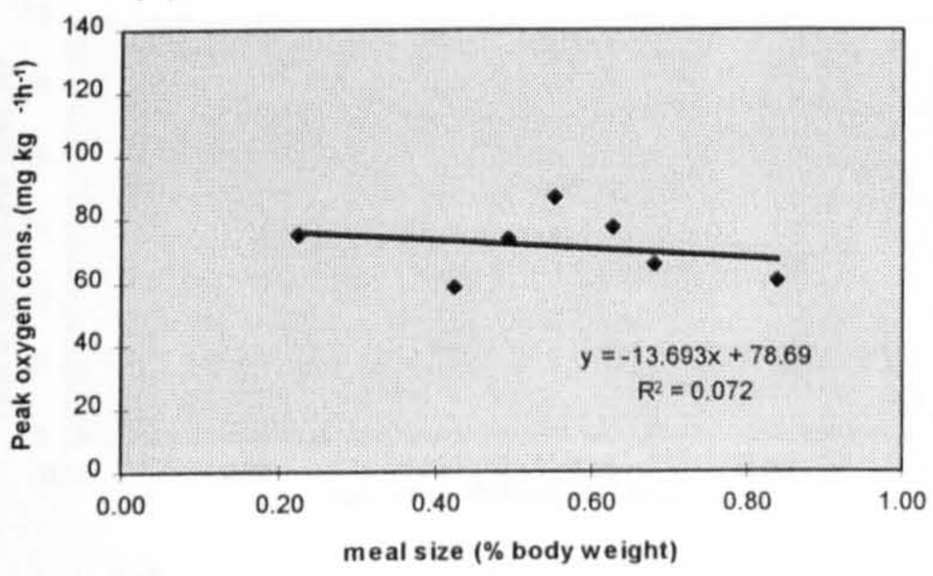
Fig. 4.17. Relationship between meal size and Energy ingested with S.D.A. coefficient in the Atlantic halibut, *Hippoglossus hippoglossus* following the ingestion of a single meal. Diet was a commercial dry pelleted feed. Temperatures are (a) 8°C, (b) 10°C, (c) 12°C and (d) 14°C. Regression equations and r^2 values are printed on individual graphs in the form $Y = a + bX$.

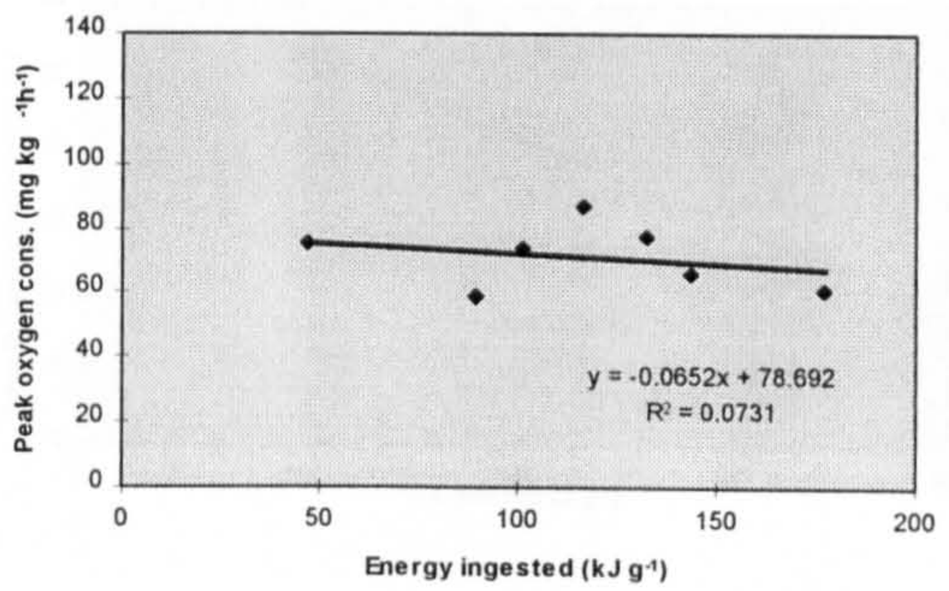
(a)



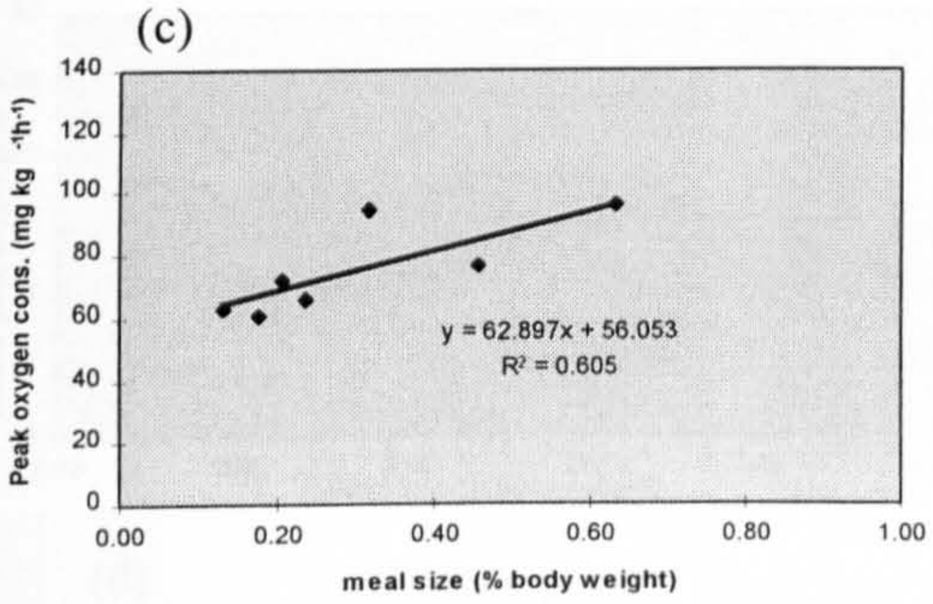


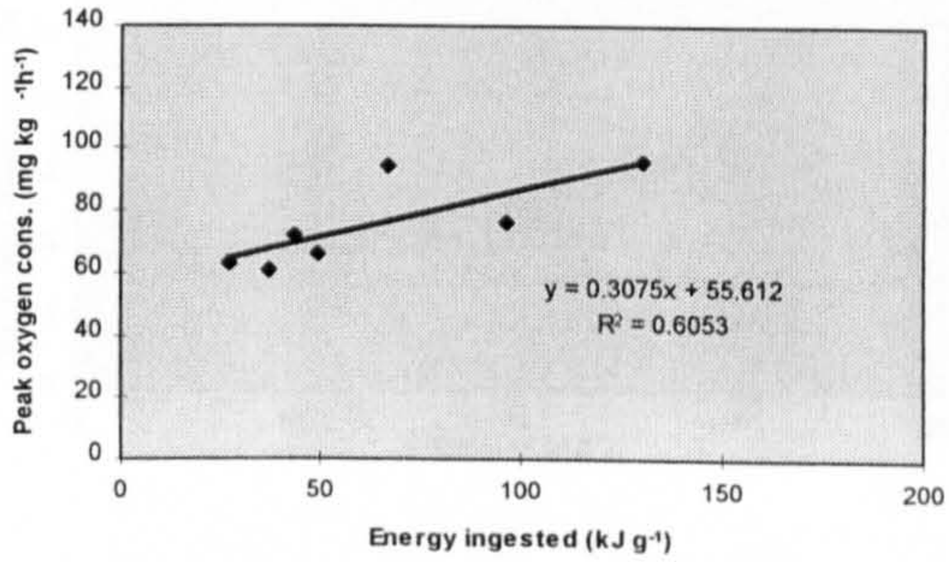
(b)



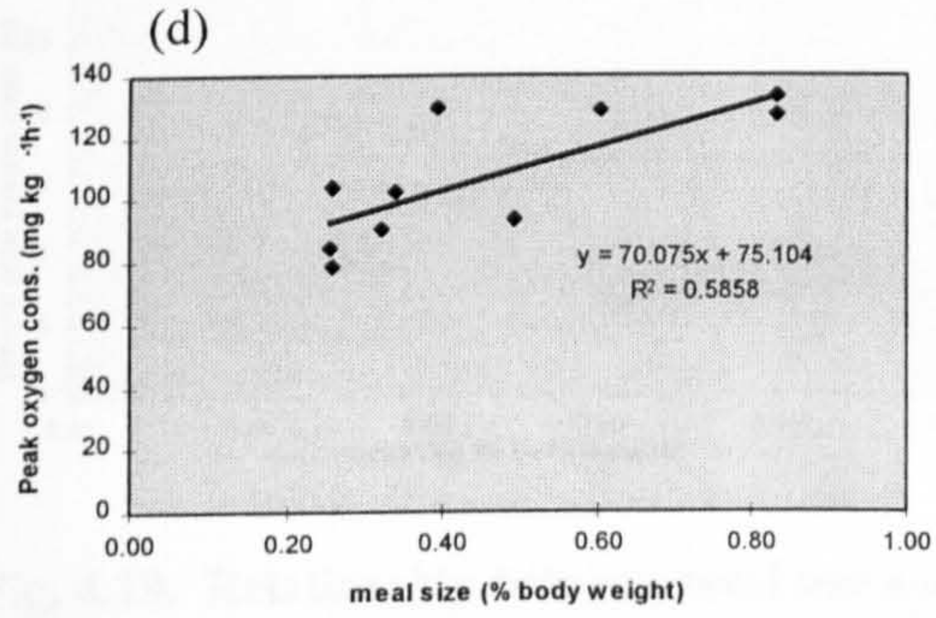


(c)





(d)



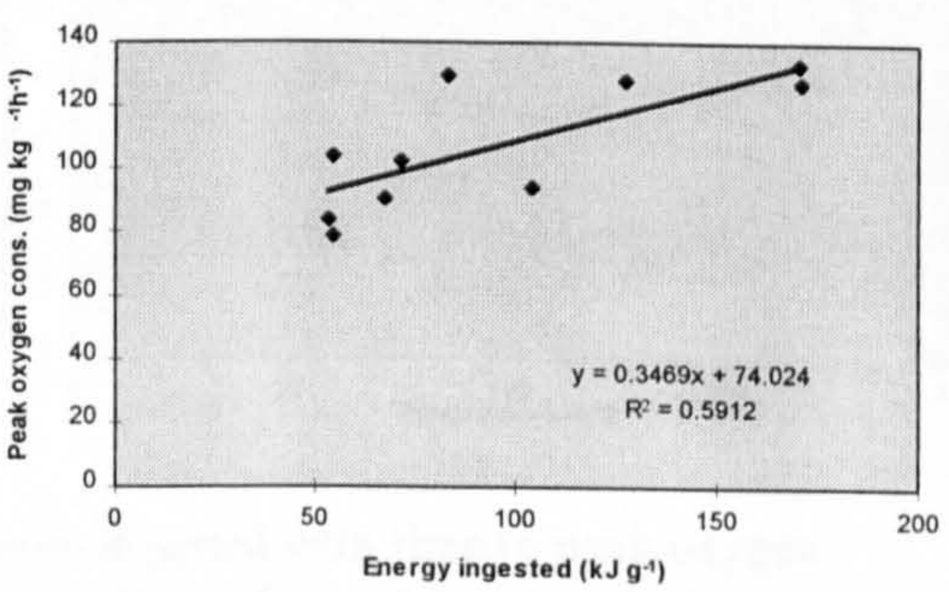


Fig. 4.18. Relationship between meal size and Energy ingested with peak post-prandial oxygen consumption rate in the Atlantic halibut, *Hippoglossus hippoglossus* following the ingestion of a single meal. Diet was a commercial dry pelleted feed. Temperatures are (a) 8°C, (b) 10°C, (c) 12°C and (d) 14°C. Regression equations and r^2 values are printed on individual graphs in the form $Y = a + bX$.

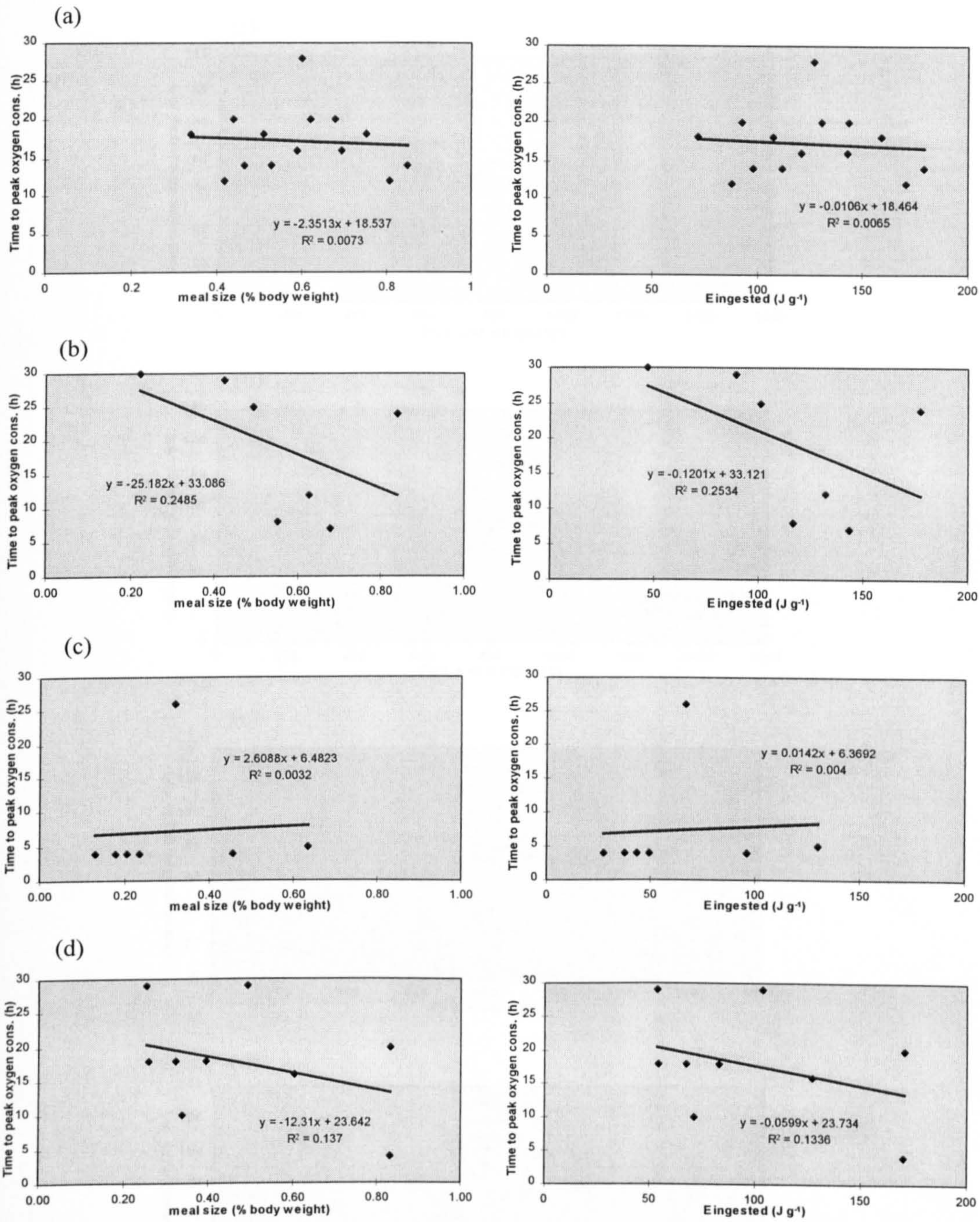
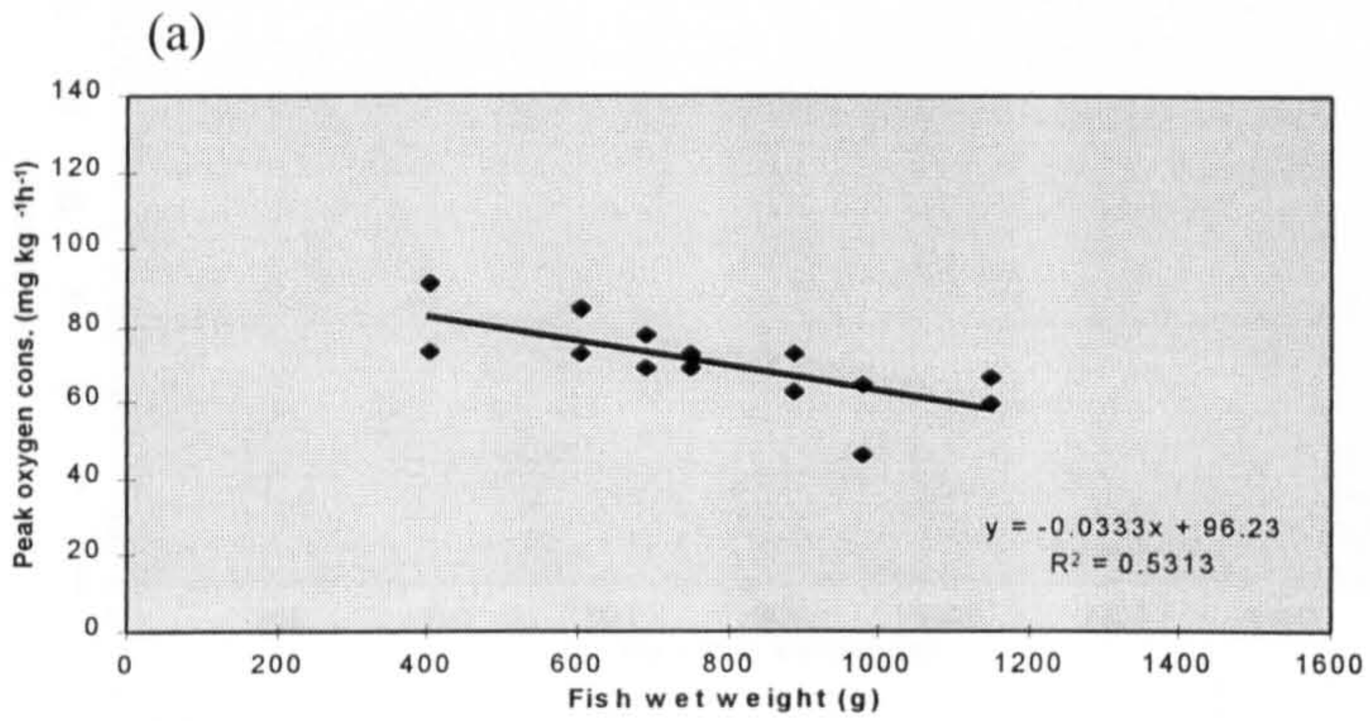
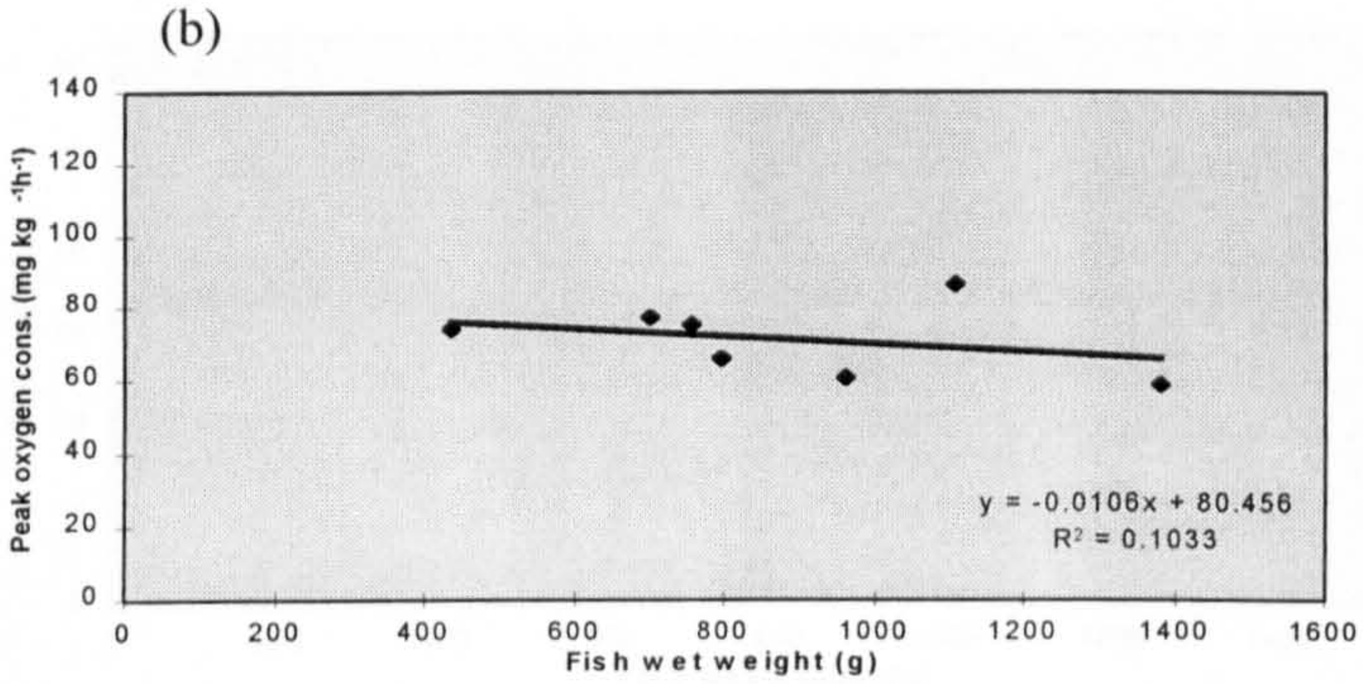
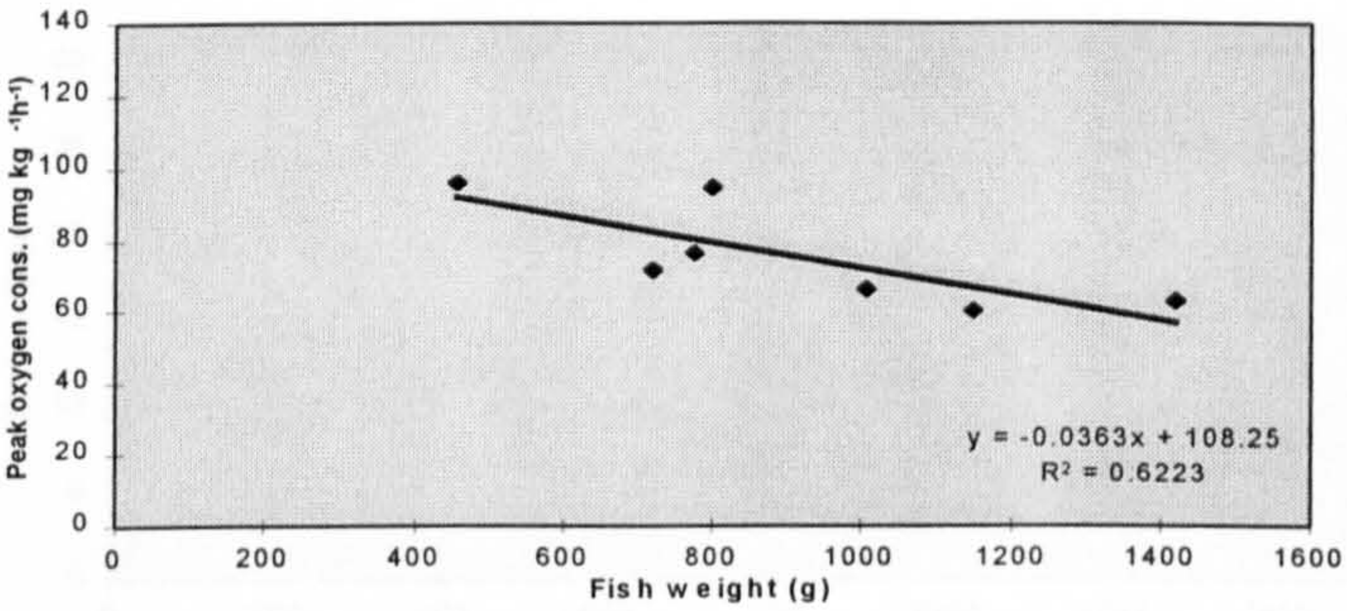


Fig. 4.19. Relationship between meal size and Energy ingested with time to peak oxygen consumption rate in the Atlantic halibut, *Hippoglossus hippoglossus* following the ingestion of a single meal. Diet was a commercial dry pelleted feed. Temperatures are (a) 8°C, (b) 10°C, (c) 12°C and (d) 14°C. Regression equations and r^2 values are printed on individual graphs in the form $Y = a + bX$.







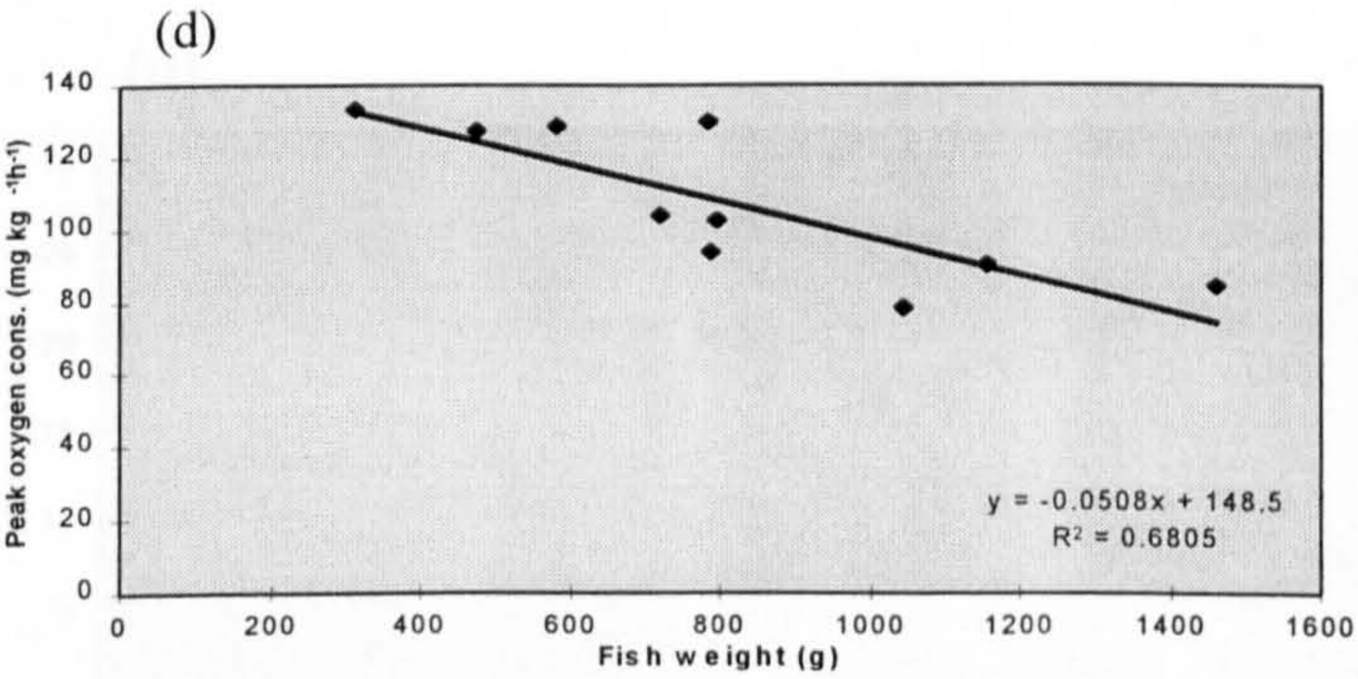
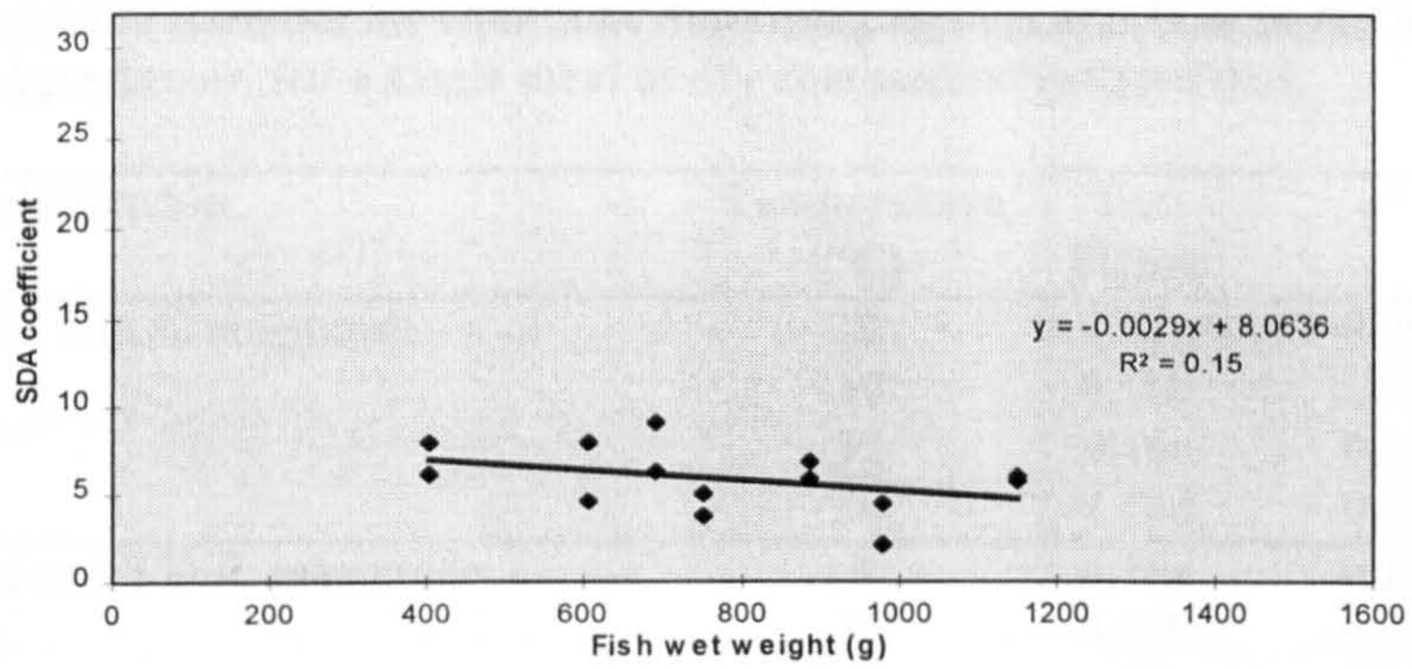
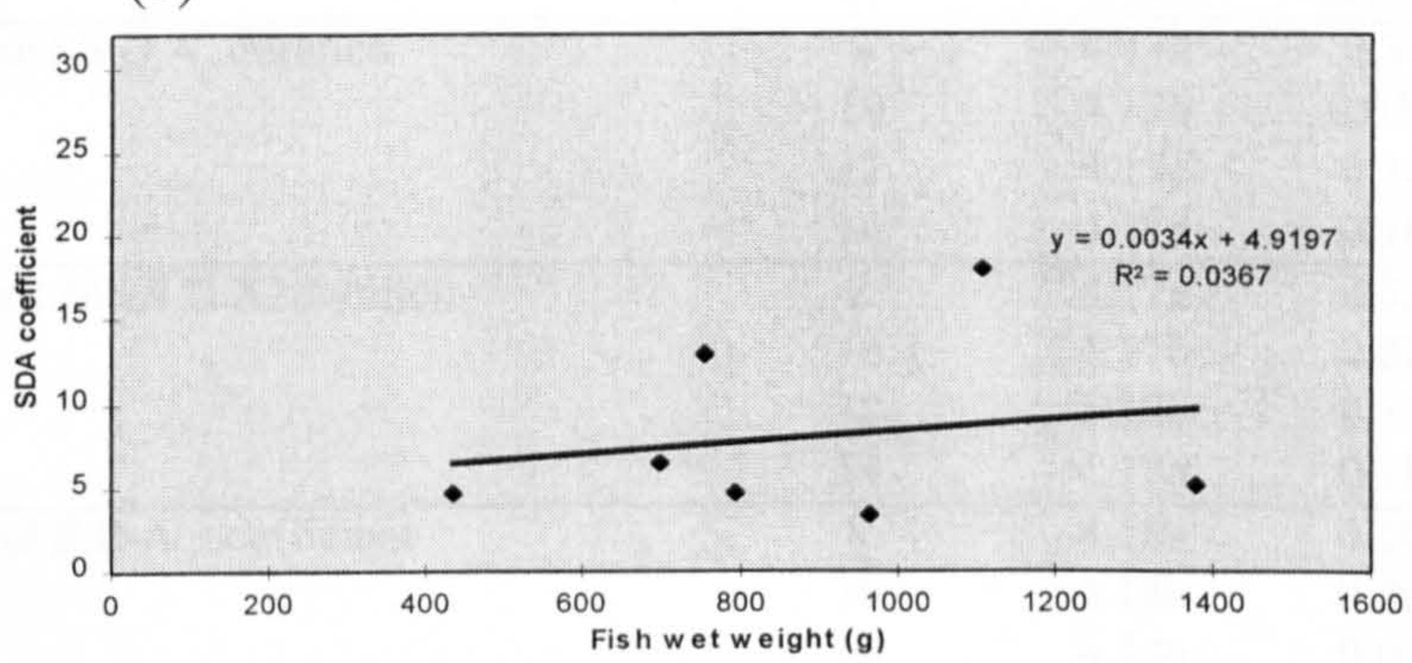


Fig. 4.20. Relationship between fish weight and peak oxygen consumption rate following the ingestion of a single meal, in the Atlantic halibut *Hippoglossus hippoglossus*. Diet was a commercial dry pelleted feed. Temperatures are (a) 8°C, (b) 10°C, (c) 12°C and (d) 14°C. Regression equations and r^2 values are printed on individual graphs in the form $Y = a + bX$.

(a)



(b)



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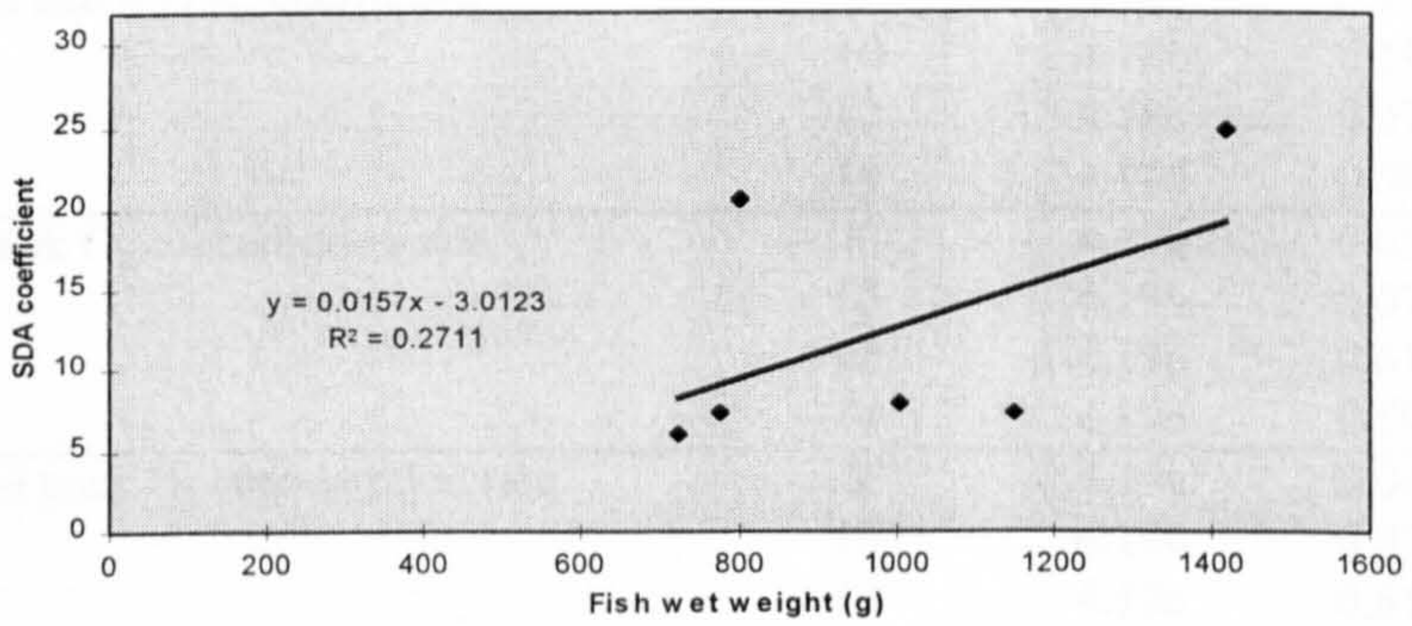


(d)

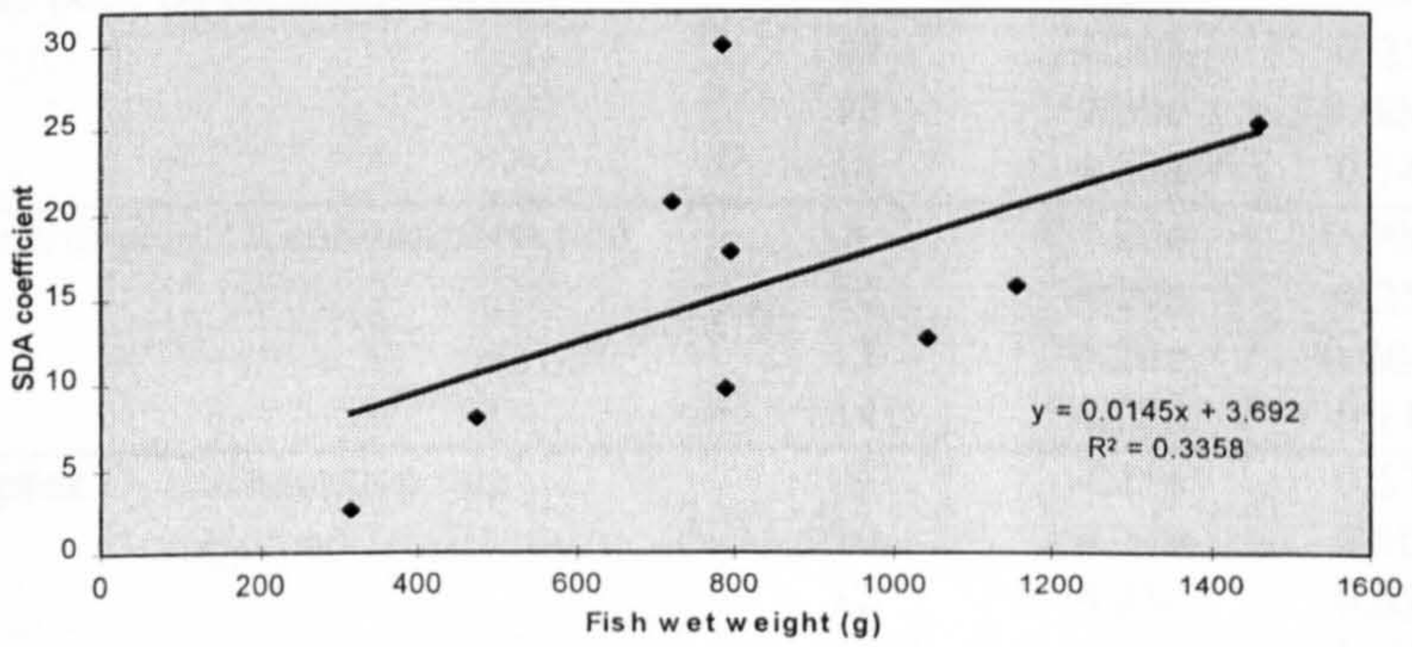


Fig. 4.21. Relationship between fish weight and S.D.A. coefficient in the Atlantic halibut, *Hippoglossus hippoglossus*, following the ingestion of a single meal. Diet was a commercial dry pelleted feed. Temperatures are (a) 8°C, (b) 10°C, (c) 12°C and (d) 14°C. Regression equations and r^2 values are printed on individual graphs in the form $Y = a + bX$.

Table 4.8. Regression statistics for equations describing aspects of S.D.A. in the Atlantic halibut, *Hippoglossus hippoglossus*, fed a single meal of dry commercial pelleted diet.

Relationship	Temperature (°C)	Text Figure	r^2	d.f.	p value
Meal size and S.D.A. magnitude	8	4.16a	0.20	12	0.104
	10	4.16b	0.007	5	0.851
	12	4.16c	0.18	4	0.392
	14	4.16d	0.04	7	0.602
Energy ingested and S.D.A. magnitude	8	4.16a	0.19	12	0.114
	10	4.16b	0.01	5	0.837
	12	4.16c	0.18	4	0.397
	14	4.16d	0.04	7	0.621
Meal size and S.D.A. duration	8	4.17a	0.34	12	0.030
	10	4.17b	0.017	5	0.787
	12	4.17c	0.14	4	0.471
	14	4.17d	0.18	7	0.234
Energy ingested and S.D.A. duration	8	4.17a	0.32	12	0.035
	10	4.17b	0.02	5	0.763
	12	4.17c	0.14	4	0.469
	14	4.17d	0.18	7	0.251
Meal size and S.D.A. coefficient	8	4.18a	0.10	12	0.262
	10	4.18b	0.20	5	0.309
	12	4.18c	0.07	4	0.610
	14	4.18d	0.50	7	0.032
Energy ingested and S.D.A. coefficient	8	4.18a	0.11	12	0.243
	10	4.18b	0.19	5	0.320
	12	4.18c	0.07	4	0.609
	14	4.18d	0.50	7	0.033
Meal size and peak O_2 consumption rate	8	4.19a	0.05	12	0.424
	10	4.19b	0.07	5	0.564
	12	4.19c	0.61	4	0.038
	14	4.19d	0.59	7	0.010
Energy ingested and peak O_2 consumption rate	8	4.19a	0.04	12	0.463
	10	4.19b	0.07	5	0.558
	12	4.19c	0.61	4	0.039
	14	4.19d	0.59	7	0.009
Meal size and time to peak O_2 consumption rate	8	4.20a	0.008	12	0.757
	10	4.20b	0.25	5	0.252
	12	4.20c	0.004	4	0.890
	14	4.20d	0.14	7	0.284
Energy ingested and time to peak O_2 consumption rate	8	4.20a	0.006	12	0.784
	10	4.20b	0.25	5	0.249
	12	4.20c	0.004	4	0.893
	14	4.20d	0.14	7	0.299
Fish weight and peak O_2 consumption rate	8	4.21a	0.53	12	0.003
	10	4.21b	0.10	5	0.482
	12	4.21c	0.62	4	0.035
	14	4.21d	0.67	7	0.003
Fish weight and S.D.A. coefficient	8	4.22a	0.15	12	0.171
	10	4.22b	0.04	5	0.681
	12	4.22c	0.27	4	0.290
	14	4.22d	0.34	7	0.102

4.3.5. *Effect of body weight and temperature on endogenous Ammonia excretion rate.*

The relationship between endogenous ammonia excretion rate with body weight and temperature was similar to that observed in routine oxygen consumption rate, with the rate per unit weight showing a decrease with increasing size, and increasing with temperature. The inverse relationship between $\log_{10}$ body weight and $\log_{10}$ endogenous ammonia excretion with temperature is represented in Fig. 4.22. and the regression statistics are shown in Table 4.9. Endogenous ammonia excretion increased with temperature over the test range 8°C to 12°C. Levels of significance were: 8°C ($r^2 = 0.96$, $p < 0.01$), 10°C ($r^2 = 0.64$, $p = 0.031$) and 12°C ($r^2 = 0.50$, $p = 0.076$).

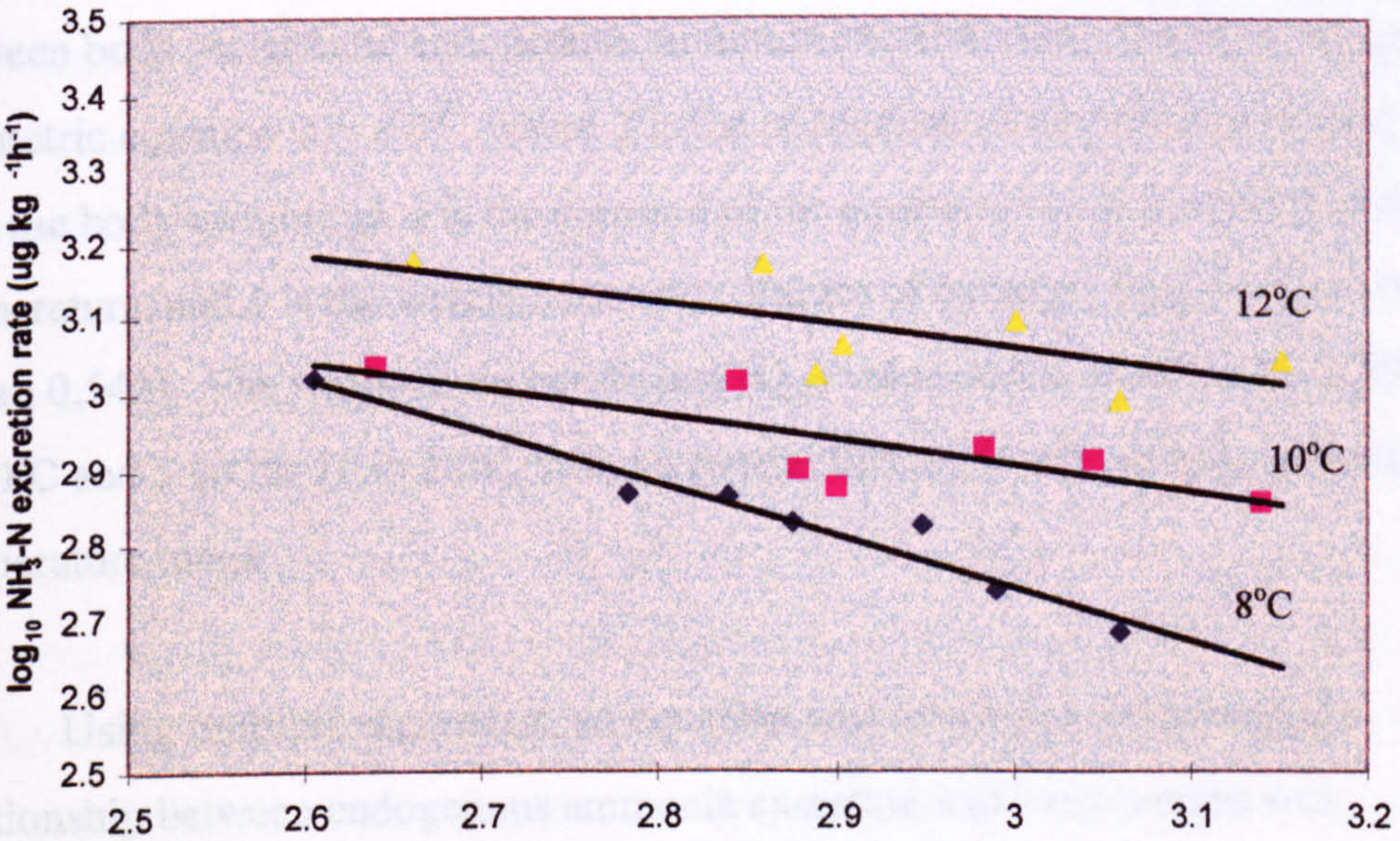


Fig.4.22. Relationship between $\log_{10}$ ammonia excretion rate and $\log_{10}$ body weight at routine levels of activity from 8°C to 12°C in tank populations of *Hippoglossus hippoglossus*.

Table 4.9. Regression statistics for the effect of temperature and body weight on endogenous ammonia excretion rate in small populations of *Hippoglossus hippoglossus* L.

Temperature (°C)	Weight range (g)	n	Regression equation and Allometric equation	r^2	Significance	Q_{10}
8	402 - 1150	7	$\text{Log}_{10} Y = 4.82 - 0.693 \text{Log}_{10} W$	0.96	$p < 0.01$	3.87
			$Y = 66.29W^{0.307}$	0.79	$p < 0.01$	
10	436 - 1380	7	$\text{Log}_{10} Y = 3.94 - 0.346 \text{Log}_{10} W$	0.64	$p < 0.05$	4.68
			$Y = 8.717 W^{0.654}$	0.86	$p < 0.01$	
12	456 - 1420	7	$\text{Log}_{10} Y = 4.01 - 0.318 \text{Log}_{10} W$	0.50	$p < 0.10$	5.66
			$Y = 10.3W^{0.682}$	0.83	$p < 0.01$	

Q_{10} values were calculated from the endogenous ammonia excretion values for a halibut of 750g body weight.

The relationship between body weight and endogenous ammonia excretion was examined at the three test temperatures, the results of which are also shown in Table 4.9. Similar to resting and routine oxygen consumption rates, the relationship between body weight and endogenous ammonia excretion was described by the allometric equation $Y = aW^b$, where Y is the endogenous ammonia excretion ($\mu g\ h^{-1}$), W is the body weight (g), a is the constant of the equation which is related to the test temperature, and b is the weight exponent. Values of b ranged from 0.307 to 0.682 (mean 0.548). The temperature coefficient (Q_{10}) values were calculated at 3.87 for 8 to 10°C and 5.66 for 10 to 12°C, with an overall figure of 4.68 covering the entire test temperature range.

Using multiple regression, an equation was developed describing the relationship between endogenous ammonia excretion and body weight with temperature over the temperature range 8°C to 12°C:

$$Y = -114 + 0.475X + 145T$$

where, Y = endogenous ammonia excretion ($\mu g\ h^{-1}$); X = fish body weight (g); T = temperature (°C).

4.3.6. *Effect of a single meal on Ammonia excretion rate.*

Ammonia excretion rates showed an increase above the endogenous excretion rate following the ingestion of a single meal in all trials. Examples of this post-prandial increase in ammonia excretion rate in tank populations of Atlantic halibut at the test temperatures of 8°C, 10°C and 12°C are shown in Figs. 4.23., 4.24 and 4.25, and the overall results for all trials are presented in Table 4.10.

All post-prandial trials showed a progressive increase in ammonia excretion rate immediately following feeding, steadily increasing to a single peak and then decreasing at a regular rate. The mean value for peak ammonia excretion as a percentage of the endogenous rate was 556 (±179) %. The length of time to peak ammonia excretion was similar for 8°C and 12°C at 20-24 hours, however this was reduced in the trials at 10°C to approximately 10 hours. Only one trial (586g halibut; 10°C; meal size 0.49% body weight) showed a complete return to endogenous ammonia excretion rates within the trial period, occurring at 28 hours post feeding. Extrapolation of the post-prandial ammonia excretion rate graphs produced in other trials was used to provide figures for the duration of elevated ammonia excretion above endogenous rate. These results are based on the assumption that there is no change in the rate of decrease of ammonia excretion within the area of extrapolation. The relationships between meal size (also energy and protein ingested) and peak ammonia excretion rate, time to peak, duration, and total ammonia excreted were explored at the test temperatures of 8°C, 10°C and 12°C. Figs. 4.26., 4.27., 4.28. and 4.29. represent these results graphically. The relationships between fish weight and peak post-prandial ammonia excretion, and fish weight and total post-prandial ammonia excretion were also explored, and the results of this analysis are presented in Figs.4.30. and 4.31. Regression statistics for the equations described by these relationships are presented in Table 4.11.

Peak post-prandial ammonia excretion rate was found to increase with increasing meal size, quantity of energy ingested, and quantity of protein ingested, and

absolute values increased with increasing temperature over the test range (Fig.4.26.). The steeper curves produced at 12°C indicate the possibility of a mass specific temperature variation in peak ammonia excretion.

The time to peak ammonia excretion varied with temperature, with the lowest values obtained at the intermediate temperature of 10°C (Fig. 4.27.). The timing of peak ammonia excretion was slightly later in halibut at 8°C than at 12°C. The curves produced at 8°C and 10°C both show a slight increase in time to peak ammonia excretion with meal size, energy ingested and protein ingested, although the results for 12°C show little influence of these factors.

Similar to time to peak ammonia excretion, the results for duration of ammonia excretion show the lowest values at 10°C, followed by 12°C and 8°C (Fig.4. 28.). The results at 10°C show an increasing time to peak ammonia excretion with increasing meal size, quantity of energy and protein ingested, and this trend is continued at 12°C although the rate of increase at this temperature is reduced. A wide variation in the data points obtained at 8°C makes determination of any relationship at this temperature difficult.

The quantity of ammonia excreted post-prandially was observed to increase with meal size, and quantity of energy and protein ingested and over the temperature range 8°C to 12°C (Fig.4.29.).

The relationship between fish weight and peak ammonia excretion was observed to change with test temperature, although the results did not follow a clear pattern (Fig.4.30.). At 8°C there appeared to be little change in peak ammonia excretion with fish weight, although at 10°C increasing fish weight was accompanied by an increase in peak ammonia excretion and at 12°C increasing fish weight was accompanied by a decrease in peak ammonia excretion. In general values for peak ammonia excretion rate showed an increase for any specific fish weight over the temperature range 8°C to 12°C.

Graphs produced for fish weight and mass-specific total ammonia excreted (Fig.4.31.) also showed a temperature related difference. At 8°C and 12°C, mass specific total ammonia excreted was found to decrease with increasing fish weight, although at 10°C the trend was for an increase with increasing fish weight.

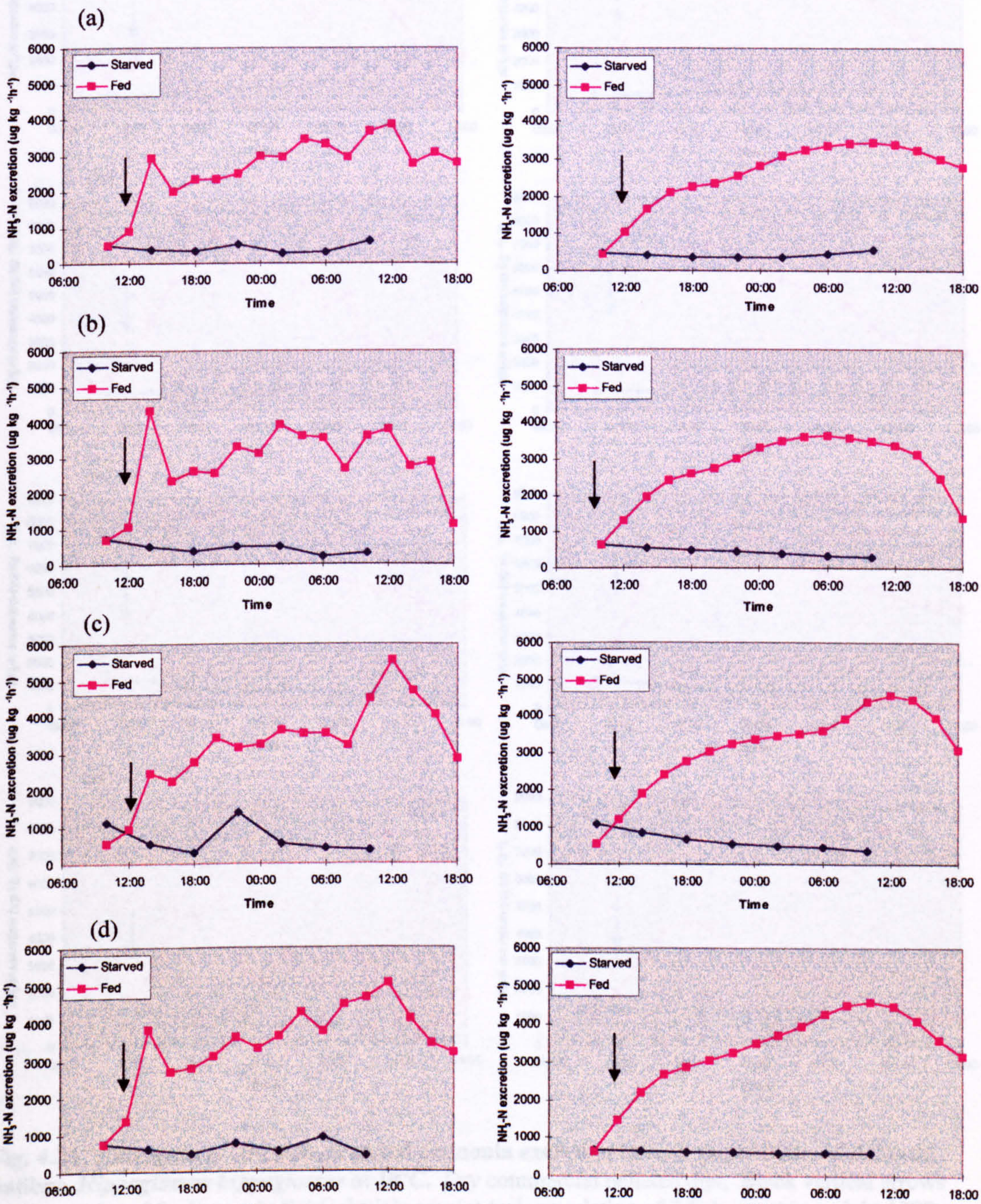


Fig. 4.23. Endogenous and post-prandial ammonia excretion in tank populations of Atlantic halibut, *Hippoglossus hippoglossus* at 8°C. Dry commercial pelleted diet. Black vertical arrows indicate point of feeding. Individual trials are: (a) tank population of 9 fish, average weight 1150g, meal size 0.47% body weight; (b) tank population of 6 fish, average weight 979g, meal size 0.60% body weight; (c) tank population of 9 fish, average weight 606g, meal size 0.85% body weight; (d) tank population of 11 fish, average weight 752g, meal size 0.81% body weight.

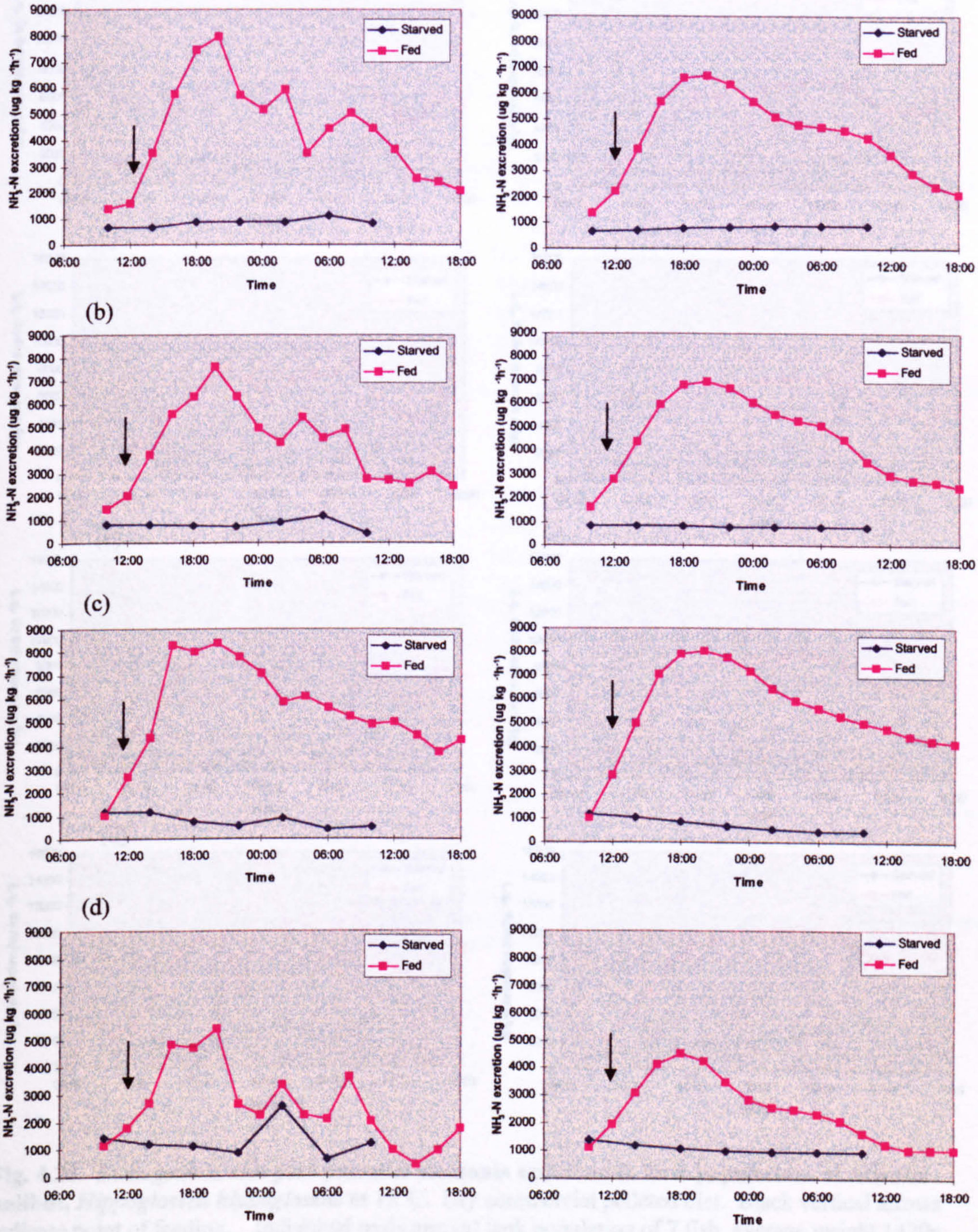


Fig. 4.24. Endogenous and post-prandial ammonia excretion in tank populations of Atlantic halibut, *Hippoglossus hippoglossus* at 10°C. Dry commercial pelleted diet. Black vertical arrows indicate point of feeding. Individual trials are: (a) tank population of 7 fish, average weight 1380g, meal size 0.42% body weight; (b) tank population of 10 fish, average weight 1109g, meal size 0.55% body weight; (c) tank population of 11 fish, average weight 795g, meal size 0.68% body weight; (d) tank population of 11 fish, average weight 436g, meal size 0.49% body weight.

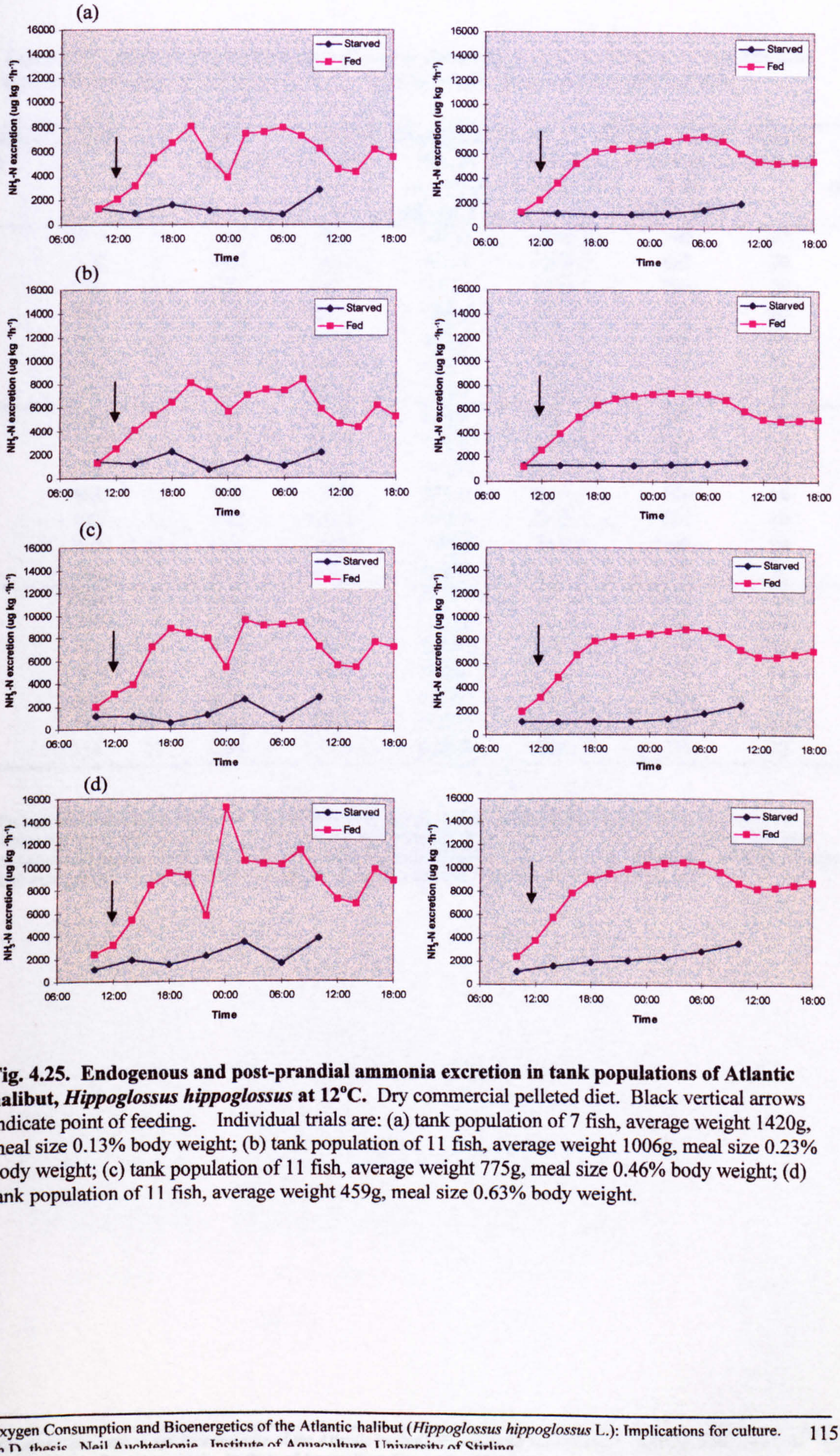


Fig. 4.25. Endogenous and post-prandial ammonia excretion in tank populations of Atlantic halibut, *Hippoglossus hippoglossus* at 12°C. Dry commercial pelleted diet. Black vertical arrows indicate point of feeding. Individual trials are: (a) tank population of 7 fish, average weight 1420g, meal size 0.13% body weight; (b) tank population of 11 fish, average weight 1006g, meal size 0.23% body weight; (c) tank population of 11 fish, average weight 775g, meal size 0.46% body weight; (d) tank population of 11 fish, average weight 459g, meal size 0.63% body weight.

Table 4.10. Analysis of post-prandial ammonia excretion in tank populations of the Atlantic halibut, *Hippoglossus hippoglossus* fed a single meal of commercial dry pelleted diet.

Temperature (°C)	wt. (g)	n	Meal (% wt.)	E ingested J g^{-1} body wt.	Endogenous NH_3-N excretion ($\mu g\ kg^{-1}\ h^{-1}$)	Peak NH_3-N excretion ($\mu g\ kg^{-1}\ h^{-1}$)	Peak NH_3-N excretion as % of endogenous	Time to peak (h)	Time to return to endogenous rate (h)
8	1150	9	0.47	98.4	532.3	3438.5	546	20	47
8	978	6	0.60	126.7	516.8	3634.5	603	20	35
8	606	9	0.85	179.4	452.7	4565.1	908	26	38
8	888	10	0.42	88.1	506.2	3817.9	654	24	43
8	751	11	0.81	170.5	485.3	4591.8	846	24	40
8	689	10	0.75	159.0	473.1	5085.3	975	24	48
8	402	11	0.70	142.7	367.4	2631.9	616	24	59
10	1380	7	0.42	89.2	849.9	6701.5	688	10	35
10	1109	10	0.55	116.5	905.8	6954.0	668	10	36
10	699	11	0.63	132.3	1072.6	6325.0	490	10	42
10	964	11	0.84	177.3	948.8	6317.7	566	16	46
10	794	11	0.68	143.3	1018.5	8015.5	687	10	44
10	757	11	0.22	47.0	1038.4	5050.4	386	10	35
10	435	11	0.49	100.9	1346.2	4537.9	237	8	26
12	1420	7	0.13	27.6	1137.9	7479.0	557	20	41
12	1150	10	0.18	37.5	1259.2	6151.1	388	18	40
12	721	10	0.21	43.4	1638.0	8725.4	433	20	41
12	1005	11	0.23	49.6	1350.9	7366.4	445	18	39
12	800	11	0.32	66.7	1537.5	8844.4	475	20	38
12	775	11	0.46	96.4	1567.7	8948.7	471	18	42
12	458	11	0.63	130.0	2220.4	10441.5	370	20	45

Endogenous ammonia excretions are calculated from the global equation relating ammonia excretion, body weight and temperature presented in section 4.3.4. Post prandial ammonia excretion rate diminished to that of endogenous ammonia excretion only in one trial (435g halibut, 10°C) after a period of 28 hours. Times to return to endogenous ammonia excretion rate were predicted in all other trials through extrapolation of the post-prandial ammonia excretion graphs.

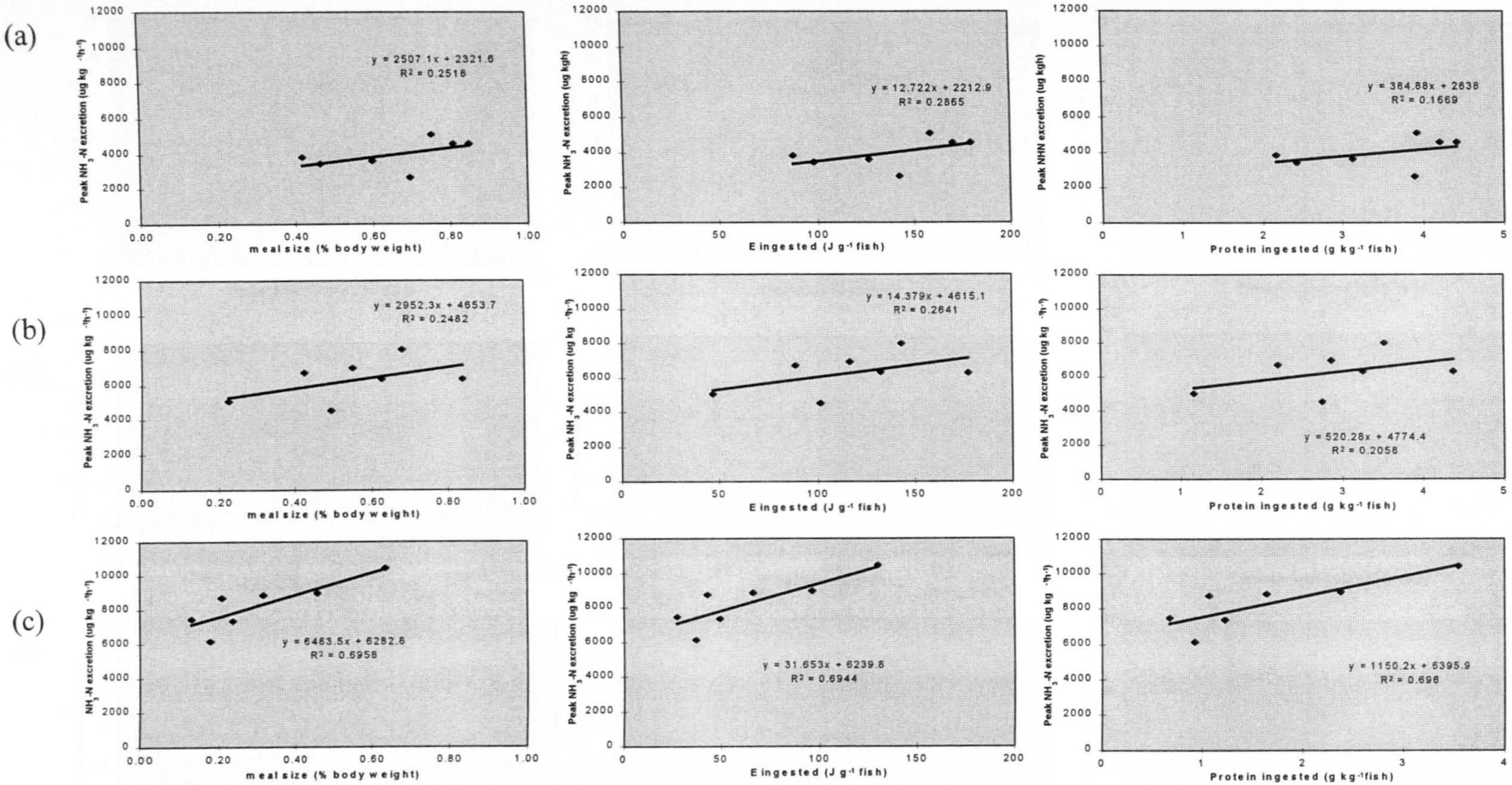


Fig. 4.26. Relationship between peak post-prandial ammonia excretion and meal size, energy ingested and protein ingested, in the Atlantic halibut, *Hippoglossus hippoglossus* following the ingestion of a single meal. Temperatures are (a) 8°C, (b) 10°C and (c) 12°C. Diet was a commercial dry pelleted feed. Regression equations and r^2 values are printed on individual graphs.

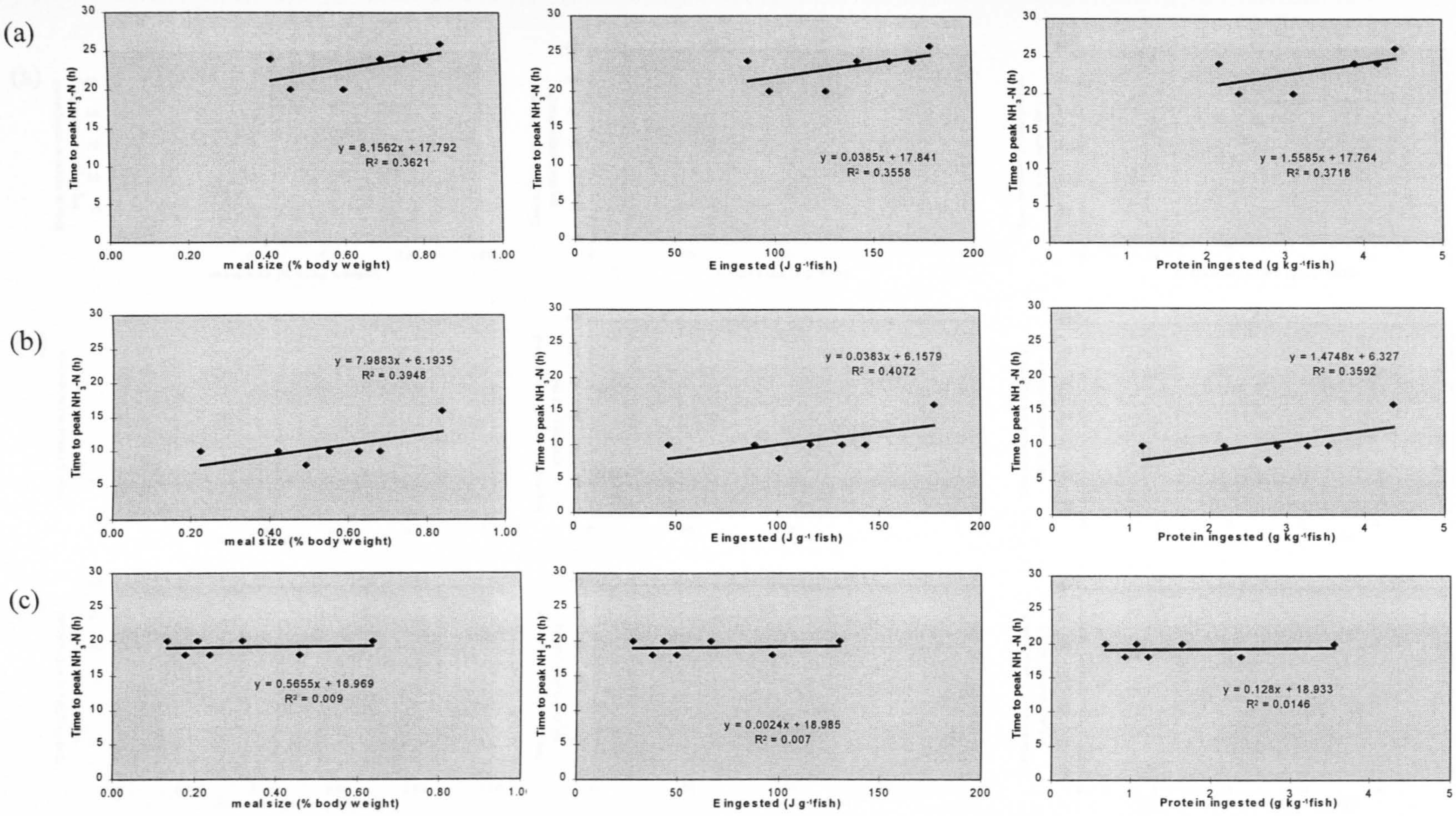


Fig. 4.27. Relationship between timing of peak post-prandial ammonia excretion and meal size, energy ingested and protein ingested, in the Atlantic halibut, *Hippoglossus hippoglossus* following the ingestion of a single meal. Temperatures are (a) 8°C, (b) 10°C and (c) 12°C. Diet was a commercial dry pelleted feed. Regression equations and r^2 values are printed on individual graphs.

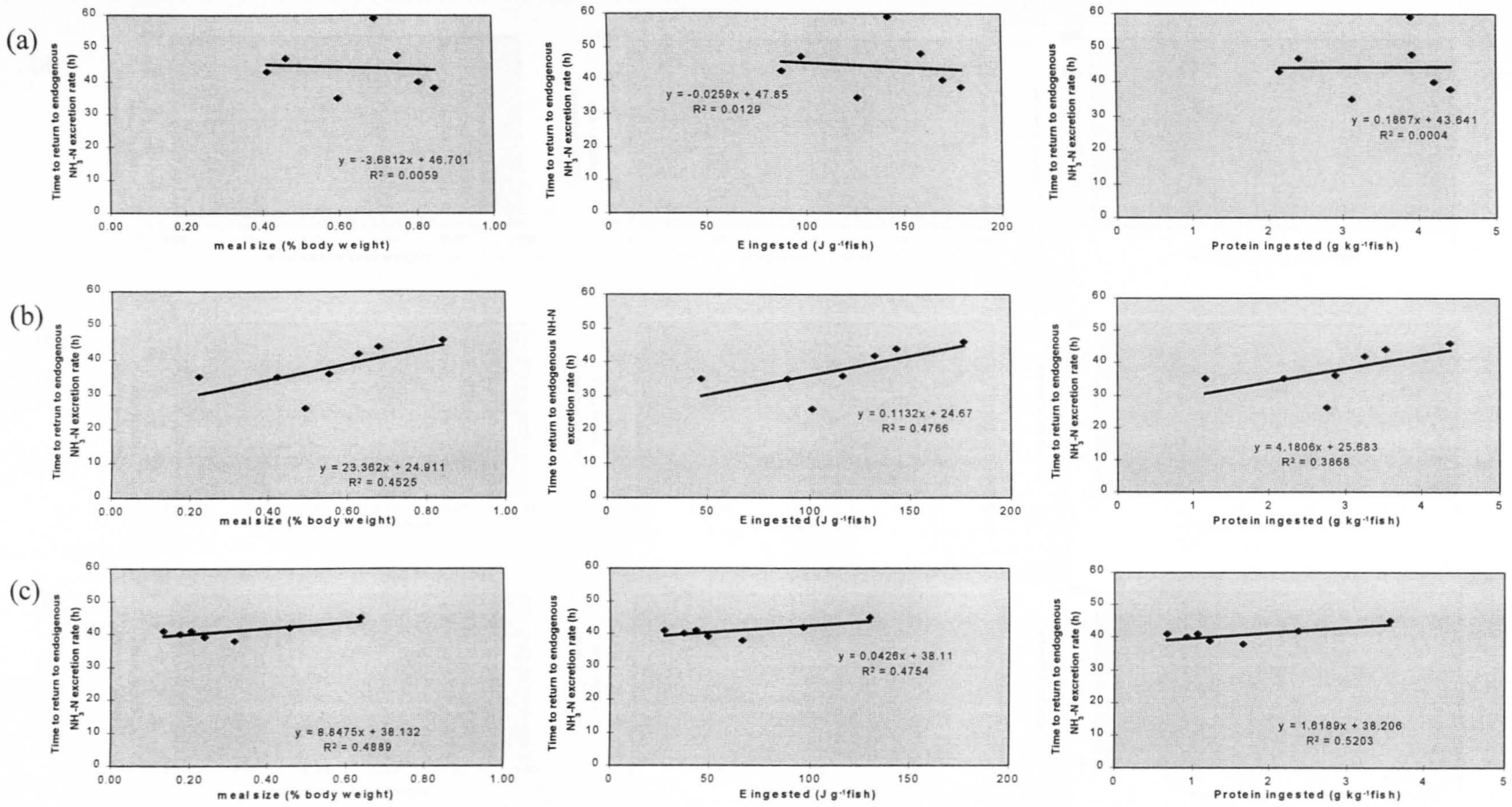


Fig. 4.28. Relationship between duration of post-prandial ammonia excretion and meal size, energy ingested and protein ingested, in the Atlantic halibut, *Hippoglossus hippoglossus* following the ingestion of a single meal. Temperatures are (a) 8°C, (b) 10°C and (c) 12°C. Diet was a commercial dry pelleted feed. Regression equations and r^2 values are printed on individual graphs.

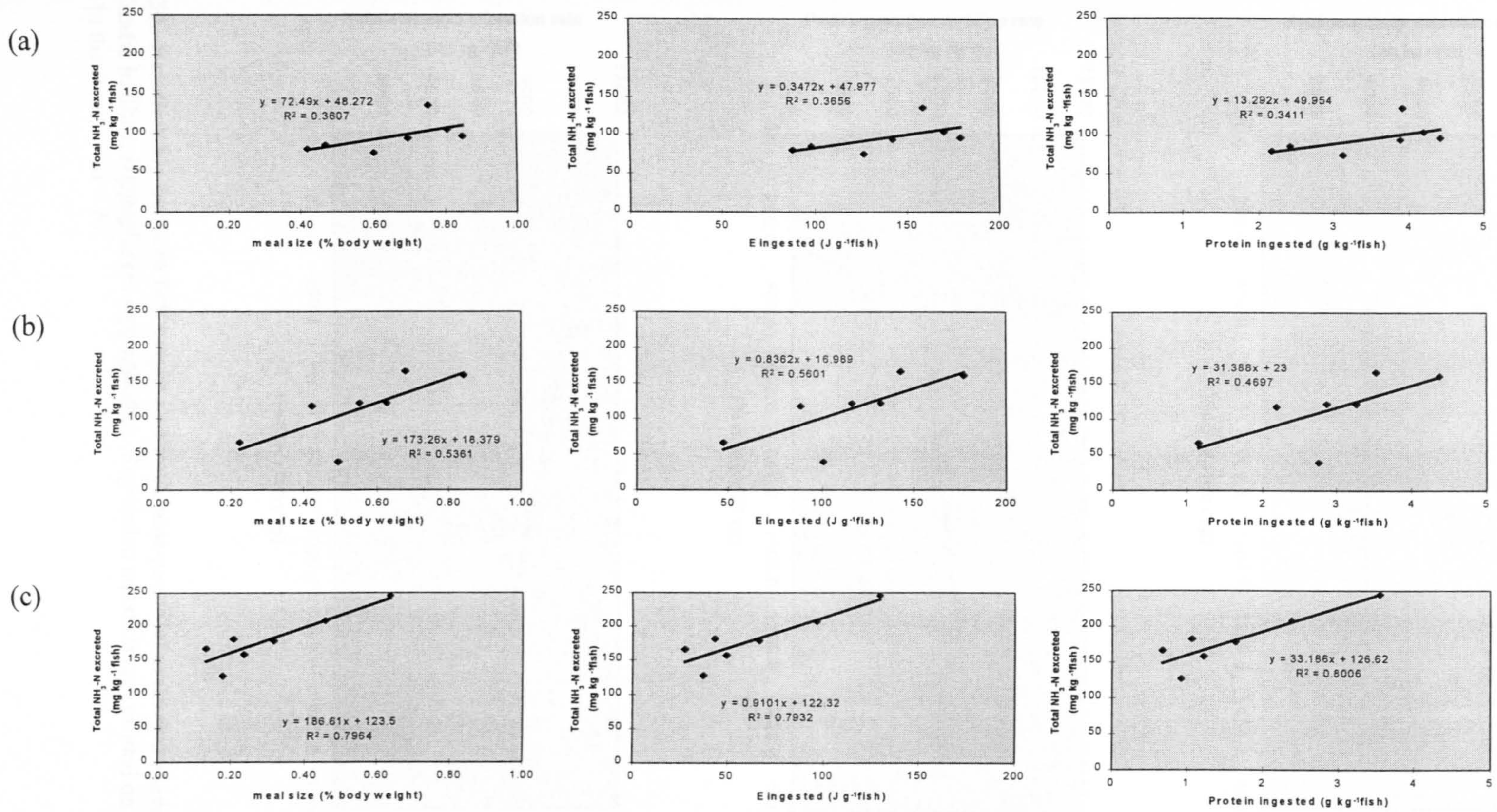


Fig. 4.29. Relationship between quantity of post-prandial ammonia excretion and meal size, energy ingested and protein ingested, in the Atlantic halibut, *Hippoglossus hippoglossus* following the ingestion of a single meal. Temperatures are (a) 8°C, (b) 10°C and (c) 12°C. Diet was a commercial dry pelleted feed. Regression equations and r^2 values are printed on individual graphs.

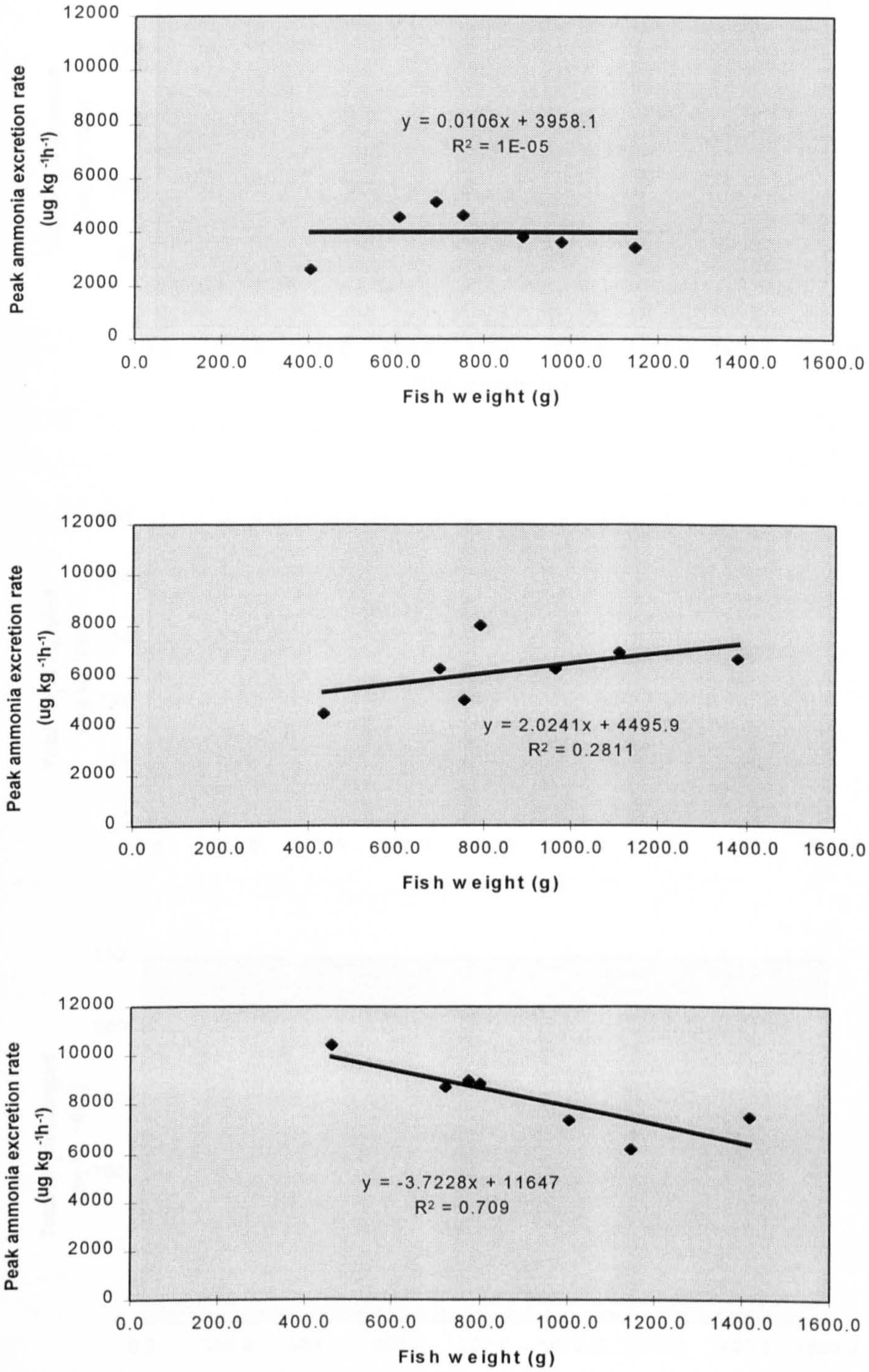
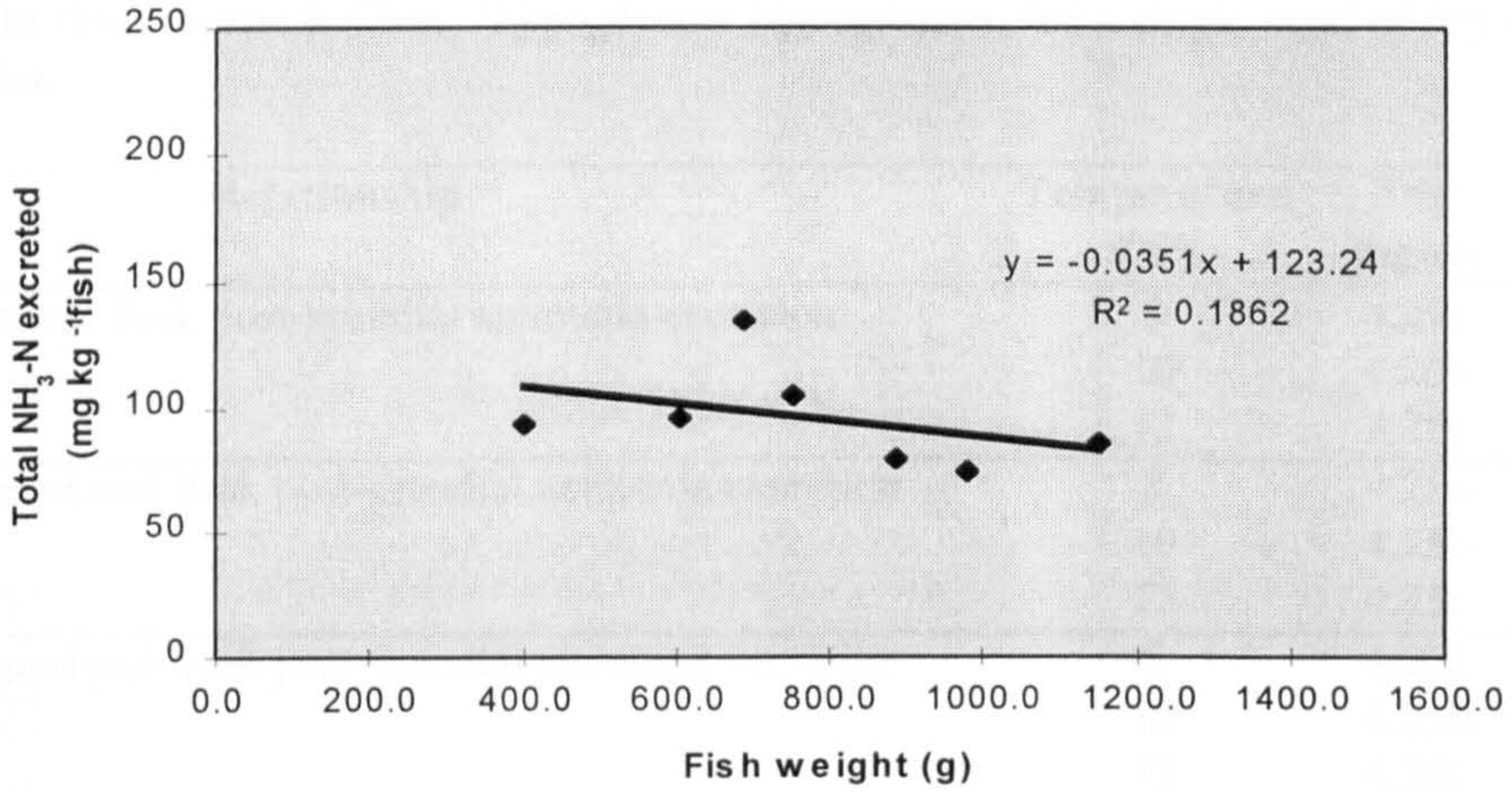
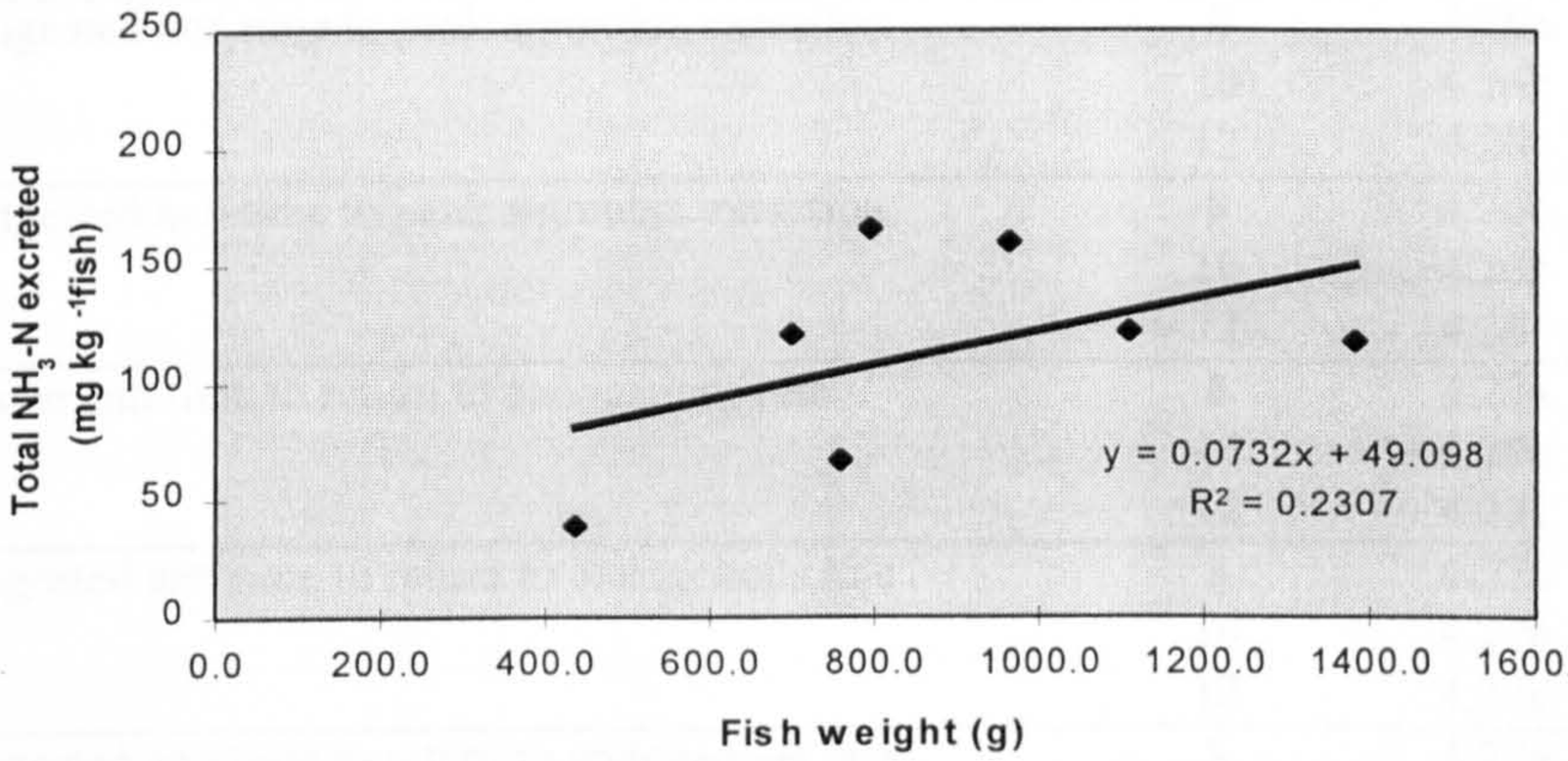


Fig. 4.30. Relationship between fish weight and Peak post-prandial ammonia excretion rate in the Atlantic halibut, *Hippoglossus hippoglossus*. Regression equations are printed on individual graphs in the form $Y = a + bX$.





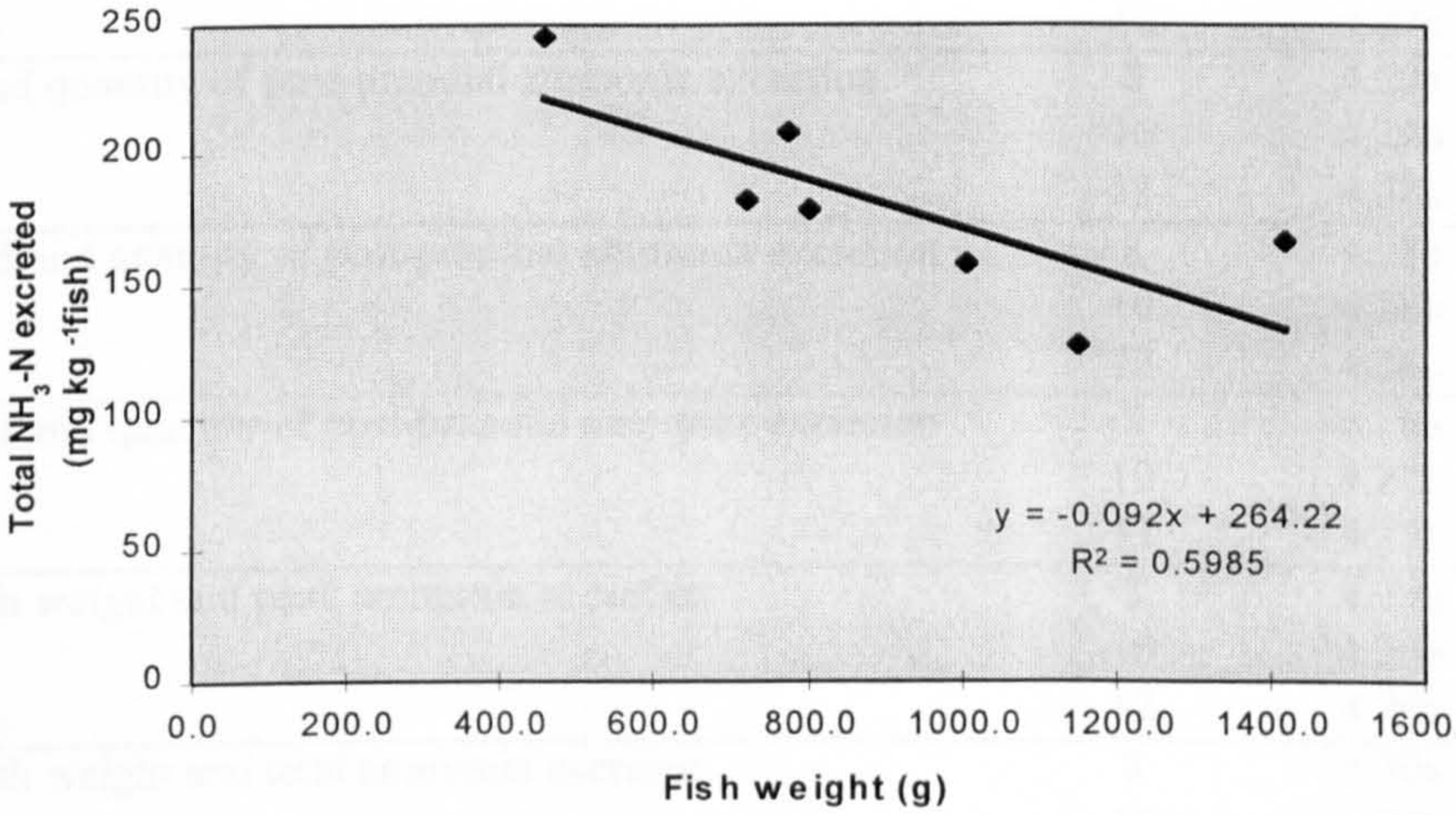


Fig. 4.31. Relationship between fish weight and total post-prandial ammonia excretion in the Atlantic halibut, *Hippoglossus hippoglossus*. Regression equations are printed on individual graphs in the form $Y = a + bX$.

Table 4.11. Regression statistics for equations describing aspects of post-prandial ammonia excretion in the Atlantic halibut, *Hippoglossus hippoglossus*, fed a single meal of dry commercial pelleted diet.

Relationship	Temperature (°C)	Text Figure	r^2	p
Meal size and Peak post-prandial ammonia excretion	8	4.25a	0.25	0.261
	10	4.25b	0.25	0.254
	12	4.25c	0.69	0.019
Energy ingested and Peak post-prandial ammonia excretion	8	4.25a	0.29	0.363
	10	4.25b	0.26	0.307
	12	4.25c	0.69	0.020
Protein ingested and Peak post-prandial ammonia excretion	8	4.25a	0.17	0.216
	10	4.25b	0.20	0.238
	12	4.25c	0.69	0.020
Meal size and time to peak ammonia excretion	8	4.26a	0.36	0.152
	10	4.26b	0.40	0.132
	12	4.26c	0.008	0.835
Energy ingested and time to peak ammonia excretion	8	4.26a	0.36	0.146
	10	4.26b	0.41	0.155
	12	4.26c	0.006	0.796
Protein ingested and time to peak ammonia excretion	8	4.26a	0.37	0.157
	10	4.26b	0.36	0.123
	12	4.26c	0.01	0.858
Meal size and time to return to endogenous rate	8	4.27a	0.006	0.880
	10	4.27b	0.45	0.096
	12	4.27c	0.49	0.082
Energy ingested and time to return to endogenous rate	8	4.27a	0.01	0.965
	10	4.27b	0.48	0.136
	12	4.27c	0.48	0.067
Protein ingested and time to return to endogenous rate	8	4.27a	0.004	0.808
	10	4.27b	0.38	0.086
	12	4.27c	0.52	0.087
Meal size and quantity of post-prandial ammonia excretion	8	4.28a	0.36	0.158
	10	4.28b	0.53	0.061
	12	4.28c	0.79	0.007
Energy ingested and quantity of post-prandial ammonia excretion	8	4.28a	0.27	0.169
	10	4.28b	0.58	0.089
	12	4.28c	0.79	0.007
Protein ingested and quantity of post-prandial ammonia excretion	8	4.28a	0.34	0.150
	10	4.28b	0.48	0.053
	12	4.28c	0.79	0.007
Fish weight and peak ammonia excretion	8	4.29a	0.001	0.995
	10	4.29b	0.28	0.325
	12	4.29c	0.71	0.041
Fish weight and total ammonia excreted	8	4.30a	0.18	0.334
	10	4.30b	0.23	0.442
	12	4.30c	0.59	0.017

In all experimental trials, n=7 (d.f. = 5).

4.4. Discussion

This study set out to provide information on the energetics of small tank populations of Atlantic halibut maintained on a standard husbandry regime, held under ambient conditions of temperature and photoperiod. In particular, the energy costs associated with feeding and excretion were investigated. The use of tanks as open system respirometers ensured that there were no changes in environmental parameters for the test animals which had been maintained in these systems for a significant period of time, and the results were gathered under conditions corresponding closely to those which the fish experienced as daily practice.

Effect of body weight and temperature on routine oxygen consumption rate

The increase in routine oxygen consumption per unit weight with a decreasing body weight, and with increasing temperature, is similar to that found in the determination of resting oxygen consumption in Chapter 3. Similar relationships have also been observed in other species such as *Mugil cephalus*, *Liza dumerili* and *L. richardsoni* (Marais, 1978) and *Scophthalmus maximus* (Waller, 1982) at routine levels. Increasing routine oxygen consumption rate with increasing temperature has also been observed in *H. hippoglossus* under very similar experimental conditions, although for much smaller fish of 20g to 90g in weight (Hallaråker *et al.*, 1995). In a recent study of routine oxygen consumption rate in other members of the *Pleuronectidae* (MacIsaac *et al.*, 1996) a large variation in results was exhibited, with two species (*Pleuronectes ferruginea* and *P. americanus*) showing elevated routine oxygen consumption rates at mid-range temperatures, and one species (*Hippoglossoides platessoides*) showing decreased values at mid-range temperatures. The work of MacIsaac *et al.* (1996) was carried out at 2°C, 11°C and 14°C, thus covering a large part of the temperature range of the work in this study. Table 4.12. presents comparative routine oxygen consumption rate data produced in other studies of routine oxygen consumption rate, together with values obtained in this study. A study by Saunders (1963) reports routine oxygen consumption rate values corresponding closely to the values produced by this study. These values, together with those recorded by Marais (1978) for three species of mullet, are within the range

Table 4.12. A summary of routine oxygen consumption rate values in a variety of fish species.

Species	Routine oxygen consumption rate (mg kg^{-1} h^{-1})	Fish size (g)	Temperature (°C)	Comments	Reference
Gadus morhua	28 - 50	220 - 2880	3	Experiments carried out on populations of 1-8 individuals	Saunders (1963)
	50 - 74	190 - 3230	10		
	61 - 153	90 - 3140	15		
Mugil cephalus	52.8	100	13	Acclimated to test temperature from ambient of 18-24°C within a 12h period.	Marais (1978)
Liza dumerili	53.2	100	13	as above	Marais (1978)
Liza richardsoni	96.1	100	13	as above	Marais (1978)
Scophthalmus maximus	33.0	100	8.1	Measurements made on groups of 5-6 individuals held in respirometers	Waller (1982)
	46.0		11.9		
	56.0		15.6		
Pleuronectes ferruginea	40.0	52-410	2	Community study, with an average stock density of 3kg m^3. Tanks used as respirometers	MacIsaac *et al.* (1996)
	292.0		11		
	91.0		14		
Pleronectes americanus	63.0	60-240	2	as above	MacIsaac *et al.* (1996)
	220.0		11		
	118.0		14		
Hippoglossoides platessoides	73.0	50-370	2	as above	MacIsaac *et al.* (1996)
	67.0		11		
	129.0		14		
Hippoglossus hippoglossus	140.0	80	10	Community study. Tanks used as respirometers.	Hallaråker *et al.* (1995)
	200.0		16		
Hippoglossus hippoglossus	46.0	500	8	Community study. Tanks used as respirometers	This study
	50.1		10		
	62.7		12		
	72.1		14		

All results quoted are for experimental work undertaken at salinities within the range 34-35‰. Values produced for this study are calculated from the equations presented in Table 4.2. The work of this study was conducted on fish of a larger body weight in comparison to the other comparative studies. A fish weight of 500g was chosen to provide values representative of the experimental size range in this study, while remaining reasonably close to the size of fish used in the other published work.

of values for flatfish, and are also comparatively low when the energetic differences between flatfish and roundfish are considered. However, the short acclimation time to the test temperature from an ambient temperature in excess of 5C° higher, could have influenced these results in the Marais (1978) study. Thermal history is important in making accurate determinations of oxygen consumption rate, with a sudden decrease in temperature generally accompanied by an artificially depressed value (Fry, 1979). The values obtained in this study are within the range of values recorded for other species of flatfish, over a similar temperature range, although they do appear slightly elevated above those of the turbot (Waller, 1982). Other values quoted for the Atlantic halibut by Hallaråker *et al.* (1995) are for individuals of 20-90g body weight, and a larger mass specific routine oxygen consumption would thus be expected. Taking size, and possibly associated differences in spontaneous activity level into account, the values of Hallaråker *et al.* (1995) correspond well with the figures of this study. A wide degree of variation is seen in the results of MacIsaac *et al.* (1996) although the origin of this range in values is difficult to determine. Overall, the results produced in this table again emphasise the importance of an identical protocol for experimental procedure if any comparison of oxygen consumption data is to be valid.

The spontaneous activity component of the total metabolic energy cost in such studies cannot be assumed to be equal throughout the temperature range, and thus may have a significant effect on the relationship between routine oxygen consumption rate and temperature. In this work, the development of an equation describing the relationship between routine oxygen consumption rate and body weight with temperature does hold good for the system under investigation, and provides a good estimate for the energy costs of spontaneous movement within such a system for a range of fish weights between 287g and 1774g over the temperature range 8°C to 14°C. The applicability of this equation to Atlantic halibut held in other facilities may not be pertinent, and caution in such application should thus be emphasised.

The value of the weight exponent is slightly lower than that produced for resting oxygen consumption rate data in Chapter 3., with an overall average of 0.66,

and again the value of this factor is sensitive to the influence of the variable activity rates observed in routine rate measurements. Given the relatively large size range of the test fish there are clearly likely to be size related differences in the 24 hour activities of these fish (explored further in Chapter 5.), which could have a subsequent impact on the routine oxygen consumption rate. It may be seen that a higher activity in the smaller size class of fish could have the effect of elevating the oxygen consumption rate relative to large fish, thus reducing the value of the weight exponent *b*, and this may account for the values produced in this study in comparison to the resting rate work in which the movement of all fish was restricted. With this in mind, it is of interest to compare the values for routine oxygen consumption rate with values for resting oxygen consumption rate determined from the work in Chapter 3, for fish of similar body weights at the same temperature. These comparisons may be summarised graphically, and are presented in Fig.4.32.

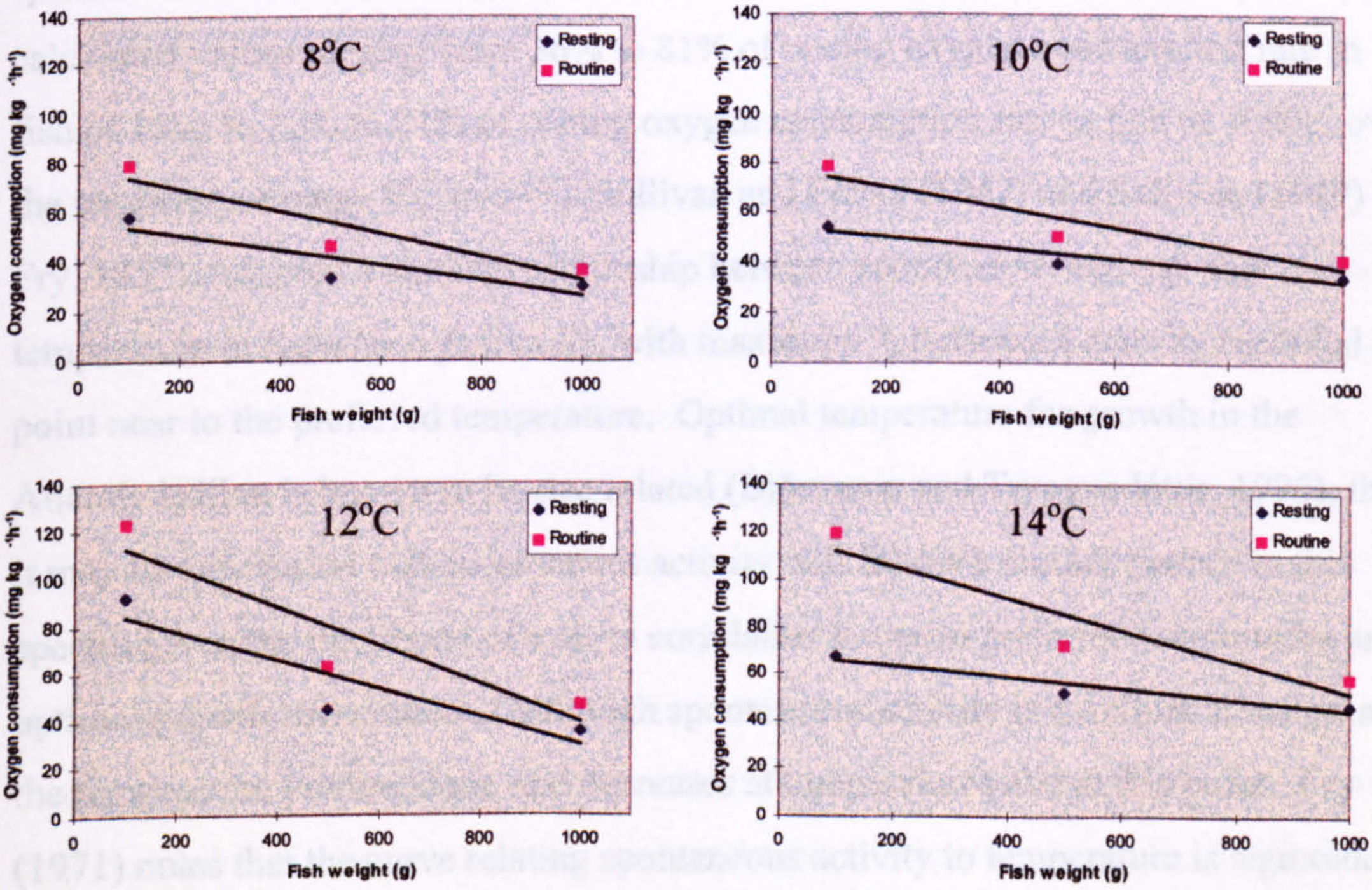


Fig.4.32. Comparison between resting and routine oxygen consumption rate in the Atlantic halibut, *Hippoglossus hippoglossus* (L). at 8°C, 10°C, 12°C and 14°C. Values are calculated from the equations produced in Chapters 3 & 4 for resting and routine oxygen consumption rate.

The results shown in Fig.4.32. show a greater divergence between the resting and routine oxygen consumption curves with decreasing body weight, indicating that there are size related differences in spontaneous activity in the Atlantic halibut. Over the temperature range 8°C to 14°C these size related differences appear to become more pronounced, and the energy allocation to spontaneous activity in 100g halibut at 14°C is proportionately far greater than that for halibut of 1000g.

Over the test temperature range in this work, there was a steady increase in routine oxygen consumption rate with temperature, and the global equation produced would appear to provide a good predictive equation in this instance. The value of routine rate measurements in energetic studies decreases as soon as an element of unpredictability enters the equation, however here the study of a fish species with a relatively sedentary nature may possess an advantage over more active species in which there may be more potential for variation in the energetic costs associated with activity. Fig.4.33. shows the overall elevation of oxygen consumption rate due to spontaneous activity to be a fraction of resting oxygen consumption rate, with calculated values ranging from 36% to 81% of resting oxygen consumption rate in fish of 100g to 20% to 24% of resting oxygen consumption rate in fish of 1000g, over the temperature range 8°C to 14°C. Sullivan and Fisher (1947) and Sullivan (1949) (in Fry, 1957) observed a similar relationship between spontaneous activity and temperature in *Salvelinus fontinalis*, with maximum spontaneous activity recorded at a point near to the preferred temperature. Optimal temperature for growth in the Atlantic halibut is known to be size related (Björnsson and Tryggvadóttir, 1996), thus it may be anticipated that spontaneous activity will follow a similar pattern in this species given the likelihood of a close correlation between preferred temperature and optimal growth temperature. Although spontaneous activity is maximal at the point of the temperature preferendum, and decreases at temperatures above this point, Fry (1971) notes that the curve relating spontaneous activity to temperature is sigmoidal, as increasing costs of homeostasis at higher temperatures offset the high costs of spontaneous activity. Reviewing experimental data obtained from the goldfish, this author also showed that peak routine oxygen consumption rate may vary from a level

close to that of the resting rate, to a level as a high as six times that of the resting rate. Resting oxygen consumption rates for the goldfish were found to be in the region of 20 - 30ml O_2 kg^{-1} h^{-1}, and routine oxygen consumption rates within the range 40 - 180ml O_2 kg^{-1} h^{-1}. Comparatively, the percentage increase of the routine oxygen consumption rate above the resting oxygen consumption rate is lower in this study, and probably reflects the relatively sedentary nature of flatfish.

Effect of photoperiod on routine oxygen consumption rate

The results of this study were quite distinct, showing a clear trend of increased routine oxygen consumption rate in the period of darkness throughout the test period. Even though the time period of this study covered a wide range of ambient photoperiods, this was pattern was retained throughout. Exceptions were few in number, and were generally fish in the small size range. The results of this work reinforce the hypothesis that there is a strong nocturnal rhythmicity in this species which is strongly related to the nocturnal activity of one of the major prey items, *Sebastes marinus* (Leim and Scott, 1966; Scott and Scott, 1988).

An increase in routine oxygen consumption rate in the dark phase over the light phase was observed in post hatchling lesser spotted dogfish (*Scyliorhinus canicula*) by Sims *et al.* (1993), and this pattern of oxygen consumption was strongly correlated with activity level. In the sole (*Solea solea*), a nocturnal rhythmicity has been established, manifest as both the period of maximal stomach distension with food in wild fish, as well as the period of greatest motor activity of fish held in aquaria (Kruuk, 1963). In the plaice (*Pleuronectes platessa*) and the flounder (*Platichthys flesus*) an increase in motor activity at night has also been observed in tank held fish, although in wild plaice maximum stomach fullness has been recorded in the daytime (Verheijen and de Groot, 1967). Edwards *et al.* (1971) studied feeding pattern in the tropical marine flatfish *Cynoglossus bilineatus*, *C. brevis*, *C. cynoglossus*, and *C. puncticeps*, and concluded that the greatest feeding period in these fish is at night-time. Sequential sampling of stomach contents throughout a 24 hour period showed that stomach fullness, expressed as wet weight of stomach contents divided by wet

weight of the fish, was maximum between the period of dusk and dawn. Unfortunately the lack of comparative oxygen consumption data taken over a 24 hour period for other species of flatfish prohibits the direct comparison of the results of this study with other species. On the understanding that there is likely to be a close link between daily activity and feeding patterns with daily pattern of oxygen consumption, an investigation into the activity pattern of the Atlantic halibut over a 24 hour period would prove beneficial. A size related difference in daylight/nocturnal activity pattern may be expected in the Atlantic halibut, given the change in feeding pattern as they increase in size and become more actively piscivorous (Haug, 1990a). A pattern of daily activity is the behavioural adaptation of the animal to its environment, and is considered to be determined by the probability of location of and capture of prey items (Manteifel *et al.*, 1978). All Atlantic halibut production currently involves the daylight feeding of fish throughout the life cycle. It would appear that a very strong inherent rhythm is operating, with the result that there is no clear modification of behaviour such that patterns of routine oxygen consumption rate correlate with this daylight feeding. In this work the test fish were routinely fed at 10.00h every day, and had been maintained on this regime throughout the period of three years of this study. Although this was the case, no evidence from the routine oxygen consumption rate results was produced for a period of anticipatory feeding behaviour and enhanced spontaneous activity prior to the daily feeding time. Sánchez-Vázquez *et al.* (1996) note the importance of time of food availability as a zeitgeber synchronising behaviour rhythms in fish, and showed a strong element of anticipation prior to feeding in goldfish held on a scheduled feeding regime. The results of this work suggest differences in feeding and activity pattern in the Atlantic halibut which merit further attention for the intensive culture of this organism.

Effect of body weight on meal size and energy intake

Temperature and body weight are known to influence ration size in fish (Braaten, 1979). The results of this study confirm the existence of an inverse relationship between both meal size and energy intake with body weight and temperature in the Atlantic halibut. Although the results for the 10°C work are variable, it appears that overall feed and energy intakes were higher at the lower temperatures of 8°C and 10°C than at 12°C and 14°C, for the size range of fish examined. In general the results are somewhat indeterminate, perhaps reflecting the relatively crude experimental procedure. The relationship between ration ingested and temperature in fish can usually be described by an asymmetrical bell-shaped curve in which the side corresponding to the upper temperature range has a much steeper decline than the incline below the optimum temperature (Jobling, 1994). A reduction in ration size at 12°C followed by an increase at 14°C in this study is difficult to explain in this regard, but could be the result of some external factor influencing appetite in the experimental fish. A slight reduction in ration size seen at 14°C over 8°C and 10°C may indicate that this temperature is slightly elevated above that for maximum ration ingestion in this size of Atlantic halibut.

The work of Davenport *et al.* (1990) used the technique of X-radiography to determine satiation meal size in the Atlantic halibut. These authors results showed that when offered a satiation meal, *H. hippoglossus* accepted a ration with an average of 11.7% of body weight at a standard temperature of 10°C, but could not determine any relationship between body weight and meal size in a sample of 13 fish of weight range 454g to 2334g. This figure is well in excess of the figures produced in this study, although Davenport *et al.* (1990) offered the fish a moist diet, which is likely to be less energy dense than a commercially prepared dry diet as used in this study. These authors presented no information on the energy content of the experimental diet, but stated that it consisted of finely minced saithe (*Pollachius virens*), hot water and gelatine. Jensen (1979) and Hislop *et al.* (1991) measured the total energy content of whole immature saithe using bomb calorimetry. A seasonal variation in saithe energy content was observed and although there was a variation in energy

content of this species associated with the liver index, a range of values of 3.94-6.27 kJ $g^{-1}$was recorded. Applying a mid range value of 5.11kJ g^{-1} to the figures of Davenport *et al.* (1990), an 11.7% body weight meal of fresh, moist saithe for a 1.5kg halibut would carry an energy ingestion of 896.8kJ. Using the equation presented above, relating ration size with fish weight and temperature a meal of 0.243% body weight would be predicted for a 1.5kg halibut at 10°C, possessing an energy intake of 76.9kJ as calculated for the test diet. These results may indicate differences in palatability between moist and dry diets in the Atlantic halibut, and the reduced energy intake observed with the dry diet does give cause for concern in an industry which is likely to be based on commercial dry pelleted feed. In a study of feed consumption and growth of Atlantic halibut averaging 422g body weight, Tuene and Nortvedt (1995) observed a wide range of feed consumption values, from close to zero up to a maximum sustainable feed intake of 0.85% body weight d^{-1} at a temperature of 8-9°C. The global equation produced in this study predicts a meal size of 0.716% body weight under equivalent conditions, which is within the range of values observed by Tuene and Nortvedt (1995) who also fed a commercial dry pelleted flatfish diet.

A study on wild stocks of the closely related Pacific halibut (*Hippoglossus stenolepis*) by Paul *et al.* (1994) showed meal sizes ranging from 4.1 (% body weight) for 100g fish to 1.1 (% body weight) for 6-8kg fish, although again these fish were also offered a moist diet. An inverse relationship between meal size (% body weight) and fish weight was observed by these authors. Given that ration intake is adjusted according to the energy requirements of the fish (Phillips, 1972; Steffens, 1989; Jobling, 1994), the reason for this disparity in the results of this study in comparison to the other relatively high values may be readily accounted for. From a bioenergetic perspective, the intake of a less energy dense diet such as a moist fish prepared feed is likely to be increased over more energy dense dry feed as the organism tries to maintain an energy balance, other factors such as temperature and fish weight being equal. Unfortunately, the lack of information on the energy content of the moist diet in the Davenport *et al.* (1990) study does not allow a comparison of energy intakes, which would prove of interest since there is an overlap between temperature and fish

weight between the two studies. The relatively high ingested energy value calculated above from energy values for the saithe are clearly subject to a large source of error, since the energy values of this species vary over such a wide range in the wild, and the proportions of saithe, gelatine and water in the manufactured test diet are not known. The apparent much higher ingestion rate of a moist diet is of interest for culture given the applicability of consumption-growth relationships in fish (Brett, 1979). The drawback to using moist diets in intensive culture of fish is the possibility of disease transmission, however such risks may be lessened to a degree during the preparation of diets with the application of new technology such as irradiation. The anatomy of the Atlantic halibut is clearly suited to the ingestion of a high-bulk, large meal, and the maintenance of this species on a high density dry pelleted diet may not provide optimum conditions for growth in this species. Further studies comparing growth efficiencies of dry and moist diets would be of interest in determining the optimum dietary type in this species, with a view to a viable Atlantic halibut farming industry.

There is little further reported literature on feed and energy consumption in the Atlantic halibut, and it is also interesting to compare the levels of feed ingested in this study with the manufacturer's suggested figures for daily feed ration. This information is presented in Table 4.13. There are clear differences between the figures obtained in this study with the suggested feeding rates provided by the manufacturer. Generally at the lower test temperatures all halibut ingested a larger ration than suggested as a daily quota, and at the higher temperatures larger halibut ingested a smaller ration and smaller halibut a larger ration than the suggested level. The intensive farming of the turbot (*Scophthalmus maximus*) is a well developed industry, and it is likely that any "flatfish" diet is geared towards the culture of that species. Clearly, there are factors which may have influenced the feed intake in this study, such as time of day of feeding (which was kept standard in this study, but may not be optimum), administration of the daily feed quota in one or several meals (one in this study), and the development of social hierarchies within such small populations of fish, which may have resulted in an unequal distribution of the feed intake among individuals within populations. The last point is regarded as significant in studies of ration intake by Jobling (1993). These facts aside, there is a general lack of

information in the area of feed intake in the Atlantic halibut and the results of this study emphasise the necessity of future research in this field. In particular, a more dedicated feeding regime tailored to the requirements of this organism will help to optimise culture in intensive systems.

Table 4.13. Comparison of ration size with manufacturer's suggested daily ration.

Temperature (°C)	body wt. (g)	meal size (% body wt.)	Manufacturer's suggested meal size (% body wt.)
8	1150	0.34	0.3
8	979	0.53	0.3
8	606	0.68	0.3
8	889	0.51	0.3
8	752	0.62	0.3
8	690	0.44	0.3
8	402	0.59	0.4
8	1150	0.47	0.3
8	979	0.60	0.3
8	606	0.85	0.3
8	889	0.42	0.3
8	752	0.81	0.3
8	690	0.75	0.3
8	402	0.70	0.4
10	1380	0.42	0.4
10	1109	0.55	0.4
10	700	0.63	0.4
10	964	0.84	0.4
10	795	0.68	0.4
10	757	0.22	0.4
10	436	0.49	0.5
12	1420	0.13	0.4
12	1150	0.18	0.4
12	722	0.21	0.4
12	1006	0.23	0.4
12	801	0.32	0.4
12	775	0.46	0.4
12	459	0.63	0.5
14	1462	0.25	0.4
14	1156	0.32	0.4
14	720	0.26	0.4
14	1045	0.26	0.4
14	788	0.49	0.4
14	797	0.34	0.4
14	476	0.83	0.6
14	315	0.83	0.6
14	786	0.39	0.4
14	581	0.60	0.4

Effect of a single meal on post-prandial oxygen consumption rate and Specific Dynamic Action (S.D.A.)

Studies of post-prandial oxygen consumption rates in fish have utilised both open (Solomon and Brafield, 1972; Vahl and Davenport, 1979; Jobling and Davies, 1980; Ross and McKinney, 1988a; Davenport *et al.*, 1990; Chakraborty, 1992; Chakraborty *et al.*, 1992c) and closed (Edwards *et al.*, 1969; Furnell, 1986; Sims and Davies, 1994) respirometer systems. This study measured post-prandial oxygen consumption rates in small populations of Atlantic halibut held in $1m^2$ tanks operated as open system respirometers, and despite the relatively unsophisticated nature of the apparatus, the post-prandial rate was found to be elevated over the routine oxygen consumption rate in all trials. The results are therefore consistent with the generally held theory of S.D.A. in which an increase in metabolic rate follows the ingestion of a meal (Rubner, 1902; Brody, 1945; Winberg, 1956; Kleiber, 1961; Brett and Groves, 1979; Jobling, 1981a, 1983, 1994). The pattern of increased oxygen consumption rate following feeding was for an increase to a single peak at a point approximately 18 hours following feeding, and then a general decline towards routine oxygen consumption levels. Hamada and Ida (1973) identified two distinct peaks in oxygen consumption in the carp following a single meal, and hypothesised that the second peak at 5-8 hours post feeding was related to nitrogenous excretion in this species. Other authors have shown a single post-prandial peak in oxygen consumption rate (Beamish, 1974; Tandler and Beamish, 1979, 1980, 1981; Jobling and Davies, 1980; Medland and Beamish, 1985; Brown and Cameron, 1991; Ross *et al.*, 1992; Chakraborty *et al.*, 1992c; Sims and Davies, 1994) in a variety of fish species, and this pattern of S.D.A. in fish now appears to be widely recognised (Jobling, 1994). The occurrence of both single and double post-prandial peaks in oxygen consumption concurrently in this study is less easy to account for, although the large variation seen within individual routine rate trials over a 24 hour period (Fig.4.4. to 4.7.) may have influenced these results.

Jobling (1981a) identifies three components of S.D.A., namely the peak level, the duration of effect, and the magnitude (represented by the S.D.A. coefficient), and

it is these parameters which provide opportunity for species, diet, weight and temperature comparisons. The development of a good experimental protocol is as inherently important for this work as it is for the measurement of resting oxygen consumption rate to ensure that such comparisons are valid (see Chapter 3.).

The peak level of oxygen consumption rate in this series of trials showed a wide variation, averaging an increase of 69.0 (± 20.9)% above the routine oxygen consumption rate. This result is similar to that presented by Jobling and Davies (1980) for work on S.D.A. in individual plaice held in respirometers, who showed a peak oxygen consumption rate ranging from 141.8 to 198.6% of resting oxygen consumption levels, with the height of the peak increasing with increasing ration. In this study a similar relationship was observed at the temperatures of 12°C and 14°C, with the results significant at the $p < 0.05$ level at 14°C, although the results at lower temperatures were less distinct. A selection of comparative values for peak post-prandial oxygen consumption is presented in Table 4.14. A comparison of the results of this study with other literature values shows peak oxygen consumption following feeding in the Atlantic halibut to be within the range of results obtained in other species of fish. The results of this study tend towards the low end of the range of published values, although it is interesting to note the similarity of the results achieved by Saunders (1963) in work on the cod.

The relationship between peak post-prandial oxygen consumption rate and fish weight is similar to that determined for both resting and routine oxygen consumption rate in this study, mass specific oxygen consumption rate decreasing with increasing fish size, and increasing over the test temperature range. A similar relationship has been determined in other species of flatfish such as the plaice and the flounder by Fonds *et al.* (1992). A study of the oxygen consumption of turbot held on an intensive fish farm, with readings timed to coincide with maximal oxygen demand by fish biomass, also showed a similar relationship for a fish size range of 4-1000g, over the temperature range 7°C to 16°C.

Table 4.14. A summary of peak post-prandial oxygen consumption rate values.

Species	Food	Temperature (°C)	Body weight (g)	Peak O_2 consumption	Comments	Reference
Gadus morhua	herring	3 - 15	220 - 3230	40 - 60% over routine rate	studies on individuals and small populations (<8)	Saunders (1963)
Oncorhynchus nerka	not listed	10-20	3-18	200-400% increase over resting rate	community studies; relationship between ration size and peak O_2 consumption determined	Brett (1976)
Oncorhynchus mykiss *Salmo salar*	Experimental 50% protein, and pruified CHO, lipid and protein diets	not listed	11-57 3.3-4.5	heat production 150-300% of routine rate	direct calorimetry; experimental studies on small populations feeding of own volition and force fed individuals.	Smith *et al.* (1978b)
Cyprinus carpio	Tubifex, grain, granular feed	10-30	15-70	5-250% increase over routine rate	Higher peak observed in fish fed finely ground meal	Jirásek and Adámek (1977)
Blennius pholis	*Mytilus edulis*	10	14.5-16.7	50-75% increase over routine rate	experiments performed on individual fish	Vahl and Davenport (1979)
Pleuronectes platessa	white fish paste & various modified diets	10-20		100% increase over resting rate	peak size not influenced by temperature	Jobling and Davies (1980)
Oncorhynchus mykiss	experimental diets of varying protein and lipid composition	15	165	100-200% increase over routine rate	mass respirometers containing 15, 35 and 50 fish	Medland and Beamish (1985)
Anoplopoma fimbria	chopped herring (ration size 5% of body weight)	8.5	993	40% increase over routine rate	experiments performed on small communities of 5 fish	Furnell (1986)
Cyprinus carpio	20-50% protein experimental diet	28	63.5-83.0	65.9-180.1% increase over resting rate	experiments performed on individual fish	Chakraborty *et al.* (1992c)
Anarhichas lupus *Gadus morhua* *Pleronectes platessa*	unknown	1.3-10.0	593-1429 40-1125 384-960	40-160% increase over routine rate	experiments performed on individual fish	Karamushko (1993)
Pleronectes platessa *Platichthys flesus*	*Mytilus edulis*	2-22	1-418	100% increase over routine rate	test populations of 1 large, 2-5 medium, or 5-10 small individuals	Fonds *et al.* (1992)
Hippoglossus hippoglossus	dry, commercial diet	8-14	315-1462	69% increase over routine rate	Study on small communities of fish.	this study

No significant relationship between meal size and energy ingested with S.D.A. magnitude was determined in this study at any of the test temperatures (Fig. 4.16. and Table 4.8.). A clear relationship between S.D.A. magnitude and ration size has been shown in the plaice (Edwards *et al.*, 1969; Jobling and Davies, 1980), carp (Hamada and Ida, 1973; Chakraborty *et al.*, 1992c), largemouth bass (Beamish, 1974; Tandler and Beamish, 1980), and the blenny (Vahl and Davenport, 1979). The results of this study at 8°C, 10°C and 12°C show a trend for increasing S.D.A. magnitude with increasing meal size, but the data points are well dispersed. Although the trend at 14°C is for a reversal of this pattern it may be seen that removal of the two outlying points at the highest ration level will reorient the regression line with a positive slope.

The time to peak oxygen consumption of approximately 18 hours seen in this study is considerably longer than that observed in studies of the feeding energetics of other fish species. Various times to peak oxygen consumption rate are recorded in the literature: 2-4 hours after feeding in the study of Vahl and Davenport (1979) in *Blennius pholis*; 2 hours in the largemouth bass (Beamish, 1974; Tandler and Beamish, 1981); 2-6 hours in rainbow trout (Medland and Beamish, 1985); 1-6 hours in *Sparus aurata* (Guinea and Fernandez, 1997); 2.5-6 hours in *Cyprinus carpio* (Chakraborty *et al.*, 1992c); 4-10 hours in *Scyliorhinus canicula* (Sims and Davies, 1994); 8 hours in *Ictalurus punctatus* (Randell Brown and Cameron, 1991a); 10-12 hours in aholehole (Muir and Niimi, 1972). Hamada and Ida (1973) found two post-prandial peaks in oxygen consumption rate in work on the common carp, one at 3-4 hours after feeding and the other at 5-8 hours. In this study only a single peak in oxygen consumption rate was observed, and although there is an overlap in timing with some of the above studies, overall this appeared at a point somewhat later than any of these studies. Time to peak was not found to be influenced by temperature in this work, which is similar to the results of Jobling and Davies (1980) in work on the plaice. The relatively large size of the Atlantic halibut stomach (classified by de Groot (1971) as a "Type I" pleuronectid possessing a large oesophagus and stomach, a simple intestinal loop, and large gill rakers - all characteristic of piscivorous fish), does suggest a long digestion time for a single meal, since a larger bolus of food present in the stomach of the Atlantic halibut will have a proportionately longer

digestion time than a bolus of food in the much smaller stomachs of fish such as carp, trout and salmon. A longer digestion time would lead to both a "smoothing out" of the S.D.A. curve, as well as an increase in the length of time to peak oxygen consumption rate following a meal. Although the values for ration ingested in this study are relatively low, there is the very real possibility that not every fish in the population consumed food, which would lead to a situation where the S.D.A. curve produced was a result from a few fish within a population consuming relatively larger meals. Clearly this could have an influence on the positioning of the peak value, the quantity of the peak value and the S.D.A. coefficient, since there may be differences in individual food consumption rates between trials, and comparisons are therefore less meaningful.

This possible inter-individual difference in food consumed may have accounted for the results of the S.D.A. coefficient in this study, which showed a degree of variability between trials. Although only 16 out of the 36 trials showed a decline to pre-feeding routine oxygen consumption rate levels, extrapolation of results produced figures for S.D.A. in a further 18 trials. As already stated, there are several assumptions associated with such data manipulation, although the mean S.D.A. coefficient value of 9.8% is well within the range of values produced by other workers. A range of values for the S.D.A. coefficient have been determined for a variety of fish species, and some representative figures are presented in Table 4.15. The results produced in this study cover a broad range, which may be linked to the assumption that the rate of decrease in extrapolated post-prandial oxygen consumption curves remains the same over the predicted time period, influencing the value for S.D.A. magnitude and hence S.D.A. coefficient. However, the mean value corresponds well with the values obtained in studies on other fish species, and is within the 5 to 20% range reported for estimates of S.D.A. coefficients by Priede (1985), although is below the range of 12 to 16% quoted by Brett and Groves (1979). The value of the S.D.A. coefficient decreased with ration size in this study, although the relationship was only significant at the $p < 0.05$ level at 14°C (Fig. 4.18 and Table 4.8.). In the study of Chakraborty *et al.* (1992c), S.D.A. coefficient was not found to be related to ration level in the carp, although was significantly correlated with protein

Table 4.15. Literature values for the S.D.A. coefficient in a variety of fish species.

Species	Food	S.D.A. coefficient (%)	Comments	Reference
Pleuronectes platessa	white fish paste	12.35 - 19.22	proportional to dietary protein content	Jobling and Davies (1980)
Cyprinus carpio	dry, pelleted	8.99 - 15.94	proportional to dietary protein content	Chakraborty *et al.* (1992)
Micropterus salmoides	Emerald shiner	14.2	combination study involving measurements in swimming fish	Beamish (1974)
Micropterus salmoides	dry, pelleted	11.3	S.D.A peak related to energy ingested and fish weight	Tandler and Beamish (1981)
Gadus morhua	pelleted	2.91 - 19.92	temperature range of 7°C to 18°C	Soofiani and Hawkins (1982)
Oncorhynchus mykiss	dry, pelleted	10.9 - 28.1	community study	Medland and Beamish (1985)
Oncorhynchus mykiss	dry, pelleted	8.0 - 12.0	influenced by dietary composition	Cho *et al.* (1976)
Hippoglossus hippoglossus	commercial, dry, pelleted	2.1-30.0	community study	This study

content of diet. Jobling and Davies (1980) also found protein content of diet to be an important factor in determining the value of S.D.A. coefficient, diets with a higher percentage protein influencing the magnitude of the S.D.A. effect and the value of the S.D.A. coefficient in the plaice. These authors also noted that to a lesser extent, the magnitude of the S.D.A. was linked to the energy content of the dietary constituents. No significant relationship between S.D.A. coefficient and fish weight was determined in the results of this study, similar to the study on largemouth bass by Beamish (1974) and the study on carp of Chakraborty *et al.* (1992c).

Overall this series of trials is characterised by a high degree of variation in results which is most likely to stem from observations on populations of fish rather than individuals. Although successful studies of S.D.A. have been achieved in fish community studies (Medland and Beamish, 1985), generally much of this work has been undertaken on single fish in individual respirometers (Vahl and Davenport, 1979; Jobling and Davies, 1980; Ross and McKinney, 1988c; Chakraborty *et al.*, 1992c). However, although a similar technique was attempted in this study utilising the respirometers described in Chapter 3., Atlantic halibut could not be trained to feed in the system, and the work was therefore carried out on small stock populations of halibut in tanks. The most obvious possibility for variation in results stems from using routine rate measurements as baseline for such work. Calculated values for routine oxygen consumption rate were used as baseline prefeeding levels of oxygen consumption rate, however this draws on the assumption that routine levels are similar throughout. Routine rate measurements may differ between pre- and post-feeding, thus affecting the calculation of the S.D.A. coefficient. A higher incidence of aggression was generally observed in starved populations which would also increase any discrepancy between starved and fed fish. Aggressive social interactions and the development of hierarchies could have influenced feed intake, and also affected routine oxygen consumption rates. Clearly future studies should centre around S.D.A. in individually held Atlantic halibut, once the production of juvenile fish has reached the stage at which the supply of a standard size of fish allows interpretation of S.D.A. in response to diet type (varying energy level and protein content) and water temperature. The respirometer system used for the determination of resting oxygen

consumption rate in this study proved successful in this regard, although the halibut could not be trained to feed when held individually within respirometers. Atlantic halibut are considered to be visual feeders (Leim and Scott, 1966), and observations on feeding individuals held in tanks showed that there is a forwards "lunge" of 0.5-1.0 body lengths immediately prior to the ingestion of a feed pellet. Halibut were allocated to respirometers according to size in order that movement was restricted and true "resting" oxygen consumption values were obtained, and restriction of this sort may be counterproductive in terms of getting the organism to feed voluntarily. Some design modification may enhance the success of feeding individual halibut in respirometers, although it is important to note that any increase in respirometer space to accommodate feeding lunges may also allow a level of spontaneous activity which will influence oxygen consumption rate.

It is interesting to compare the values and pattern of post-prandial oxygen consumption rate produced in this study with other species which possess anatomical and life history similarities. Obvious comparisons may be drawn with the cod, *Gadus morhua*, which occupies a similar ecological niche in temperate marine ecosystems, and also possesses a relatively low resting oxygen consumption rate (see Chapter 3.) and a large stomach. Moreover, there is a wide overlap in temperature preferenda for these species, and the cod is also regarded as showing potential for culture in temperate regions (Tilseth, 1990). In his study, Saunders (1963) found oxygen consumption of cod to remain elevated for a considerable length of time (7 days at 10°C; 4-5 days at 15°C) when fed large meals of herring, with peak values obtained at a point between 12 and 24 hours post feeding. In another study on the cod, Soofiani and Hawkins (1982) determined the energetic costs of feeding over a range of temperatures between 7°C and 18°C at five different feeding levels. These authors found that peak oxygen consumption rate increased with size of ration, and that this relationship was statistically significant for all test temperatures. Overall the increase in post-prandial oxygen consumption rate was slow, with the decline dependent on temperature and ration size, and these authors hypothesised that there is a slow rate of assimilation in this species with the associated energetic costs having a wide temporal distribution. A comparison of fed metabolic rate with active metabolic rate (values

produced from cod exercised in swim-system respirometers) produced very similar values, indicating that in fed cod a large portion of the metabolic scope is involved in assimilation of a meal, consequently limiting the scope for activity. The cod appears to be unusual in this respect, with values for M_F approaching and sometimes exceeding those of M_A. This phenomenon has been explored further, and is discussed by Priede (1985). More recently, the results of Schurmann and Steffensen (1997) on active metabolic rate suggest that the metabolic scope in the cod may be slightly higher than was first hypothesised, and these authors concluded that the cod possesses a similar respiratory physiology to other species of fish, contrary to earlier reports. From a bioenergetic perspective the cod is of interest, not least because in sharing a similar natural life history pattern to the Atlantic halibut it provides good comparative information on the partitioning of metabolic energy, and also shows potential as another marine species for culture in temperate regions. An investigation into the swimming energetics of the halibut would prove of considerable interest as a comparison with the cod, since it is unlikely that the M_A component is large given the results of other studies into flatfish activity (Priede and Holliday, 1980; Duthie, 1982), and therefore metabolic scope in this species may also be limited.

Effect of body weight and temperature on endogenous Ammonia excretion rate.

An inverse relationship between endogenous ammonia excretion rate and body weight in the Atlantic halibut was determined from the results of this work. The rate of endogenous ammonia excretion was observed to increase with increasing temperature over the test range of 8°C to 12°C. This is a similar pattern to that observed with routine oxygen consumption rate, body weight and temperature. The mean weight exponent of 0.548 determined in this study agrees with the figure of 0.54 of Gerking (1955) who determined a similar weight dependent relationship for endogenous nitrogen excretion in the bluegill sunfish. Endogenous ammonia excretion did not exhibit any degree of rhythmicity over a 24 hour period.

Endogenous ammonia excretion arises from the catabolism of proteins and represents a loss of energy to the organism (Pandian, 1987). The condition factor of

the fish is of importance in such studies, since there may be preferential metabolism of fat in well conditioned fish, followed by a proportional increase in metabolism of somatic protein as the fish progressively loses condition over the starvation period. In this study, starvation periods were maintained constant, with fish only having just entered the postabsorptive phase. In the absence of any nutritional stressor, trends in level of metabolic rate are likely to be reflected in nitrogenous excretion rates as protein are used as a primary source of energy in fish (Jobling, 1994).

A similar relationship was observed by Jobling (1981b) in the plaice, who found total ammonia nitrogen to account for 75-85% of the nitrogenous excretion in juvenile fish. A weight exponent of 0.67 was determined for the allometric equation describing endogenous ammonia excretion. In this study the level of significance of the relationship was reduced at 10°C, although a single value produced at the small end of the size range could clearly have influenced this result. Discounting the results for 10°C, the common weight exponent for endogenous ammonia excretion in the Atlantic halibut is 0.62, a figure close to that produced for plaice by Jobling (1981b).

A summary of values for endogenous ammonia excretion rate in a variety of fish species is presented in Table 4.16. The results of this study are towards the low end of the range of values produced for other species, but there is close agreement with the results of Davenport *et al.* (1990) for Atlantic halibut, Jobling (1981b) for the plaice, and Gaumet *et al.* (1994) for the turbot. Davenport *et al.* (1990) measured the excretion rate of total ammonia nitrogen in Atlantic halibut placed in shallow vessels by the sequential extraction of water samples over a period of 6 hours. No relationship between fish weight and endogenous ammonia excretion rate was determined. Using the equation relating endogenous ammonia excretion, body weight and temperature produced in this study, a value of 0.85 mg kg^{-1} h^{-1} is provided for a 1383g fish at 10°C, which is clearly somewhat lower than the figure of Davenport *et al.* (1990). Possible reasons for this discrepancy centre around the difference in protocol between the two studies, with this study providing information from open system respirometers and Davenport *et al.* (1990) utilising what were effectively closed system respirometers, and again emphasise the importance of a common

Table 4.16. A summary of endogenous ammonia excretion rate values in a variety of fish species.

Species	Endogenous ammonia excretion rate (mg kg^{-1} h^{-1})	Fish size (g)	Temperature (°C)	Comments	Reference
Oncorhynchus nerka	7.27	28.6	15	Measurements on small populations of fingerling sockeye in fresh water	Brett and Zala (1975)
Oncorhynchus mykiss	2.67	130	15-18	Small numbers (3-15) fish maintained in a circular trough	Kaushik (1980)
Cyrpinus carpio	2.16	350	16-18		
Pleuronectes platessa	1.13	5-90	10	Experiments on individual fish	Jobling (1981b)
Gadus morhua	6.52	199.3	14	Ammonia excretion continuously monitored in small popualtions of fish before and after feeding	Ramnarine *et al.* (1987)
Hippoglossus hippoglossus	2.32	454-2334	10	Measurements made on sequentially extracted water samples from shallow vessels containing individual halibut	Davenport *et al.* (1990)
Cyprinus carpio	4.09-5.20	65	28	Individual fish held in metabolism chambers	Chakraborty *et al.* (1992b)
Scophthalmus maximus	2.00	80	16	Measurements made on small population of fish over a 10 day period	Gaumet *et al.* (1994)
Hippoglossus hippoglossus	0.48-1.50 (mean 0.93)	402.3-1420.0	8-12	Measurements made on small populations of fish over a 24 hour period	This study

Endogenous ammonia excretion was measured at resting levels in the carp by Chakraborty *et al.* (1992b) and in the Atlantic halibut by Davenport *et al.* (1990); In all other studies presented, endogenous ammonia excretion was measured at routine levels.

protocol in fish energetic work. Particularly the elements of stress, and the effect of increasing levels of metabolites in closed system respirometry are areas which may influence results. This study produced values from Atlantic halibut held under conditions which were very close to the daily regime.

Jobling (1981b) produces a regression equation for the endogenous excretion of total ammonia at 10°C in the plaice. For a body weight of 1383g this equation predicts an endogenous ammonia excretion rate of 0.78 mg kg^{-1} h^{-1}. This is in close agreement with the work of this study, however the extrapolation of Jobling's (1981b) equation to this body weight may not be valid since it was conducted on fish of a much smaller size range.

Clearly the values produced in this work for the Atlantic halibut are relatively low when compared to other species, although there is little data allowing comparisons of closely related species at a similar body weight and temperature. Further studies on halibut held individually in respirometers will provide more exact data on endogenous ammonia excretion in this species, and further elucidate the relationship with body weight and temperature.

Effect of a single meal on Ammonia excretion rate.

Ammonia is the predominant compound of nitrogenous excretion in teleosts, although urea and trimethylamine oxide may also contribute to this process in marine species (Brett and Groves, 1979). In freshwater species ammonia is considered to comprise 80-98% of nitrogenous excretion (Brett, 1962; Forster and Goldstein, 1969; Solomon and Brafield, 1972), and in marine species 75-85% (Jobling, 1981a). Only ammonia was examined in this study, however both the endogenous and exogenous fractions of ammonia excretion were examined, which provides useful information in the determination of an energy budget.

The trend observed in this study was for ammonia excretion to rise steadily to a single peak following feeding, and then decline over a longer period of time. This

pattern of post-prandial ammonia excretion has also been observed in other fish species such as the cod (Ramnarine *et al.*, 1987), the plaice (Jobling, 1981b), the turbot (Gaumet *et al.*, 1994), sockeye salmon (Brett and Zala, 1975), eel (Heinsbroek *et al.*, 1993), and carp (Kaushik, 1980; Chakraborty *et al.*, 1992b; Heinsbroek *et al.*, 1991). The time taken for post-prandial ammonia excretion rate to decline to pre-feeding levels has been determined by some authors, and some of these values are compared in Table 4.17. The response closely followed the pattern of S.D.A., and examples of time series curves for the temperatures 8°C, 10°C and 12°C are presented in Fig. 4.34. Peaks in oxygen consumption rate preceded ammonia excretion peaks by between 3 and 15 hours.

A wide range of values is reported for the duration of elevated ammonia excretion following feeding, with the complexities of temperature, fish size, feed type and ration size drawing caution over any direct species comparisons without full knowledge of the experimental conditions. The results of this work provide information on post-prandial ammonia excretion in the Atlantic halibut which is of interest in determining the energy cost of the *U* component of the bioenergetic equation, and also for predicting the effects of feeding on tank environment water quality in intensive culture. These figures are of particular interest for recirculation technology, in which ammonia excretion rate is a limiting factor in the effective operation of enclosed systems.

In this study, relationships between meal size, quantity of energy and protein ingested, with value of peak post-prandial ammonia excretion, time to peak ammonia excretion, time to return to endogenous ammonia excretion rate, and quantity of post-prandial ammonia excretion were determined at varying levels of significance for the temperatures 8°C, 10°C and 12°C. In addition, relationships between fish weight and value of peak ammonia excretion rate, and fish weight and quantity of ammonia excreted were also determined for these temperatures. Similar to the S.D.A. results, the value of the correlation coefficient, r, showed a wide variation between temperatures within the same determinations (Table 4.10.), although the significance of this variability is unclear.

Table 4.17. Literature values for post-prandial ammonia excretion in several fish species.

Species	Food	Temperature (°C)	Time to peak NH_3-N excretion (h)	Increase over endogenous rate	Time to decline to pre-feeding levels (h)	Comments	Reference
Oncorhynchus nerka	moist commercial pellet	15	4.5	5		Community study	Brett and Zala (1975)
Cyprinus carpio *Oncorhynchus mykiss*	experimental diet - herring meal based	16-18 15-18	2-10 4-11	1-10 1-10	24 24	Community studies	Kaushik (1980)
Pleuronectes platessa	minced white fish	10		2 - 11	48 - 96	Peak and duration of effect increased with amount of nitrogen absorbed from food	Jobling (1981b)
Gadus morhua	moist, lab prepared	14	6.5 - 27	1.89 - 6.32	19 - 95	Increase in meal size increased time to peak, and duration	Ramnarine *et al.* (1987)
Hippoglossus hippoglossus	minced saithe fillets	10	24	2		shallow water vessels used for measurements on individual fish	Davenport *et al.* (1990)
Cyprinus carpio	prepared diet	28	5-7	9 - 17	17 - 22	Peak rate of excretion and duration increased with increasing ration level	Chakraborty *et al.* (1992b)
Anguilla anguilla	extruded pellet	19.8-25.0	8	4.8	24	Continuous measurement	Heinsbroek *et al.* (1993)
Cyprinus carpio	extruded pellet	19.8-25.0	7.5	6	18	Continuous measurement	Heinsbroek *et al.* (1993)
Scophthalmus maximus	experimental pellet	16	9	8		Continuous measurement	Gaumet *et al.* (1994)
Scophthalmus maximus	expanded dry pellet	8-20	6-7	2-5	19-20	ammonia excretion rate related to temperature	Burel *et al.* (1996)
Hippoglossus hippoglossus	dry commercial pelleted diet	8-12	8 - 26	2 - 9	26-59	Community study	This study

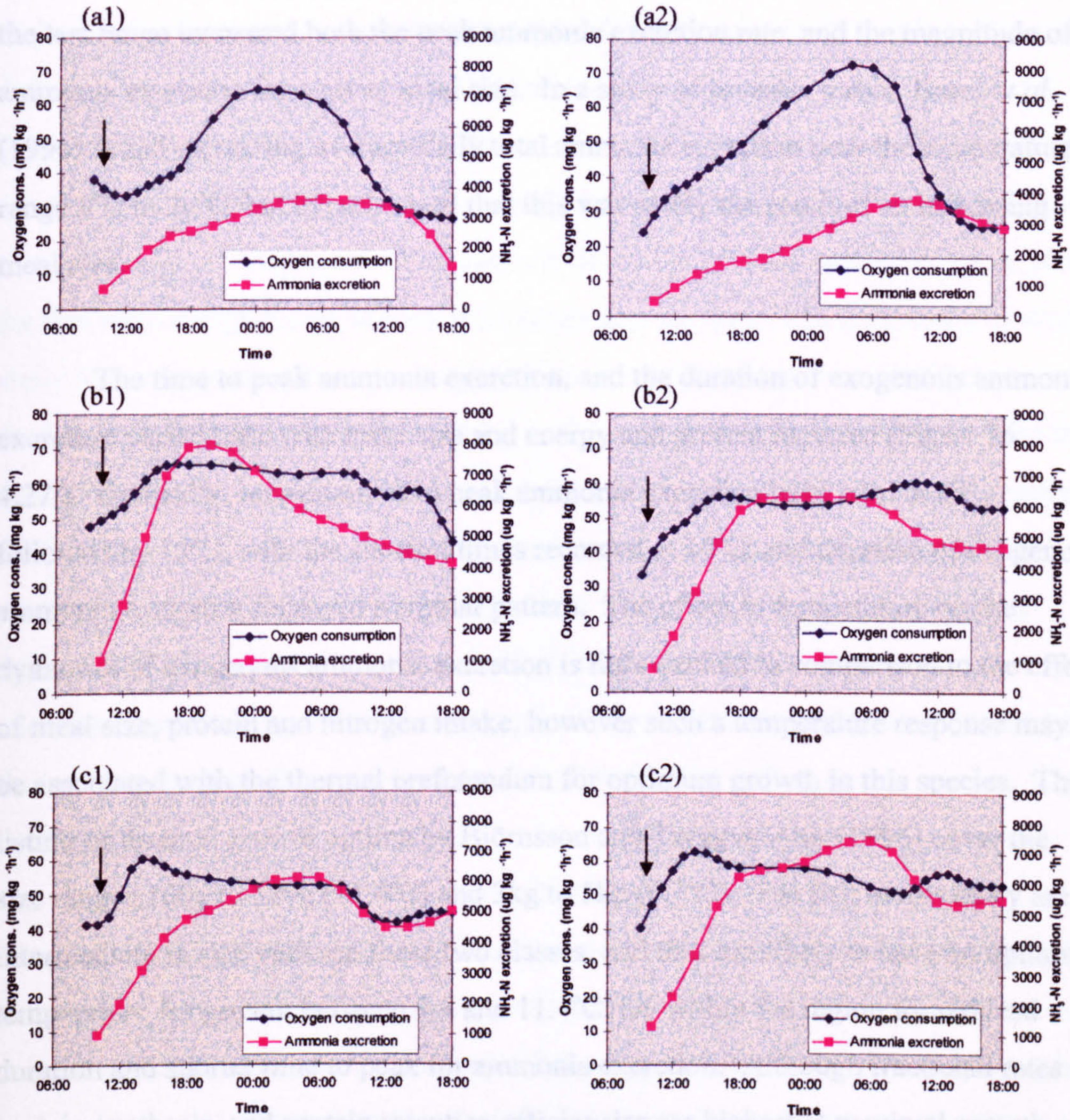


Fig. 4.34. Pattern of post-prandial oxygen consumption and ammonia excretion in tank populations of Atlantic halibut, indicating timing of peaks. Temperatures are (a) 8°C, (b) 10°C and (c) 12°C. Tank populations are: (a1) 6 fish, 979g average weight, meal size 0.60% body weight; (a2) 10 fish, 889g average weight, meal size 0.42% body weight; (b1) 11 fish, 795g average weight, meal size 0.68% body weight; (b2) 11 fish, 964g average weight, meal size 0.68% body weight; (c1) 10 fish, 1150g average weight, meal size 0.18% body weight; (c2) 7 fish, 1420g average weight, meal size 0.13% body weight. Black vertical arrows indicate time of feeding.

In the study of Chakraborty *et al.* (1992b), ammonia excretion was observed to increase with dietary protein content, and the results of this study are similar with both peak ammonia excretion rate (Fig.4.25.) and quantity of post-prandial ammonia excretion increasing with increasing meal size, and quantity of energy and protein

ingested at the test temperatures of 8°C, 10°C and 12°C. Increasing temperature over the test range increased both the peak ammonia excretion rate, and the magnitude of ammonia excretion for a given meal size. In a study of juvenile turbot, Burel *et al.* (1996) found increasing average daily total ammonia excretion over the temperature range 8°C to 20°C, but hypothesised that this was solely the result of an increasing meal size.

The time to peak ammonia excretion, and the duration of exogenous ammonia excretion varied little with meal size and energy and protein ingested (Figs.4.26, 4.27.). Generally, longest times to peak ammonia excretion were seen at 8°C, followed by 12°C, with the shortest times recorded at 10°C, and duration of exogenous ammonia excretion followed a similar pattern. The effect of temperature on the dynamics of exogenous ammonia excretion is little studied in comparison to the effect of meal size, protein and nitrogen intake, however such a temperature response may be associated with the thermal preferendum for optimum growth in this species. The listing of thermal growth optima by Björnsson and Tryggvadóttir (1996) cover the size ranges 100g to 500g (11.4°C) and 3kg to 5kg (9.7°C). The fish in this study are intermediate in size between these two classes, and thus are likely to have an optimum temperature for growth between 9.7 and 11.4°C, i.e. within the region for reduced duration and shorter time to peak for ammonia excretion. Although fractional rates of protein synthesis, and protein retention efficiencies are highest at maximal growth rates, an anabolic increase in proteolysis with growth rate has been recognised in mammals, and is considered to be true for fish (Houlihan, 1991). An increased rate of protein turnover could have the effect of reducing the time to peak ammonia excretion, and the overall duration of response, and the temperature differences observed in this study may be linked to this phenomenon.

A comparison of literature values for post-prandial ammonia excretion in a variety of fish species is presented in Table 4.17. The results of these experiments fall within the values of other researchers working with a variety of roundfish and flatfish species, and it is of interest to note the similarity of the results of Ramnarine *et al.* (1987) for work on the cod, with those of this study. The peak excretion rate observed

in turbot (Gaumet *et al.*, 1994) also falls within the range of this study, and provides an interesting comparison with another cultured flatfish species. The only comparative data for Atlantic halibut is produced by Davenport *et al.* (1990), who determined a figure of 5.08 mg kg^{-1} h^{-1} ammonia nitrogen excreted in halibut fed a single moist diet meal at 10°C. These authors results for fed Atlantic halibut utilised a similar method to that which they used for the measurement of endogenous ammonia excretion, and the fish were sampled only at a point 24 hours after feeding. The results of this study show the location of peak ammonia excretion rate to be between 8 and 26 hours post feeding, and although there is an overlap with the time of sampling, there remains the possibility that the ammonia excretion rate had declined by the point of measurement by Davenport *et al.* (1990). Meal size also showed a considerable variation in the study, ranging between 3.63 and 16.98% body weight for a moist diet. With this study confirming a significant relationship between meal size and peak ammonia excretion rate, there is a clear possibility that the published results were influenced by these parameters, leading to the production of such a low value.

In contrast to the results for the S.D.A. study, no significant relationship between fish weight and peak ammonia excretion rate could be determined (Fig. 4.31, Table 4.11.). This result is surprising given the relationships between fish weight and endogenous ammonia excretion rate, and fish weight and meal size determined earlier in this chapter. Houlihan (1991) also notes that liver protein synthesis rates show an increase associated with the increased oxygen consumption rate due to S.D.A., and any related deamination of proteins would be likely to result in a parallel increased ammonia excretion.

The results for post-prandial ammonia excretion observed in this study, and figures produced for other species such as cod, plaice and turbot would appear somewhat analagous to the pattern of post-prandial oxygen consumption in the cod hypothesised by Soofiani and Hawkins (1982) to relate to slow digestion rates. It is not altogether surprising that the pattern of ammonia excretion should mimic that of oxygen consumption following feeding, given the relationship to protein metabolism and the production of energy and growth in fish. The similarities between the Atlantic

halibut and the cod are of great interest, given the potential of both these species for temperate marine aquaculture (Tilseth, 1990). The results of this study have direct application in the intensive farm production of Atlantic halibut, allowing the prediction of oxygen consumption and ammonia excretion rates in culture situations. This is particularly pertinent for recirculation systems in which the precise calculation of oxygen uptake and ammonia loading within the system is of importance in maintaining the functioning of the system and the health of the organisms.

Energy Budget Calculation

The results of Chapters 3 and 4 in this thesis provide information on the energy costs associated with resting metabolic rate, spontaneous activity and S.D.A., as well as exogenous and endogenous ammonia excretion in the Atlantic halibut. This knowledge provides a base for the generation of a predictive energetic model, taking into account the partitioning of energy within the M_M, M_A, M_F and U components of the energy budget equation. Drawing on the equations predicting resting and routine oxygen consumption rate, the application of the oxycalorific coefficient13.56J mg^{-1} O_2 (Brett and Groves, 1979) supplies an energy equivalent for the M_M and M_A components. Using the mean S.D.A. coefficient of 9.8, and the predictive meal size and energy intake equations, a value for the M_F component may also be identified. This assumes the daily intake of a single meal of the quantity determined in this study. An energetic equivalent of 0.62 cal mg^{-1} (= 13.73J mg^{-1} NH_3-N) for carnivorous ammoneotelic fish is provided by Elliott and Davison (1975), and this factor may also be used to predict the energy costs associated with the U component, in association with the equations predicting endogenous ammonia excretion and post-prandial magnitude with body weight. Berge and Storebakken (1991) and Berge *et al.* (1991) carried out digestibility determination on captive Atlantic halibut fed moist diets, and found the apparent digestibility of lipid to be within the range 85-95%, and protein 84-86%. Digestibility of carbohydrate was not determined in these studies. There is a lack of information on the digestibilities of dry diets in this species, and faecal energy loss was not determined in this study. By applying values for lipid digestibility of 90% and protein digestibility of 85%, an approximate value for digestible energy may

be determined. These values are also identical to the proportions of these nutrients in turbot faeces in animals fed a semi-moist diet predicted by Nijhof (1994), who also gave a value of 65% digestibility of carbohydrate in that flatfish. Assuming that these digestibilities are applicable to Atlantic halibut fed a dry pelleted diet, and by applying energy equivalents for protein, lipid and carbohydrate, a figure of 16.444 kJ g^{-1} digestible energy is produced. This value is 77.93% of gross energy, and predicts an energy loss of 22.07% in the *F* component. Drawing from this information, the predicted percentage daily energetic requirements for 100g, 500g, 1000g and 1500g Atlantic halibut at a temperature of 10°C may be calculated. These values are tabulated in Table 4.18. and are presented schematically in Fig.4.35.

Table 4.18. Percentage daily energy allocation to energy budget components in tank held populations of the Atlantic halibut fed a commercial dry pelleted diet at 10°C.

	Fish size (g)			
Energy Budget component	**100**	**500**	**1000**	**1500**
E ingested kJ $fish^{-1}$ d^{-1}	16.88	67.52	92.84	75.96
M_M	10.4	9.5	12.0	20.4
M_A	15.1	12.1	14.5	23.7
M_F	9.8	9.8	9.8	9.8
U endogenous	0.3	0.2	0.3	0.5
U exogenous	0.9	1.0	1.2	1.8
F	22.1	22.1	22.1	22.1
P	41.4	45.4	40.1	21.8

Energy budget component values are expressed as daily energy allocation to the component as a percentage of the daily ingested value. The M_A component relates to spontaneous activity within a $1m^2$ tank.

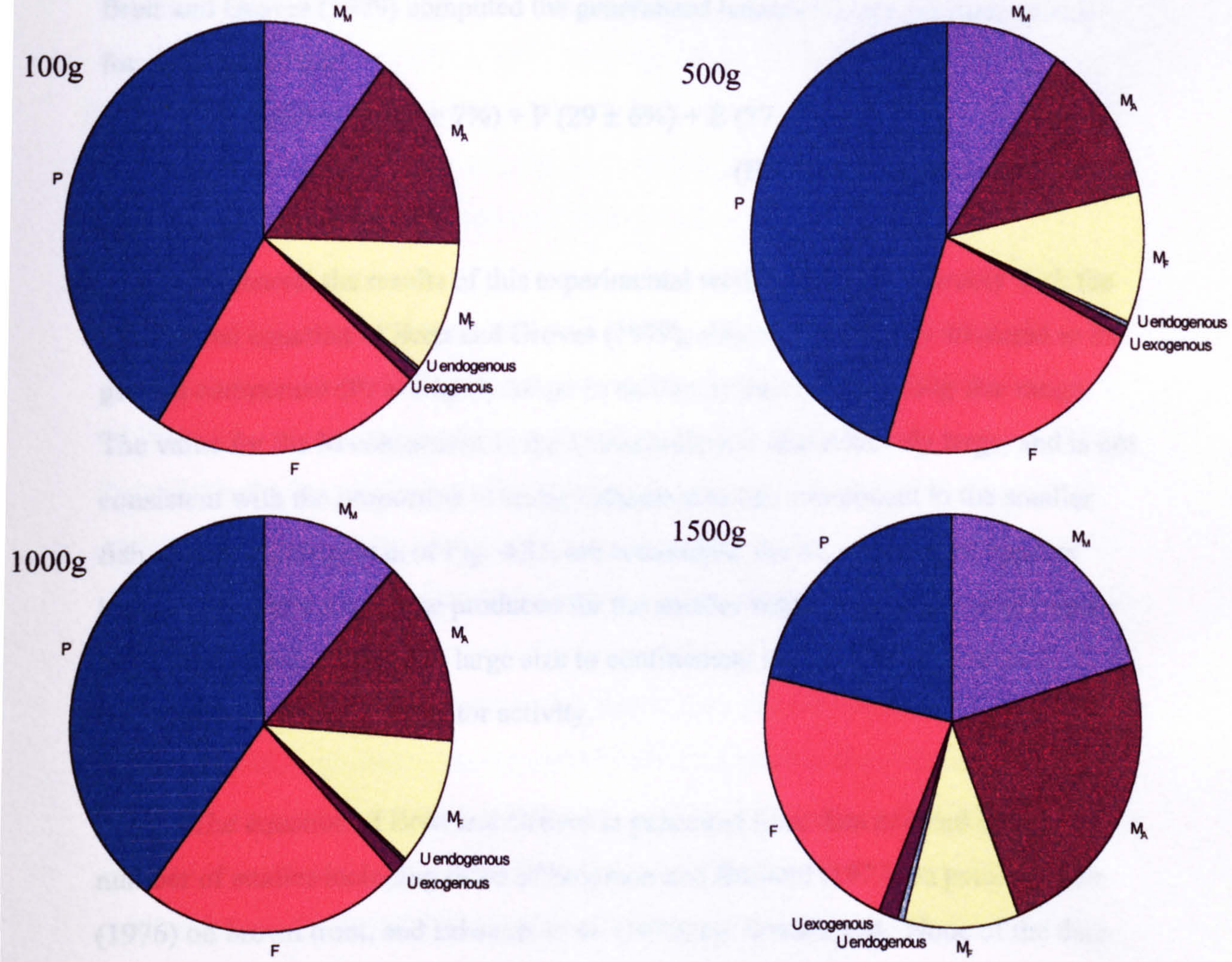


Fig. 4.35. Schematic representation of percentage daily energy allocation within energy budget components in tank held populations of Atlantic halibut fed a dry commercial pelleted diet, for fish of 100g, 500g, 1000g and 1500g at a temperature of 10°C. The M_A component relates to spontaneous activity within a $1m^2$ tank.

When expressed in the form of the energy budget equation, R = F + U + M + P, the following balanced equations are produced:

100g halibut R (100%) = F (22.1%) + U (1.2%) + M (35.3%) + P (41.4%)

500g halibut R (100%) = F (22.1%) + U (1.2%) + M (31.4%) + P (45.4%)

1000g halibut R (100%) = F (22.1%) + U (1.5%) + M (36.3%) + P (40.1%)

1500g halibut R (100%) = F (22.1%) + U (2.3%) + M (53.9%) + P (21.8%)

Brett and Groves (1979) computed the generalised balanced energy budget equation for carnivorous fish:

$$R\ (100\%) = M\ (44 \pm 7\%) + P\ (29 \pm 6\%) + E\ (27 \pm 3\%)$$

(E = F + U components)

In general the results of this experimental work compare favourably with the generalised equation of Brett and Groves (1979), although the energy allocated to the growth component (P) is slightly larger in halibut of the 100g to 1000g size range. The value for the M component in the 1500g halibut is also relatively large, and is not consistent with the proportion of energy allocated to this component in the smaller fish. When the diagrams of Fig. 4.35. are considered, the M_A component appears large in comparison to those produced for the smaller halibut, and may reflect a poor response of halibut of such a large size to confinement in small tanks with a corresponding increase in motor activity.

The equation of Brett and Groves is generated from data collated from a number of studies including those of Solomon and Brafield (1972) on perch, Elliott (1976) on brown trout, and Edwards *et al.* (1972) on Atlantic cod. None of the data includes data on flatfish, and the differences between the equations summarising the work of this study and the equation of Brett and Groves (1979) may reflect this. It is of note that the values for the M component in this study for 100g to 1000g halibut are at the extreme lower end of the range of values in the generalised carnivorous fish energy budget. This may illustrate comparative energetic savings in metabolism in flatfish relative to differences in physiology, morphology and behaviour over roundfish species. Any reduction of the energetic costs allocated to the M component allows the contribution of a greater proportion of metabolisable energy towards growth. In a relatively sedentary flatfish species this is likely to be advantageous in the natural environment, where a greater premium will be placed on growth and the attainment of a large size, rather than on scope for activity.

Chapter 5.

Diurnal Pattern of Activity in the Atlantic halibut (*Hippoglossus hippoglossus* L.).

5.1. Introduction

Intensive aquaculture systems are ideally positioned to benefit from the development of bioenergetic models, with the practical application of such models facilitating optimal production of cultured species (Knights, 1985; Ross *et al.*, 1988c). Essentially the use of bioenergetic models allows the prediction of growth under a variety of conditions through detailed knowledge of the physiological energetics of the cultured species, principally resulting in a more efficient utilisation of feed inputs to the system. Whilst a great deal of research effort has been devoted to resting metabolism and specific dynamic action within bioenergetic models for cultured species, the M_A component has received relatively little attention, although it is likely to comprise a significant portion of the energy budget. Rowan and Rasmussen (1996) estimate that the metabolic costs of activity in wild fish may be as large as 40% of consumption, with considerable variation between populations of fish. Laboratory studies of swimming energetics in fish are relatively few in number in comparison to studies of resting metabolism and S.D.A. Brett's studies on the energetic costs of swimming activity in the sockeye salmon (Brett, 1964; 1965; Brett and Glass, 1973) are of great historical importance in this field, and the techniques employed by this author have provided the basis for much later work covering a range of fish species. Locomotion through the aquatic medium is energetically costly, and Jobling (1993) states that as a general cross-species guide, active metabolic rate in fish forced to swim for prolonged periods shows an elevation of between four and seven times the resting metabolic rate.

Studies on the M_A component in energy budgets of fish have generally involved the measurement of active oxygen consumption rate when a fish has been forced to swim against a water current of known velocity (Brett, 1964, 1965; Kutty, 1968; Farmer and Beamish, 1969; Webb, 1971; Brett, 1972; Brett and Glass, 1973;

Priede and Holliday, 1980; Duthie, 1982; Bushnell *et al.*, 1994). When conducted over a range of flow velocities, these studies provide information on the metabolic requirements of individual fish swimming at known speeds, and if knowledge on resting metabolism has been acquired estimates of the "metabolic scope" may be predicted (Priede, 1985). The concept of metabolic scope was conceived by Brody (1945) for application to the production of homeotherms, and it has been defined by Fry (1947) for fish studies as the difference between resting metabolism, and "active" metabolism (calculated as the maximum aerobic metabolic rate), and is the metabolic range within which the animal's metabolic processes must be regulated in order that it may function. The maximum aerobic metabolic rate is regarded as that level of activity which may be sustained by the fish for a period of one hour (Braaten, 1979), although Brett (1964) quotes a time duration in excess of 200 minutes for such studies of sustained performance. This level of swimming activity is known as " maximum sustained swimming" (Brett, 1964). Similarly to the situation regarding measurements of resting metabolic rate, it is clear that a well defined protocol must be adhered to in activity studies in order that species comparisons may be made without undue influence of uncontrolled variables.

In a review of the metabolic scope in fish, Priede (1985) drew an analogy with industrial machines which must operate components and power levels well below maximum levels to ensure reliability in day to day use. This author states that within fish a positive energy balance must be maintained, with the rate of energy flow within the system defined by the metabolic scope. Enforced activity above or below the limits of the metabolic scope implies the certain death of the organism (Priede, 1977).

The prediction of the energy costs associated with activity has posed a problem in studies of both cultured and wild fish. The technique of radio-telemetry has been successfully applied to field studies involving fish in the natural environment (Holliday *et al.*, 1974; Priede and Tytler, 1977; Priede and Young, 1977; Stewart *et al.*, 1983), and useful bioenergetic models for fish living under such conditions have been developed from information gathered by this method (Priede, 1985). More recently, video activity monitoring systems have been utilised to provide information

on fish activity levels in both wild and tank held populations (Koch and Wieser, 1983; Sims *et al.*, 1993; Trudel and Boisclair, 1996; Ross and de Rooi, unpublished results), making it possible to monitor activity of groups of fish, or individuals within groups, and assess daily activity. Kaufmann (1983) notes that the separation of the M_M and M_A components within the energy budget is a complex issue, and this author developed a video activity monitoring processor which allowed long term monitoring of small populations of fish. Such long term monitoring reduces the possibility of sample bias linked to single point sampling, giving a clearer view of daily activity patterns. Three important points for the study of fish activity were emphasised by Kaufmann (1983), namely that the movement of several objects be simultaneously recorded; that the system is suitable for animals of different shapes and sizes; and that minimal disturbance to the animals under investigation is achieved.

The energetic costs of the activity of fish held in culture conditions are likely to be influenced by the social interactions of individuals within a single population. The work of Feldmeth (1983) on the species *Cyprinodon nevadensis amargosae*, *C. pecosensis* and *Oncorhynchus mykiss* (Walbaum) produced estimates for metabolic costs of social interactions by combining data for field and laboratory studies, although the results of this study suggest that such interactions are not as energetically costly as may be predicted. The application of physiological data produced from fish swimming under conditions of forced activity to general energy budgets where the activity is spontaneous, has been brought into question by Boisclair and Tang (1993). These authors examined published data on oxygen consumption rate of spontaneously active fish, and fish forced to swim at speed from a total of thirteen studies carried out between 1964 and 1990. Energetic costs of between 1.6 to 9.4 times greater than forced swimming experiments were estimated for conditions of spontaneous activity, in ten roundfish species of similar size and at similar swimming speeds. However, these authors conceded that currently there is little alternative but to apply forced swimming data to field studies given the lack of data on spontaneous activity energy costs governing a range of fish weights and swim speeds. These studies imply an element of caution in the cross-application of data produced under different experimental conditions.

In a critique of observational studies of behaviour, Altmann (1974) identifies seven different types of sampling method and the recommended use for each category. This author notes the importance of ensuring that there is no element of bias within the sample, either inherently associated with sample timing or generated through the sampling process. Concurrent multiple sampling achieved through monitoring of a whole social group of animals, and subsequent analysis of the behaviour into several categories, is viewed as the most efficient method of research into group behaviour. This type of work has a clear application in studies of the bioenergetics of cultured fish, in which the daily estimates of motor activity energy costs may be assessed and accounted for within the energy budget.

Studies into the existence of behavioural rhythms in fish activity patterns are a relatively recent phenomenon, although the application of the results of such studies have clear implications for aquaculture. Eriksson (1978), who notes that the study of circadian rhythms in fish increased substantially from 1960 onwards, states that light is the main environmental variable affecting such rhythms, although the results of many studies are indistinct. Collating data from a series of trials, Eriksson (1978) provided evidence for a diurnal rhythm, a nocturnal rhythm, and dualistic behaviour in the brown trout (*Salmo trutta*), under varying conditions of photoperiod. He concluded that this species possesses a "dual phasing capacity" in diel locomotor activity, with the phasing of the activity pattern considered to be modified through the light regime. It was argued that this confers an adaptive advantage for an opportunistic species within the ecological niche.

Many fish species exhibit diurnal variations in activity with a related rhythmicity in oxygen consumption rates, and a bimodal crepuscular rhythm has been shown in rainbow trout (Kausch, 1972 in Hepher, 1988). In a study on juvenile Atlantic salmon, Hirata (1973) observed diurnal peaks of oxygen consumption, feeding and activity in fish held under ambient, 6L:6D and 12L:12D photoperiods. Under conditions of constant light, or constant dark, no rhythmicity was evident. The silver hake (*Merluccius bilinearis*) occupies a principal predatory role in the north-

west Atlantic, a role similar to that of the Atlantic halibut, and has been found to feed at night mostly between the hours of dusk and midnight (Bowman and Bowman, 1980).

An investigation into the daily feeding rhythm of sea caged Atlantic salmon by Kadri *et al.* (1991) showed an increase in fish activity in the early morning and early evening, with a strong correlation between fish activity and feeding response. Such work is directly applicable to the farming of any species, where feed presentation may be timed according to the inherent rhythm of the organism. Work by Sánchez-Vázquez *et al.*, (1995a,b) on cultured sea bass (*Dicentrarchus labrax*), demonstrates dualism in a species of fish which is routinely maintained on a diurnal husbandry regime. The locomotor activity in rainbow trout showed a phase adjusted vertical distribution in tanks, with activity closer to the water surface in the scotophase, although demand-feeding activity was confined to the photophase (Sánchez-Vázquez and Tabata, 1998). Clearly diurnal patterns of activity are species specific, and an opportunity therefore exists to maximise the efficiency of culture systems once patterns of activity have been identified in any cultured species. A strong photoperiod and seasonality component appears to be linked to circadian activity rhythms in fish, with the stage of the life history a further factor adding complexity to the situation. Greater emphasis on the optimisation of contemporary aquaculture units from a financial and environmental perspective necessitates further work in this area to elucidate rhythmicity in other cultured species under different photoperiods, and a variety of other environmental conditions.

The importance of flatfish species such as the plaice to the wild fisheries of temperate countries has directed some research to patterns of rhythmicity in these fish, generally in order to maximise catch techniques. Much of this work suggests rhythmic activity linked to tidal phases (Gibson, 1973; Greer Walker *et al.*, 1978; Weihs, 1978; Harden Jones, 1979, 1980; Ansell and Gibson, 1990), although there is evidence to support increased nocturnal activity patterns in *Solea solea* (Kruuk, 1963) and for *Pleuronectes platessa* and *Platichthys flesus* (Verheijen and de Groot, 1967) held in tanks. Nocturnal activity patterns were also observed by Edwards *et al.*

(1971), who found evidence for a diel feeding rhythm in some tropical flatfish species. Generally however, there is little documentation on activity and feeding rhythmicity in species of flatfish in the wild or in culture. *H. hippoglossus* is considered to be a mid-water piscivore in the Atlantic (Nelson, 1984), and one of the halibut's main prey items, *Sebastes marinus*, (Leim and Scott, 1966) shows increased open water activity at night, suggesting that wild halibut may share the nocturnal patterns of behaviour which have been observed in other flatfish species.

The energy costs associated with locomotion in various species of flatfish have been determined under conditions of forced swimming (Priede and Holliday, 1980; Duthie, 1982) and in comparison to roundfish these appear relatively large, with optimum swimming speeds close to critical swimming speeds, and consequently close to the limits of the metabolic scope. This has been argued by Priede (1985) to reflect the life history strategies of these species, with an "all or nothing" swimming strategy advantageous in fish species which have opportunities to rest for long periods on the ocean floor recouping any oxygen debt incurred.

The anatomical closeness of the Atlantic halibut to other members of the *Pleuronectidae* suggests a commonality with the energetics of these species. Consequently, it may be hypothesised that the energy costs associated with locomotion are likely to be relatively high in the Atlantic halibut, and metabolic scope may be relatively small in comparison to more active roundfish species. Although flatfish species may make energetic "savings" in terms of a reduced resting metabolic rate (see Chapter 3.), a relatively small metabolic scope will impose some restrictions on energy expenditure. This may be manifest as a low level of motor activity, since the M_A component may be more readily regulated by the organism than the M_M component which is fixed, and the M_F component which will depend on food availability.

One study of adult Atlantic halibut in a cage system utilising an underwater camera system to monitor behaviour (Martinez-Cordero *et al.*, 1994), does provide data on the activity of this large flatfish when held in commercial cage systems.

These authors found that significantly more fish were observed on the cage bottom than within the water column, although a few curious individuals would swim to the surface of the cage when approached by husbandry staff. The water column between the cage floor and the surface was relatively unused by the halibut. Overall it was concluded that this species adapted well to such a rearing environment, although on the occasions when rough weather affected the stability of the cage floor the behaviour of the halibut altered with significantly fewer fish observed resting on the cage floor.

The aim of this work was to examine activity patterns in small populations of Atlantic halibut over a 24 hour period using a video monitoring technique, in order to determine the occurrence of a diurnal/nocturnal pattern, which may be correlated to the nocturnal peaks in oxygen consumption rate observed in many of the trials with individual fish and tank populations (see Chapters 3 and 4). The possible influence of stocking density in determining levels of activity through social interactions was also examined. Where stock densities were sufficiently high to allow operation of the equipment, routine rate respirometry was also undertaken with the aim to correlate oxygen consumption rate peaks with peaks in fish activity.

5.2. Materials and Methods

5.2.1. Fish

Atlantic halibut of the 1994 and 1995 year classes were acclimated to the experimental conditions in the test tank for 4 weeks prior to the commencement of trials. General husbandry was as described in Chapter 2., except during trial periods. Experiments were carried out on fish in the postabsorptive phase (starvation periods as described in Chapter 2 were applied).

5.2.2. Facilities

The video unit was a modified $1m^2$ tank, and a diagrammatic representation of this facility is shown in Fig. 5.1. The unit was shrouded in light-proof black polythene sheet and illumination was provided by a single 100w bulb, controlled by timer providing a 12L:12D photoperiod (on at 08.00h). A single 40w red bulb attached to a dimmer switch operated continuously providing just sufficient light to film in the scotophase. Light levels within the unit were 150 lux for the photophase, and 2-3 lux for the scotophase. A single 90 x 130cm mirror was fixed at a 45° angle at a height of 1m above the water surface. A Sony CCD-V700E video camera recorder supported on a tripod and positioned to one side of the tank received the image of the tank floor and the halibut from this mirror. Disturbance was minimised through the arrangement of black plastic sheeting around the camera. Water temperature was not controlled, and experimental work was timed to coincide with ambient mid range temperatures within the range 8°C to 12°C.

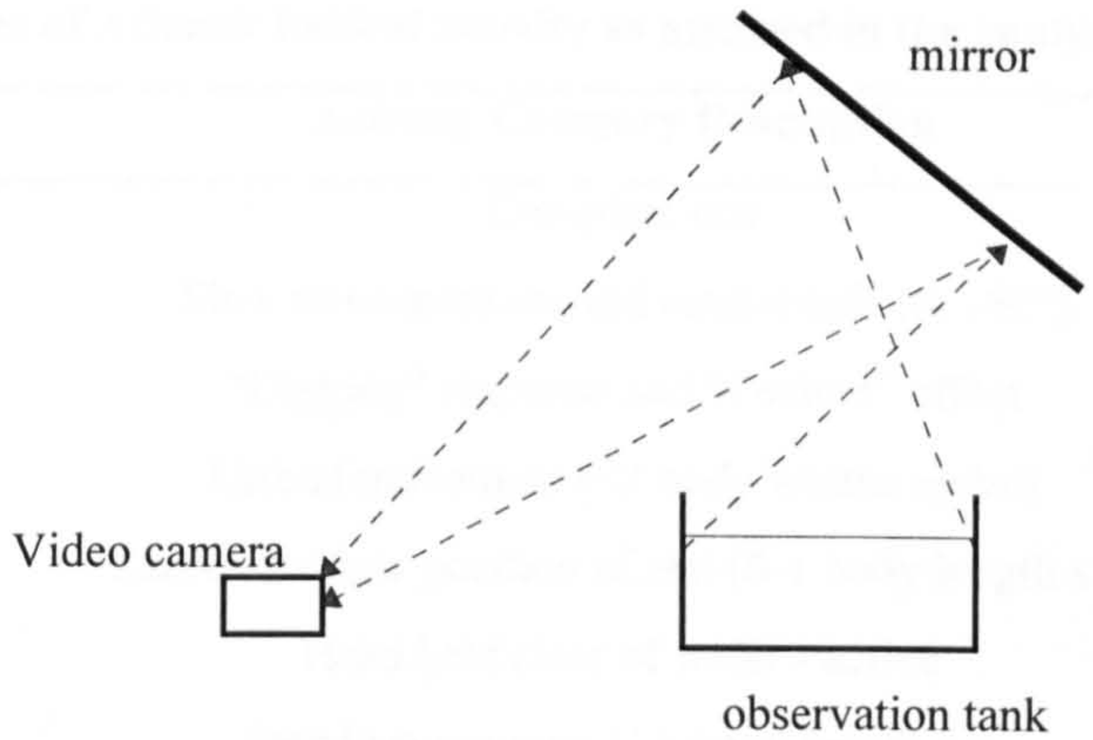


Fig. 5.1. Diagrammatic representation of the activity monitoring unit.

5.2.3. Trial Protocol

In total, twelve activity trials were conducted between 26 April 1997 and 9 November 1997. Nine trials were conducted on 1995 year class halibut, comprising triplicate trials at 3 stocking densities (n = 18, n = 9 and n = 5). The average weight of fish in these trials was 290g, 312g and 332g respectively . The remaining three trials were triplicates of one stocking density for 1994 year class halibut (n = 5, average weight 1774g). Recording was conducted for a 6 minute period in every hour throughout a 24 hour period, commencing at 08.30h.

Routine oxygen consumption experiments were carried out in association with the highest stock density 1995 year class and the 1994 year class trials following the protocol as described in Chapter 3.

5.2.4. Video Tape Analysis

Preliminary analysis of video tape from a 24 hour trial revealed various consistently identifiable classes of halibut activity. These categories, which are summarised in Table 5.1., then provided the basis for the subsequent analysis of the activity levels for the various social groups.

Table 5.1. Categories of Atlantic halibut activity as assessed in the analysis of video tapes.

Score	Activity Category Description	Activity level
0	Complete rest	
1	Slow movement around central axis (to 180°)	LOW
2	"Digging" response and "Posture" effect	↓
3	Lateral movement (<2 body widths move)	↓
4	Search for new position of rest (0-1 body length s^{-1})	increasing
5	Head held clear of water - active	↓
6	Steady swimming (1+ body length s^{-1})	↓
7	Aggression/Response to aggression	HIGH

5.2.5. Data Analysis

Video tapes from individual trials were analysed, and the activity level of the total fish population in each experimental period assessed. Each 6 minute time period was split into 10 second intervals, and activity within this period was categorised according to the levels shown in Table 5.1. for each individual fish within a trial population. The categorised activity of individual fish within ten second assessment periods was assumed a duration of ten seconds, and this assumption is central to the ultimate calculation of the energy allocation to activity over a 24 hour period. The validity of this assumption was checked through a more detailed analysis of two six minute time periods (one large fish trial and one small fish trial with n = 5) where the activity of individual fish was assessed for each category and time spent in each category timed exactly. When compared with the extrapolated data for the entire populations, this more detailed analysis showed a close correlation in the length of time allocated to each individual activity category, and these results are shown in Table 5.2. As a percentage, total actual time allocated to activity category was 2.5-30% lower for each activity category than the time extrapolated from assumed duration figures. Overall, accurate timing showed a slightly reduced allocation of time to activity of 6.4% in comparison to the assumed figures from extrapolation of the ten second interval results.

Table 5.2. Comparison between individually assessed activity duration and population estimates in tank populations of 5 small and 5 large Atlantic halibut, within one 6 minute measurement period.

	Activity category						
	1	**2**	**3**	**4**	**5**	**6**	**7**
Small	0	7	0	29	312	0	0
	0	10	0	30	330	0	0
Large	96	76	214	39	22	0	0
	110	80	220	40	30	0	0

Values are times allocated to activity category (s), with the upper values for each size the sum of actual timed values for individuals within the population, and the lower values the extrapolated values assuming a 10s duration for each recorded activity within the time period.

Raw data was entered into spreadsheets (Microsoft EXCEL 7.0) for further analysis of the timing and pattern of activity categories. Activity frequency was represented as activities $fish^{-1}$. This facilitated comparison between trials of different stocking density, and different size of fish. Oxygen consumption data was analysed according to the protocol of Chapter 4. Activity levels were assigned oxygen consumption rates relative to values previously published for swimming flatfish (Priede and Holliday, 1980; Duthie, 1982). Recorded and estimated (from activity levels) oxygen consumption rate values were converted to energy equivalents (using the value of 13.56 Jmg^{-1} O_2 as calculated by Brett and Groves (1979) for a fish respiring its own tissues) and compared over 24 hour experimental periods for similarity in pattern. Calculations of the total activity generated by an individual fish within a 24 hour period were determined from the mean values for activity category frequency in replicates of all stock density and fish size trials.

5.3. Results

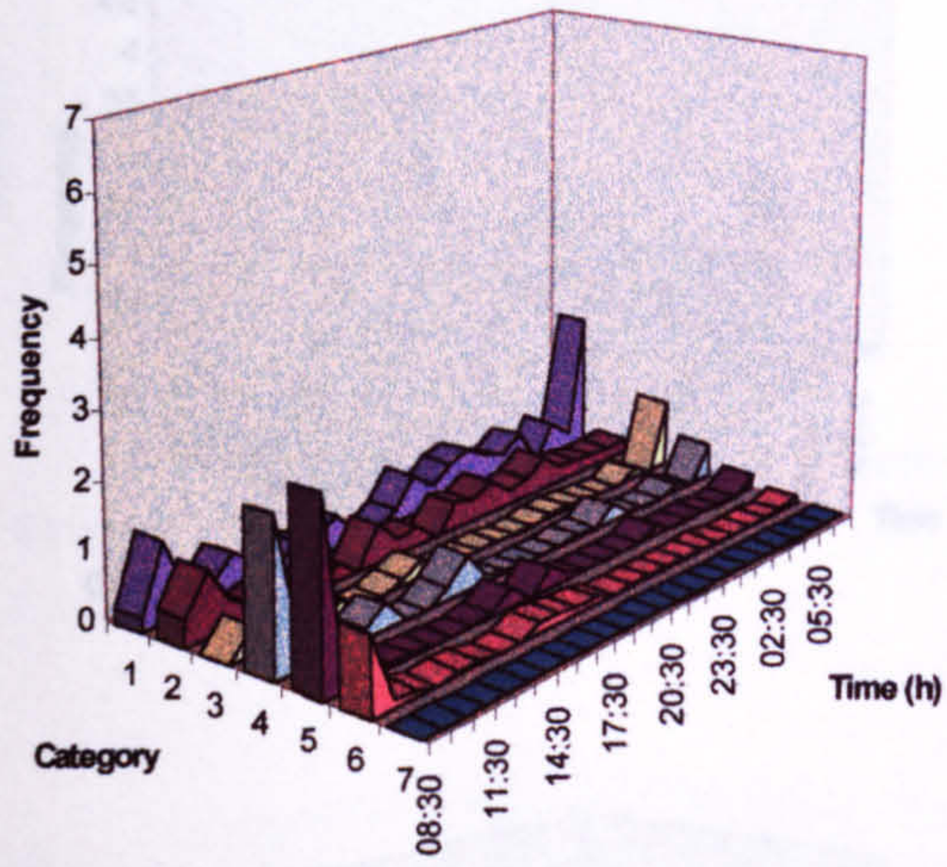
5.3.1. Diurnal Pattern of Activity

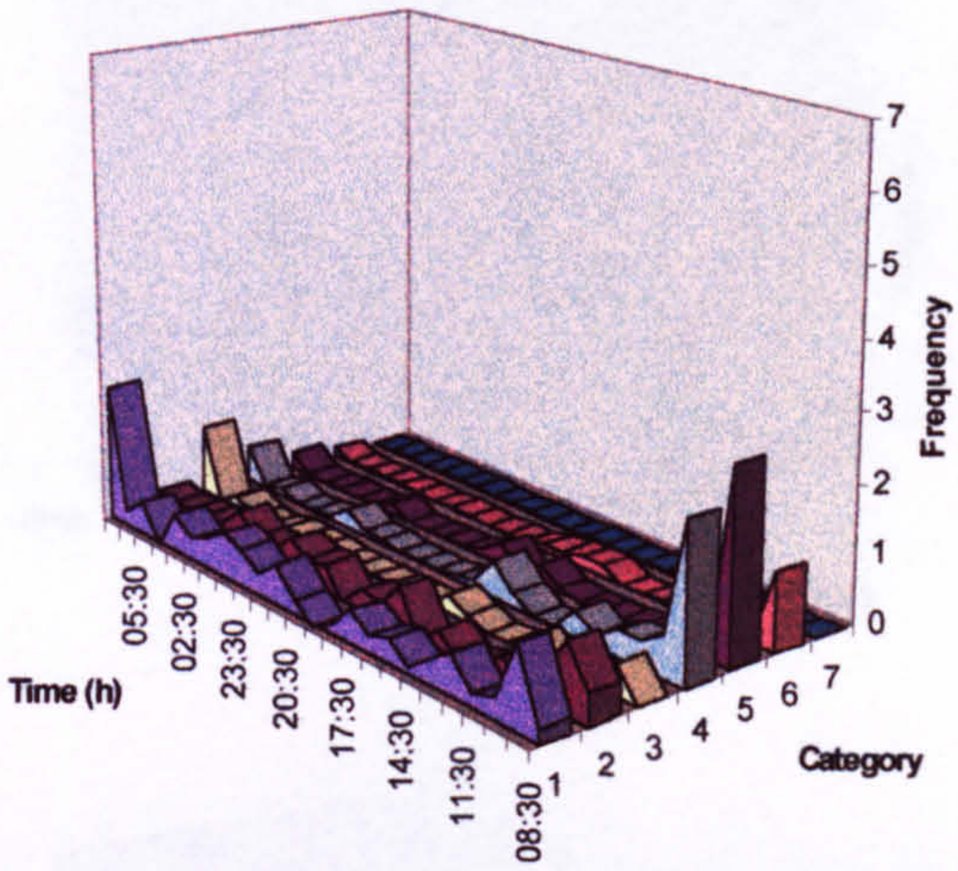
The categories of activity used in this work may be regarded as episodes in the social behaviour and interactions of a group of Atlantic halibut operating under conditions where spontaneous activity is not restricted. The analysis and interpretation of the results in this way may introduce an element of error into the study, since overall the interactions are dynamic with no clear break between categories. This fact was recognised by Altmann (1975) who suggested that over the course of a study the true pattern of behaviour will emerge regardless of these difficulties in analysis. The validity of the results of this work and the interpretation of the data so obtained, are based on this assumption.

The results for the analysis of daily activity pattern in all stock density trials are represented graphically in Fig. 5.2. (small fish, 100% stock density), Fig. 5.3. (small fish, 50% stock density), Fig. 5.4. (small fish, 25% stock density) and Fig. 5.5. (large fish). These graphs show frequencies of individual activity categories (represented as activities $fish^{-1}$) within trial populations throughout the 24 hour trial periods.

The primary aim of this work was to investigate the daily pattern of activity in the Atlantic halibut, correlating changing activity levels with the change in oxygen consumption rate in the dark phase which had previously been observed in resting and routine rate experiments (see Chapters 3 and 4). No significant phase location of activity patterns is indicated in Figs. 5.2.-5.5., with the halibut observed to be active in both the light and dark phases. Overall, it would appear that there is a slight decrease in motor activity in the dark phase for all sizes and all stock densities examined, though the significance of this effect is difficult to determine solely on the results of this work. The results suggest an element of dualism operating in the daily activity patterns of these Atlantic halibut, at stocking densities equivalent to commercial production.

(a)





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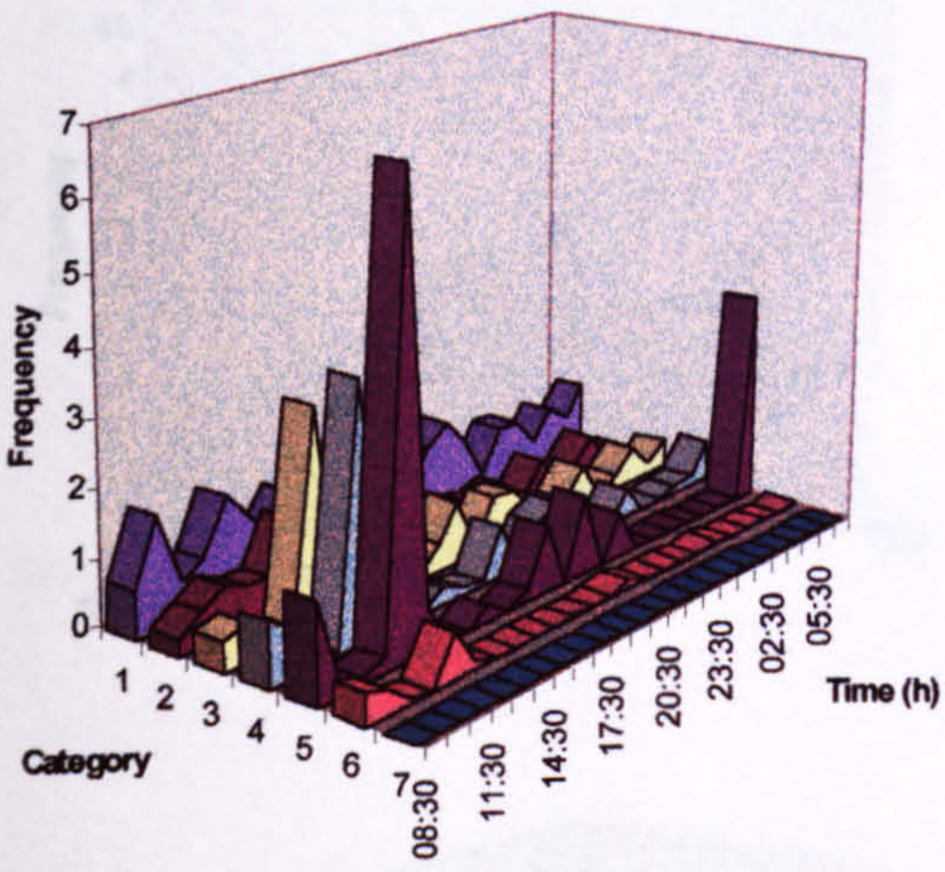

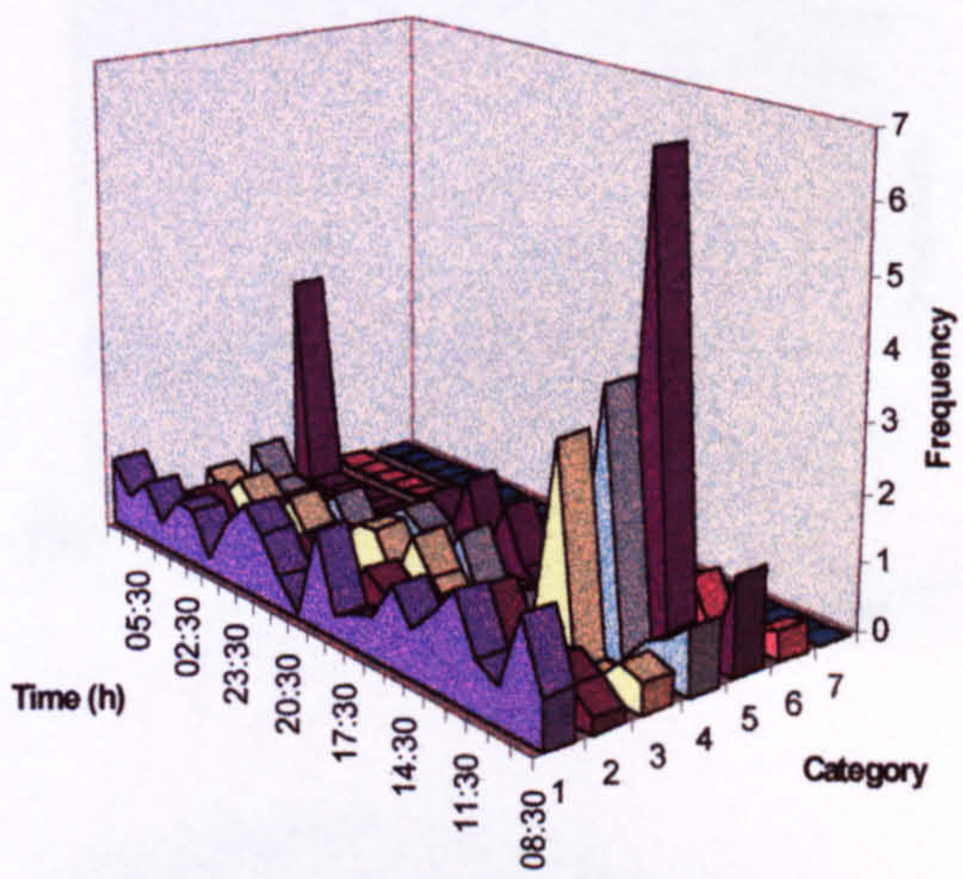

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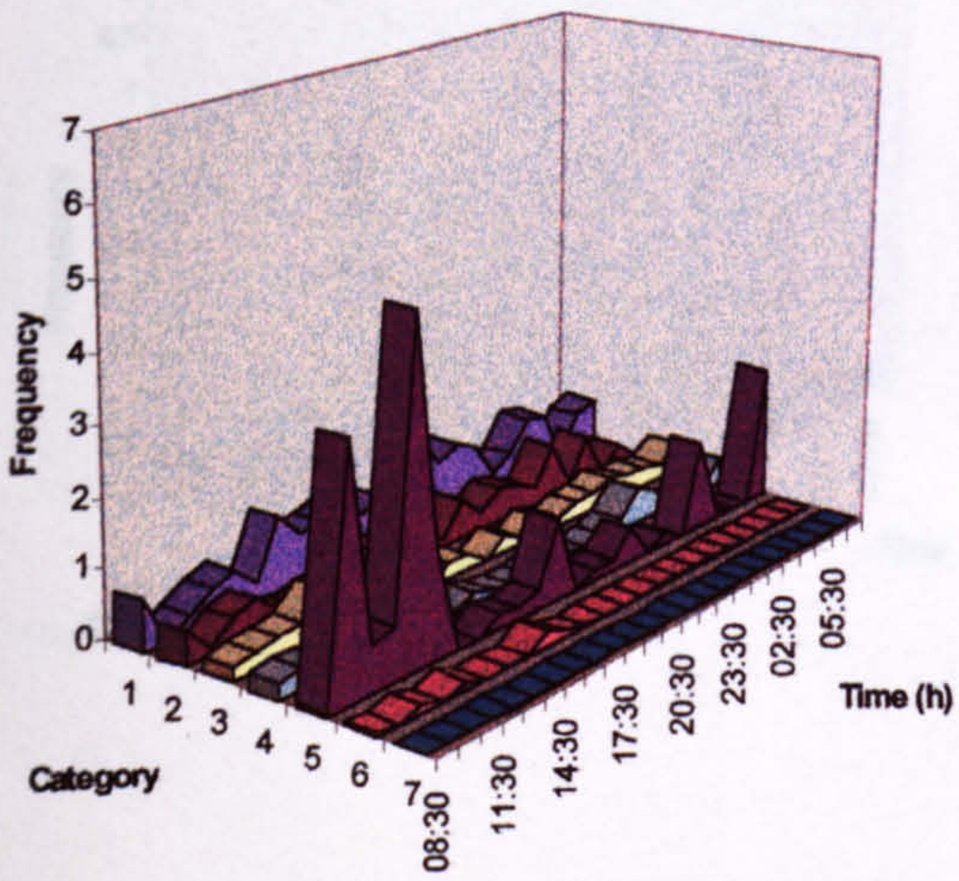


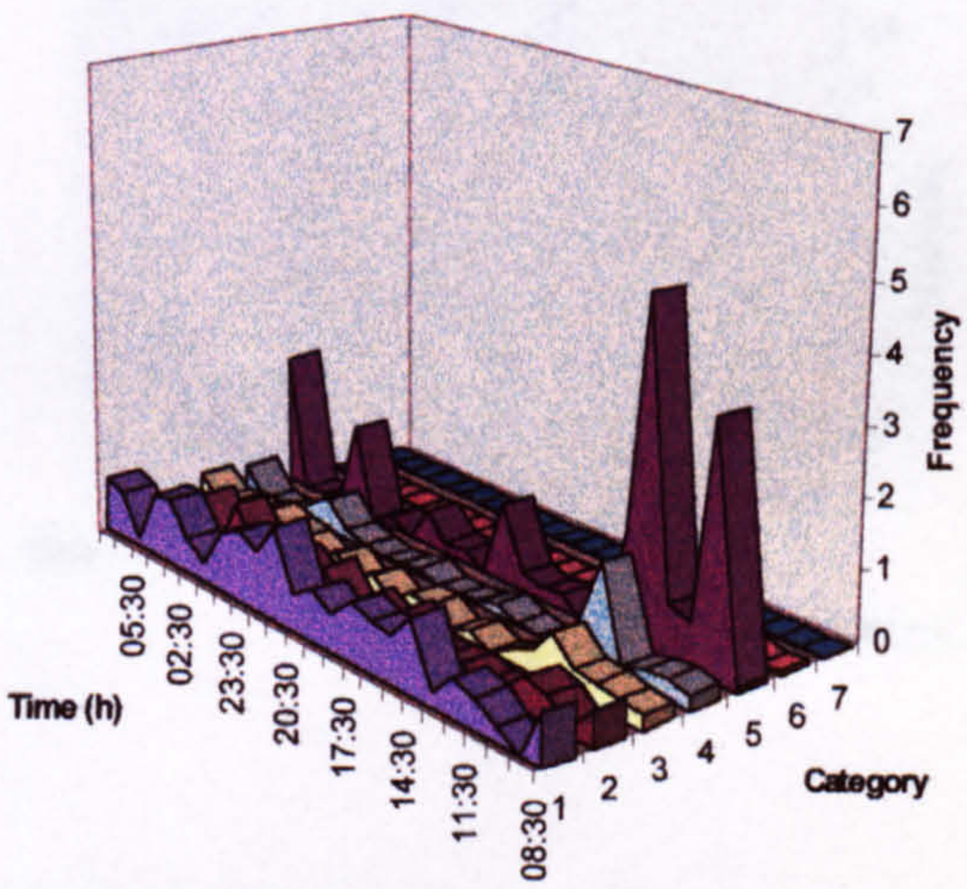
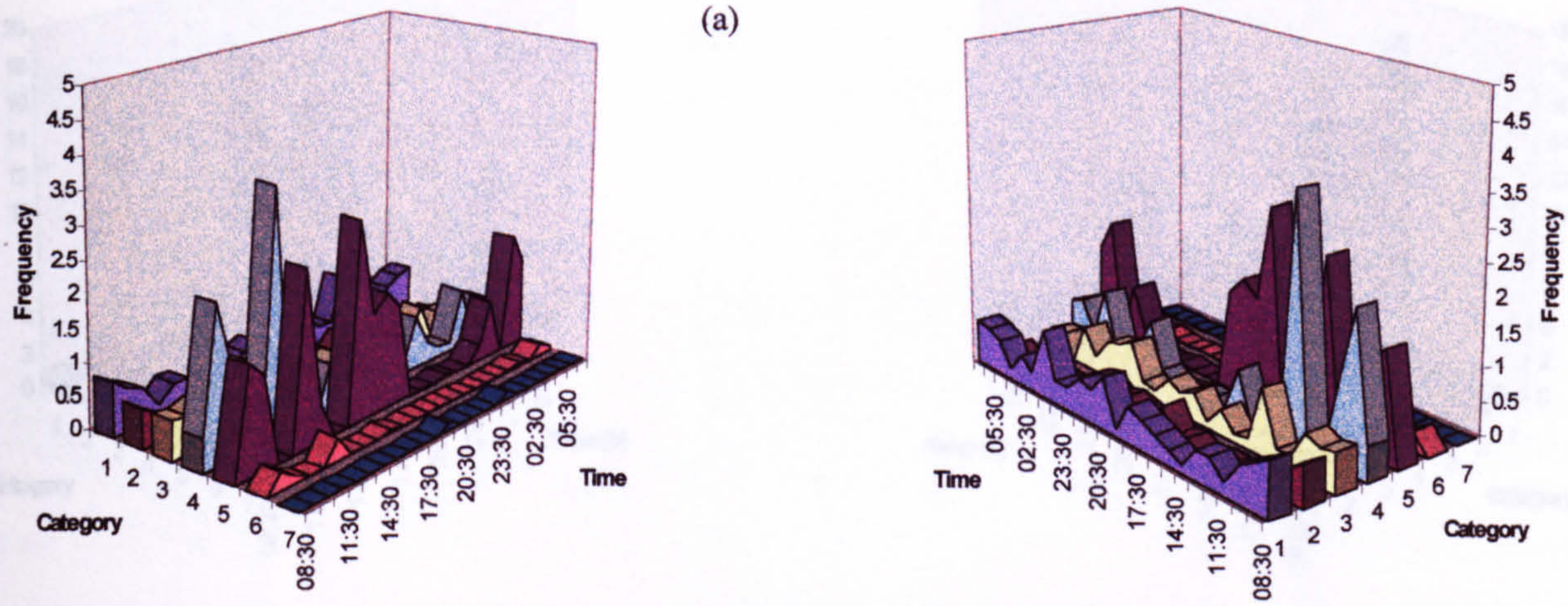


Fig. 5.2. (a-c) Diurnal Pattern of Activity in Atlantic halibut. Replicates of small fish, 100% stock density trials. Categories of activity as defined in Table 5.1. Right hand side and left hand side images of graphs are presented for clarity. Photoperiod 12L:12D (08.00h:20.00h).

(a)



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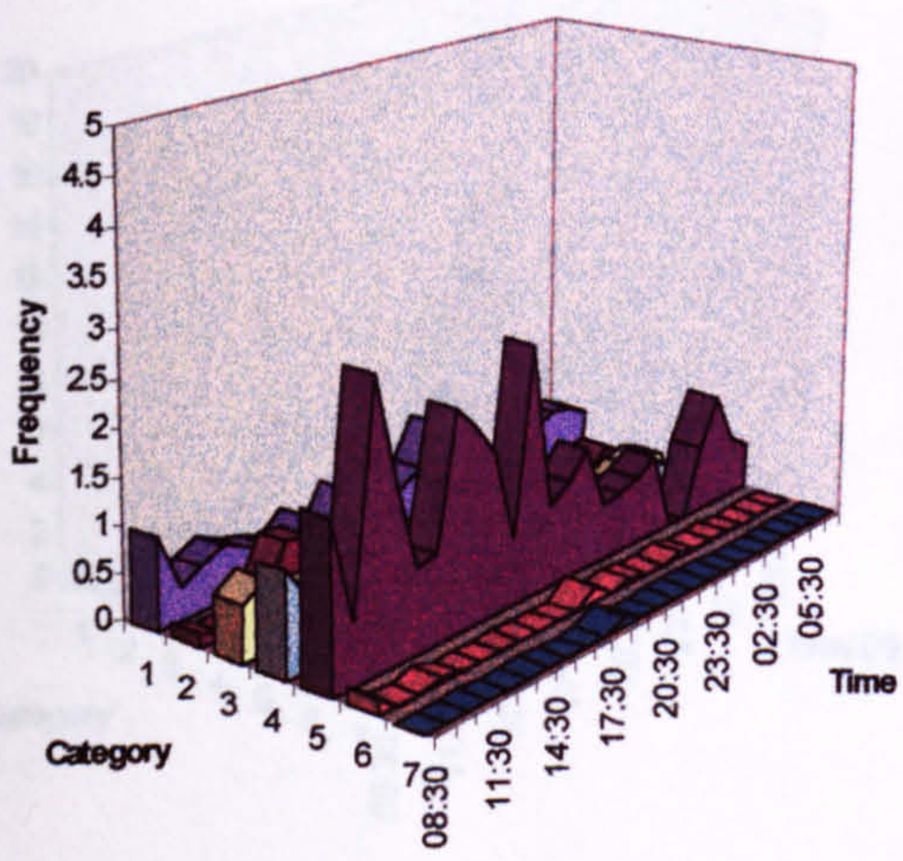

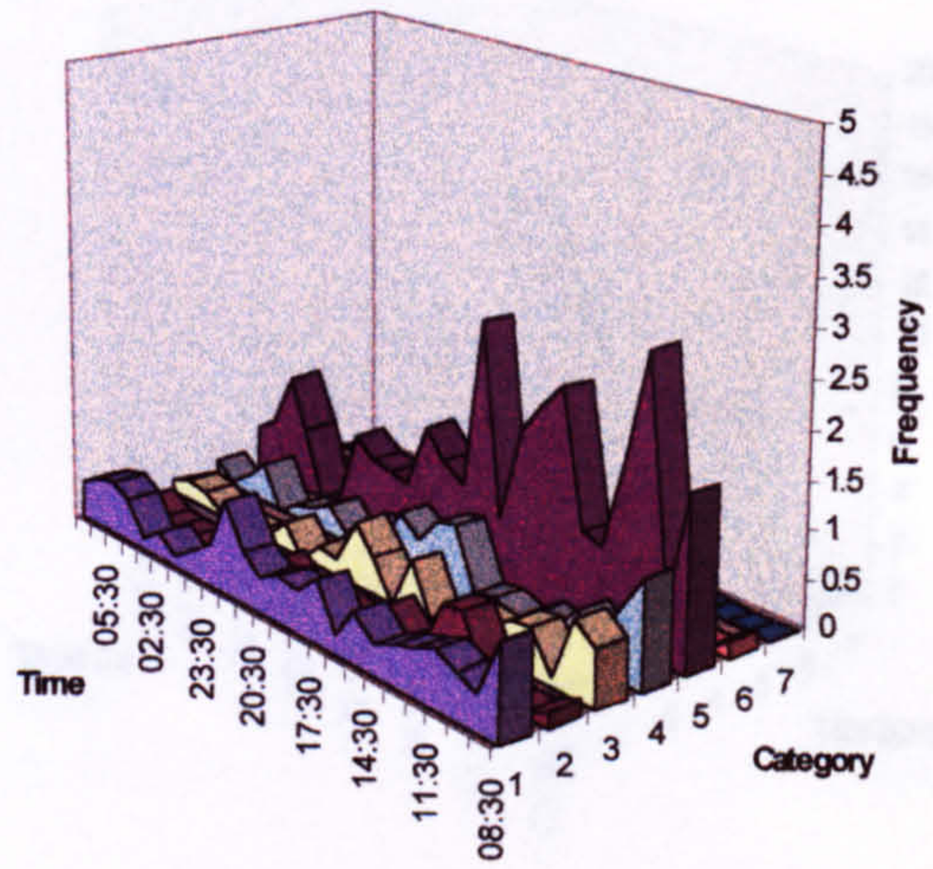

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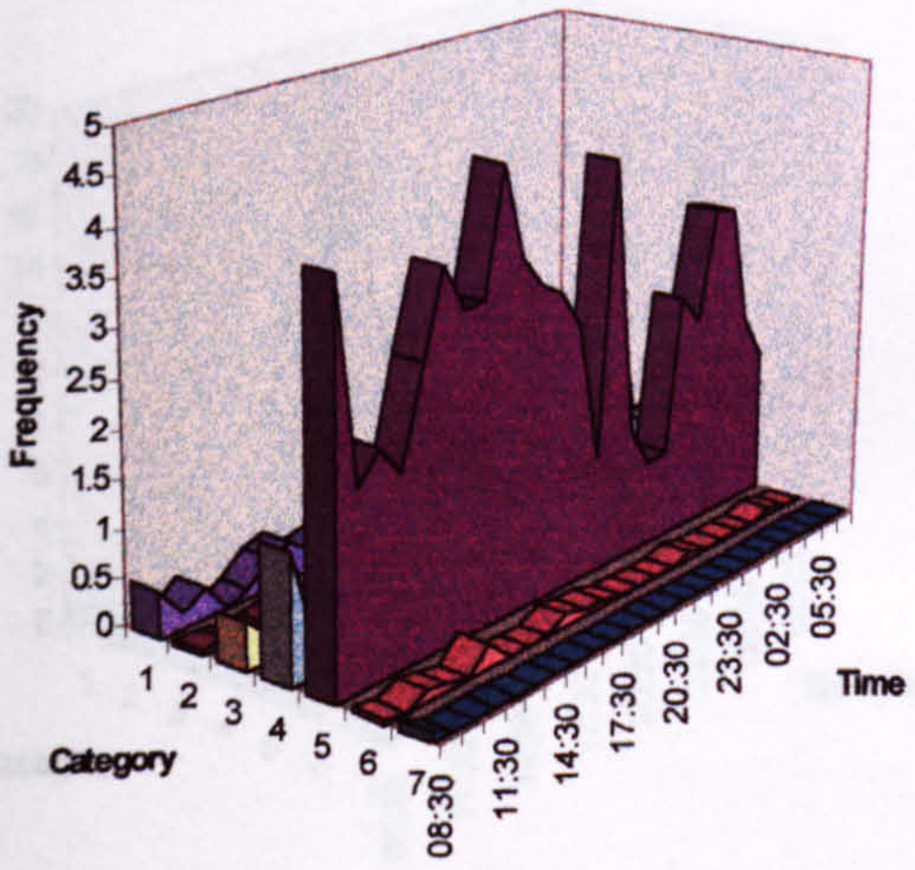


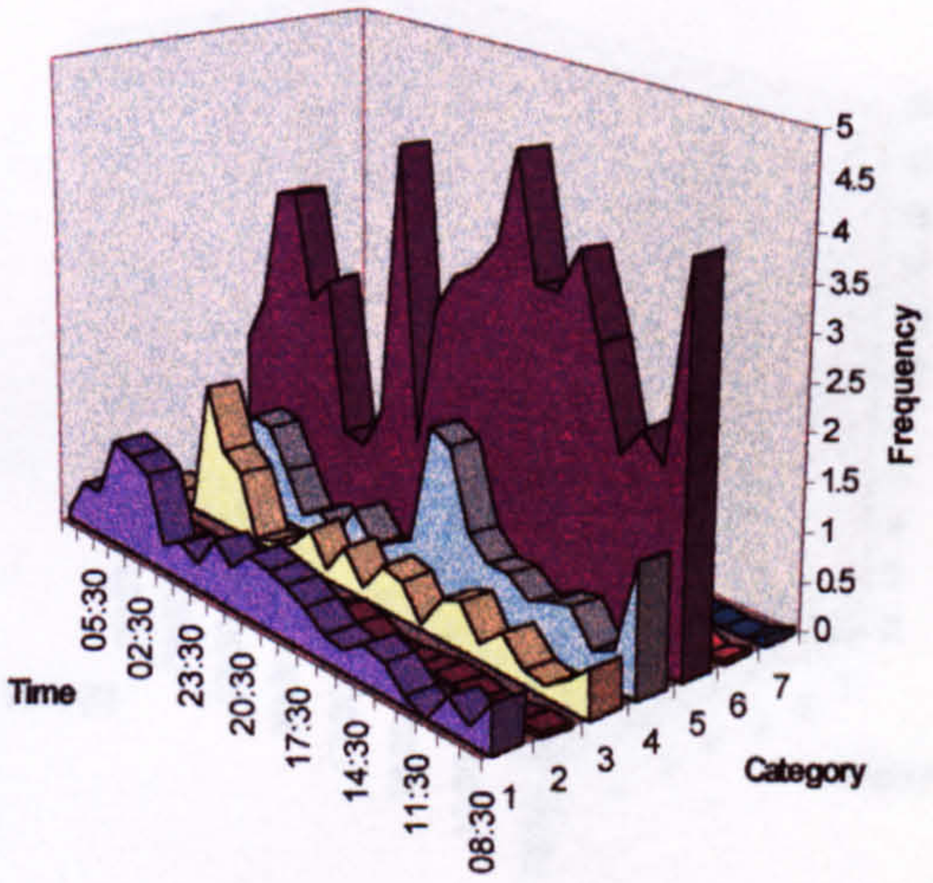


Fig. 5.3. (a-c) Diurnal Pattern of Activity in Atlantic halibut. Replicates of small fish, 50% stock density trials. Categories of activity as defined in Table 5.1. Right hand side and left hand side images of graphs are presented for clarity. Photoperiod 12L:12D (08.00h:20.00h).

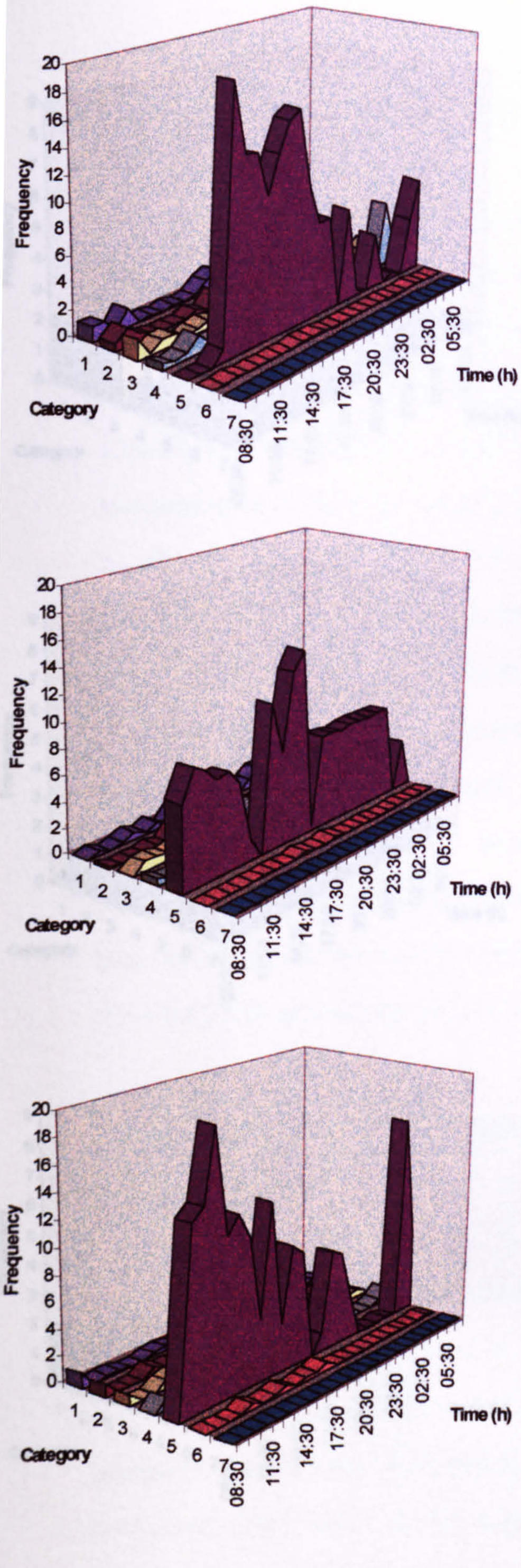
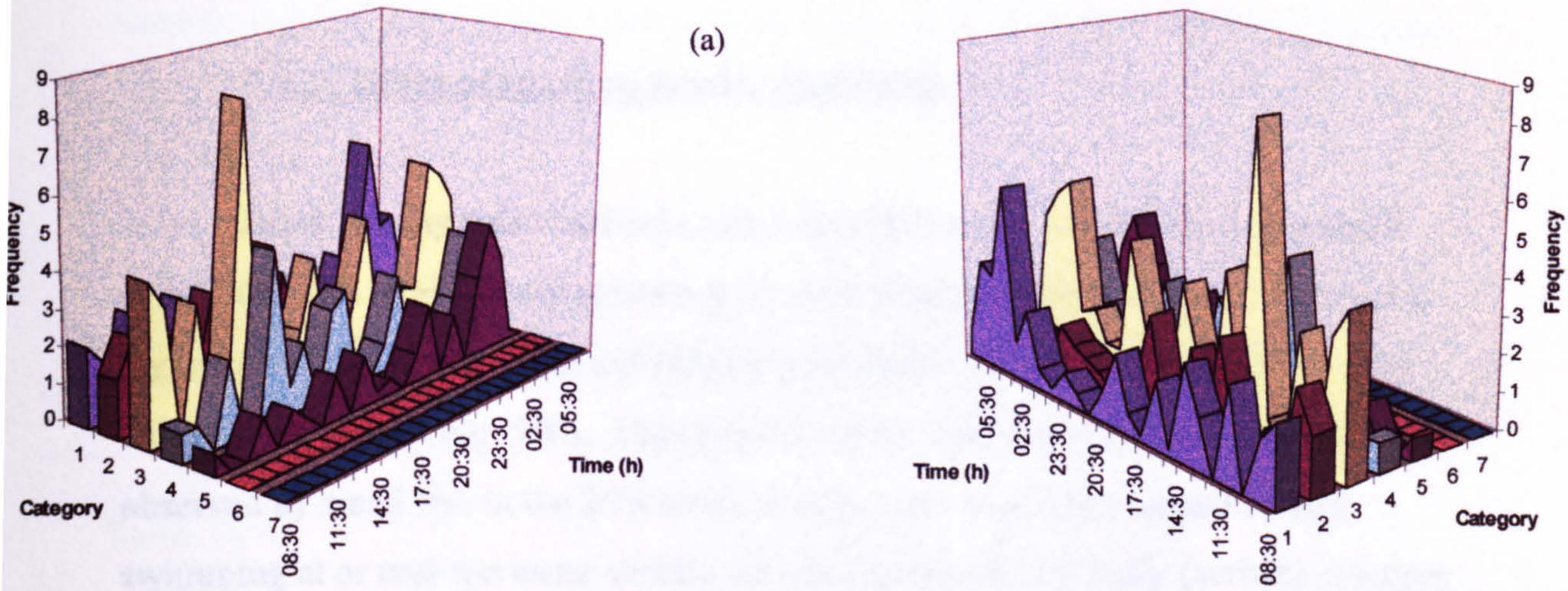


Fig. 5.4. (a-c) Diurnal Pattern of Activity in Atlantic halibut. Replicates of small fish, 25% stock density trials. Categories of activity as defined in Table 5.1. Right hand side and left hand side images of graphs are presented for clarity. Photoperiod 12L:12D (08.00h:20.00h).

(a)



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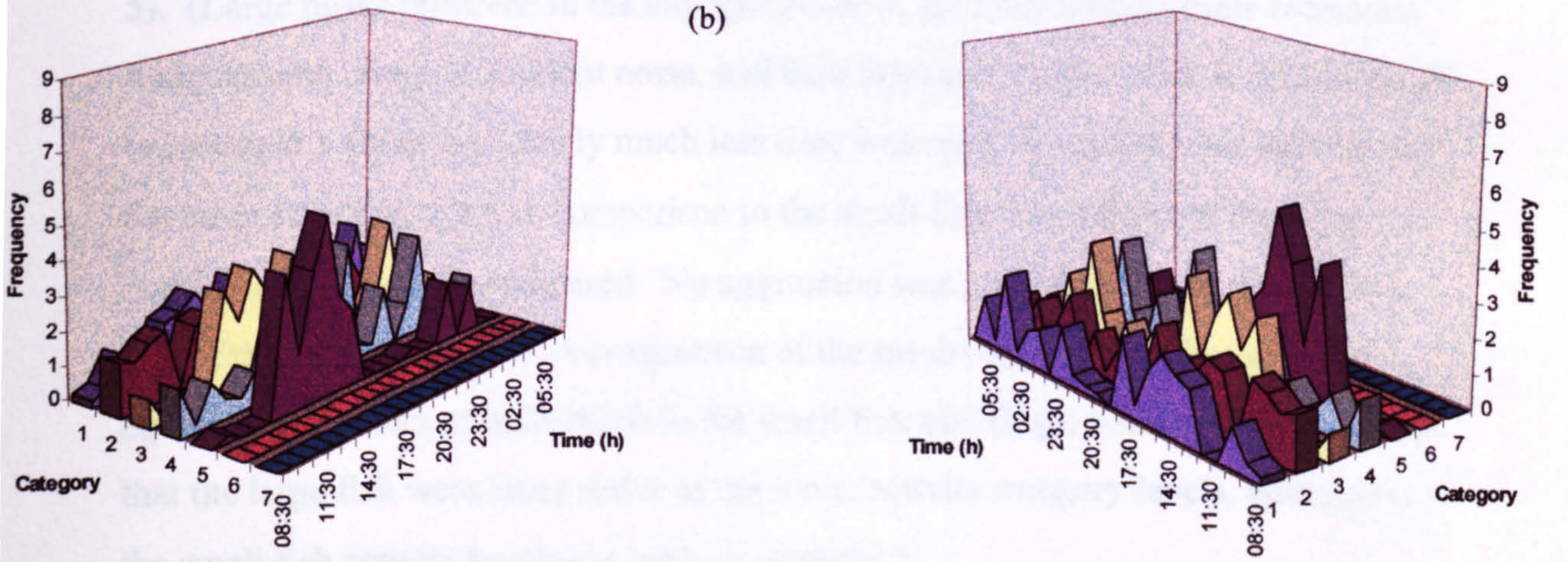

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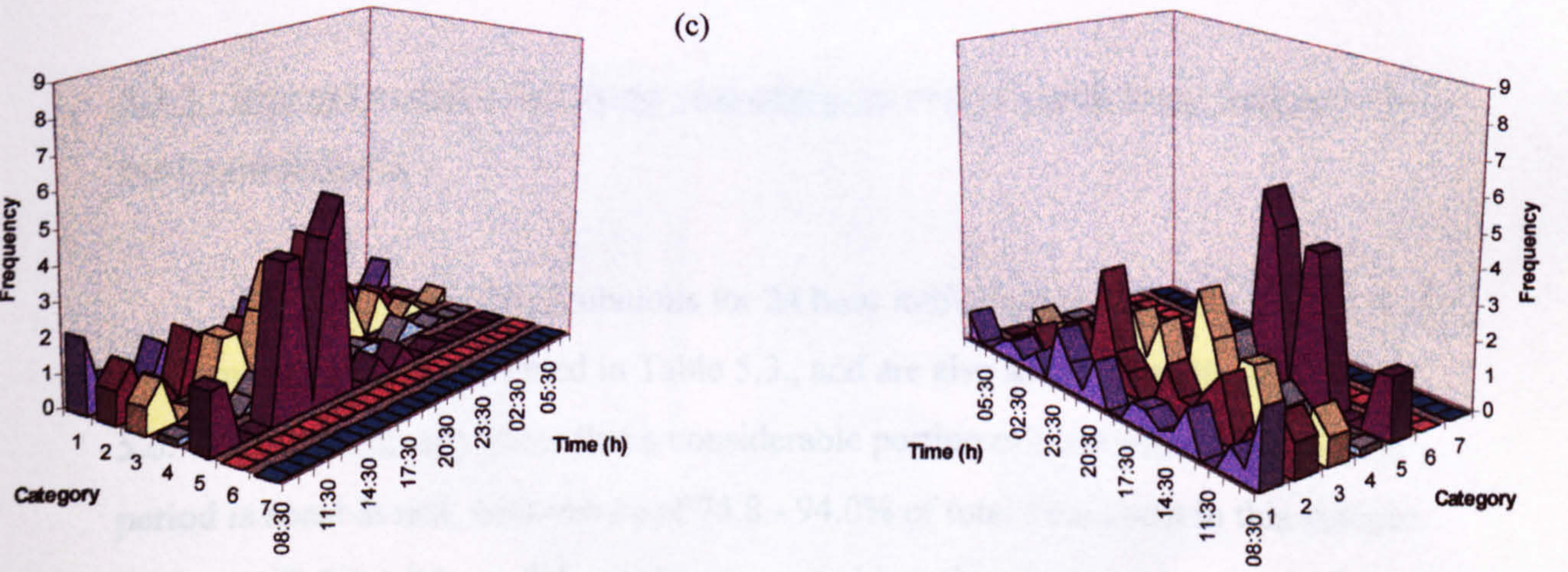

Fig. 5.5. (a-c) Diurnal Pattern of Activity in Atlantic halibut. Replicates of large fish trials. Categories of activity as defined in Table 5.1. Right hand side and left hand side images of graphs are presented for clarity. Photoperiod 12L:12D (08.00h:20.00h).

5.3.2. Effect of stocking density on activity

Stock density was observed to have an effect on motor activity in the small fish trials, with total activity increasing as stock density decreased (Fig. 5.2. to 5.4.). Aggressive episodes between individuals were observed only in the small fish 50% stock density trials (Fig. 5.3.). The proportional increased frequency of activity observed by small fish in the 25% stock density trials was characterised by fish swimming at or near the water surface with the body held vertically (activity category 5). (Large peaks observed in the daylight phase in the 100% smalls trials coincided with extreme levels of ambient noise, and their relevance to this work is thus difficult to ascertain.) Correspondingly much less time was spent at the water/air interface by the large fish (Fig. 5.5.), in comparison to the small fish, when trials of the same number of animals are compared. No aggression was noted between individuals in any of the large fish trials. A comparison of the results for large halibut with the equivalent numbers of individuals in the small fish trial (Figs. 5.4. and 5.5.) indicates that the large fish were more active at the lower activity category levels, with much of the small fish activity located in activity category 5.

5.3.3. Diurnal activity and associated energetic costs in individual halibut held in tank populations.

The results of the calculations for 24 hour individual activities of halibut in the trial populations are presented in Table 5.3., and are also shown graphically in Fig. 5.6. The results clearly show that a considerable portion of time within any 24 hour period is spent at rest, with values of 75.8 - 94.0% of total time spent in this manner. In the small fish trials, as fish number decreased less time was spent at rest and motor energy expenditure increased, with much of the increased activity observed in the lower stock density trials located in activity category 5 - "head held clear of water - active". This increase in activity was very marked when compared with higher stock densities in the small fish trials. The large fish spent less time at complete rest than the two higher stock density trials with small fish, and the activity of these fish was

located evenly around the lower activity categories 1-5. In contrast, category 5 tended to predominate in small fish activity, with less time observed between complete rest and this level of activity.

Table 5.3. Time allocated to the various categories of activity in a 24 hour period, by an individual Atlantic halibut held within the trial populations.

		Activity Category						
Trial	**Rest**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Smalls 100% density	1354.0 (94.0)	25.5 (1.8)	13.6 (0.9)	11.1 (0.8)	12.4 (0.9)	20.8 (1.4)	2.5 (0.2)	0.0 (0.0)
Smalls 50% density	1321.5 (91.8)	18.4 (1.3)	4.0 (0.3)	12.9 (0.9)	20.2 (1.4)	61.4 (4.3)	1.3 (0.1)	0.2 (0.02)
Smalls 25% density	1091.0 (75.8)	18.1 (1.3)	12.2 (0.8)	24.6 (1.7)	29.6 (2.1)	262.6 (18.2)	2.0 (0.1)	0.0 (0.0)
Large 100% density	1242.3 (86.3)	36.4 (2.5)	36.2 (2.5)	57.3 (4.0)	30.9 (2.1)	36.8 (2.6)	0.0 (0.0)	0.0 (0.0)

Values are duration, expressed as minutes allocated to each activity category within a 24 hour period. Percentage time allocated to each activity category is given in brackets.

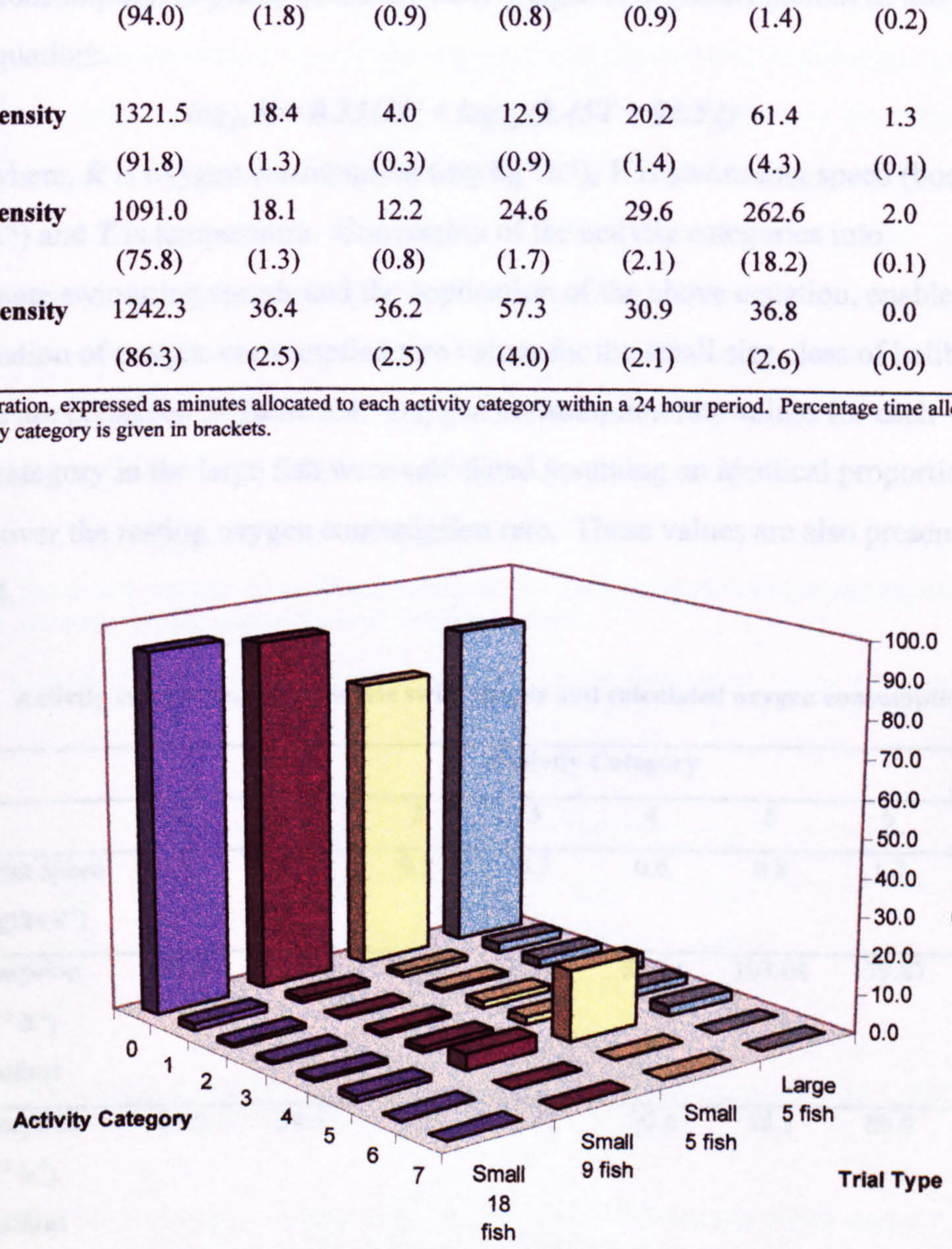

Fig. 5.6. Percentage of time allocated to activity categories within a 24 hour period in tank populations of Atlantic halibut.

The values for duration of activity within a 24 hour period may be used to calculate the energetic costs associated with the observed level of spontaneous activity in the tank system. In Chapter 3 an equation relating oxygen consumption rate with body size and temperature in resting Atlantic halibut was derived. The value for the energetic cost at rest may thus be calculated and incorporated into an energy budget determining spontaneous activity in the Atlantic halibut held in tank systems. There is little information on the energy costs of locomotion in flatfish, although Priede and Holliday (1980) described the relationship between specific swimming speed and oxygen consumption in plaice of similar body weight to the small halibut in this study by the equation:

$$\log_{10} R = 0.3318V + \log_{10}(2.45T + 26.52)$$

where, R is oxygen consumption (mg kg^{-1} h^{-1}), V is swimming speed (body lengths s^{-1}) and T is temperature. Conversion of the activity categories into approximate swimming speeds and the application of the above equation, enable the determination of oxygen consumption rate values for the small size class of halibut, and these are presented in Table 5.4. Oxygen consumption rate values for each activity category in the large fish were calculated assuming an identical proportional increase over the resting oxygen consumption rate. These values are also presented in Table 5.4.

Table 5.4. Activity categories, approximate swim speeds and calculated oxygen consumption rate values.

	Activity Category							
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Approx. swim speed (body lengths s^{-1})	0	0.1	0.2	0.3	0.6	0.8	1.2	2.0
O_2 consumption (mg kg^{-1} h^{-1}) 311g halibut	55.5	60.36	65.15	70.33	88.44	103.04	139.87	257.7
O_2 consumption (mg kg^{-1} h^{-1}) 1774g halibut	31.7	34.5	37.2	40.2	50.6	58.9	80.0	147.3

Swimming energy expenditure calculations are derived from the equation of Priede and Holliday (1980) for swimming plaice. The work of these authors was carried out on plaice of 288g to 330g body weight, corresponding closely to the weights of the small size class halibut in this study. The resting oxygen consumption rate values (activity category zero) are derived from the equation of Chapter 3., for fish of 310g and 1774g body weight at 12°C. Swimming oxygen consumption rates for a 1774g fish are calculated assuming an identical proportional increase with activity category to the 311g halibut.

Through application of the Brett and Groves (1979) oxycalorific coefficient value of 13.56 J mg^{-1} O_2 for fasting fish to the values produced in Table 5.3. and incorporating the times spent within each activity category presented in Table 5.2., the mean daily energy expenditure within each activity category, and the total daily energy expenditure associated with spontaneous motor activity may be calculated for the fish in this study. The lack of information on the energetic cost of swimming activity in large flatfish means that estimates of daily motor energy expenditure are more difficult to obtain for the large halibut in this study, and it cannot be assumed that the energetic costs of locomotion are similar throughout the size range. However, utilising the values produced for routine oxygen consumption rate in Chapter 4., the energetic cost of total motor activity may be estimated as the difference between this value and the resting oxygen consumption rate value produced from the equation in Chapter 3. This figure may then be used to calculate a value for total oxygen consumed by the population on a daily basis, over and above that of resting rate metabolism and attributable to motor activity. Again, use of the oxycalorific coefficient produces a value for the energy allocated to motor activity in a small population of large fish on a daily basis. The results are presented in Table 5.5.

Table 5.5. Total daily energy expenditure in spontaneous activity, and mean energy expenditure by activity category in tank populations of Atlantic halibut.

	Mean daily energy expenditure by activity category (J kg^{-1} d^{-1})								Total daily mot energy expenditu (J kg^{-1})
	0	1	2	3	4	5	6	7	
ll (18)	16983	348	200	177	247	485	80	0	1538
all (9)	16575	251	59	206	405	1429	41	14	2405
all (5)	13684	247	180	390	591	6114	63	0	7585
ge (5)	8909	261	260	411	222	264	0	0	1417 (1765*)

*Daily motor energy expenditure in the large fish calculated from the equations describing routine and resting oxygen consumption rate produced in Chapters 3 & 4, for halibut of 1774g at 12°C.

Daily motor energy expenditure increased with decreasing stock density in the small fish trials, and this increase was especially large in the trials involving the fewest number of fish. The energy expenditure of large fish in daily motor activity was lower than that of small fish at any stock density.

Through application of the Brett and Groves (1979) oxycalorific coefficient value of 13.56 J mg^{-1} O_2 for fasting fish to the values produced in Table 5.3. and incorporating the times spent within each activity category presented in Table 5.2., the mean daily energy expenditure within each activity category, and the total daily energy expenditure associated with spontaneous motor activity may be calculated for the fish in this study. The lack of information on the energetic cost of swimming activity in large flatfish means that estimates of daily motor energy expenditure are more difficult to obtain for the large halibut in this study, and it cannot be assumed that the energetic costs of locomotion are similar throughout the size range. However, utilising the values produced for routine oxygen consumption rate in Chapter 4., the energetic cost of total motor activity may be estimated as the difference between this value and the resting oxygen consumption rate value produced from the equation in Chapter 3. This figure may then be used to calculate a value for total oxygen consumed by the population on a daily basis, over and above that of resting rate metabolism and attributable to motor activity. Again, use of the oxycalorific coefficient produces a value for the energy allocated to motor activity in a small population of large fish on a daily basis. The results are presented in Table 5.5.

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Small (18)	16983	348	200	177	247	485	80	0	1538
Small (9)	16575	251	59	206	405	1429	41	14	2405
Small (5)	13684	247	180	390	591	6114	63	0	7585
Large (5)	8909	261	260	411	222	264	0	0	1417 (1765*)

*Daily motor energy expenditure in the large fish calculated from the equations describing routine and resting oxygen consumption rate produced in Chapters 3 & 4, for halibut of 1774g at 12°C.

Daily motor energy expenditure increased with decreasing stock density in the small fish trials, and this increase was especially large in the trials involving the fewest number of fish. The energy expenditure of large fish in daily motor activity was lower than that of small fish at any stock density.

5.3.4. Diurnal activity pattern and routine oxygen consumption rate

The small fish 100% stock density and the large fish trials involved the simultaneous recording of routine oxygen consumption rate, as well as the assessment of activity. Through application of the oxygen consumption rate equivalents related to swimming speed shown in Table 5.4. to the activity results for each hour within a 24 hour period, oxygen consumption rate values were calculated for the replicates of both trials. Routine oxygen consumption rate data was further converted to energy equivalents, expressed as J fish h^{-1}, and the results of this work are presented in Figs. 5.7 and 5.8. In both the small and large fish trials, there is less hourly variation in the energy consumption estimated from activity than that calculated from routine oxygen consumption rate data. In all three small fish trials, the estimated energy consumption from activity is consistently higher by approximately 50-75 J fish h^{-1} over the 24 hour period. In the large fish trials there is better agreement between the estimated and calculated energy consumption values over the 24 hour period, although the greater degree of variability in oxygen consumption rate observed in these trials is not matched by variability in activity level, and thus estimated energy consumption. Overall there is no clear correlation in pattern between energy consumption estimated from activity levels, and energy consumption calculated from routine oxygen consumption rate.

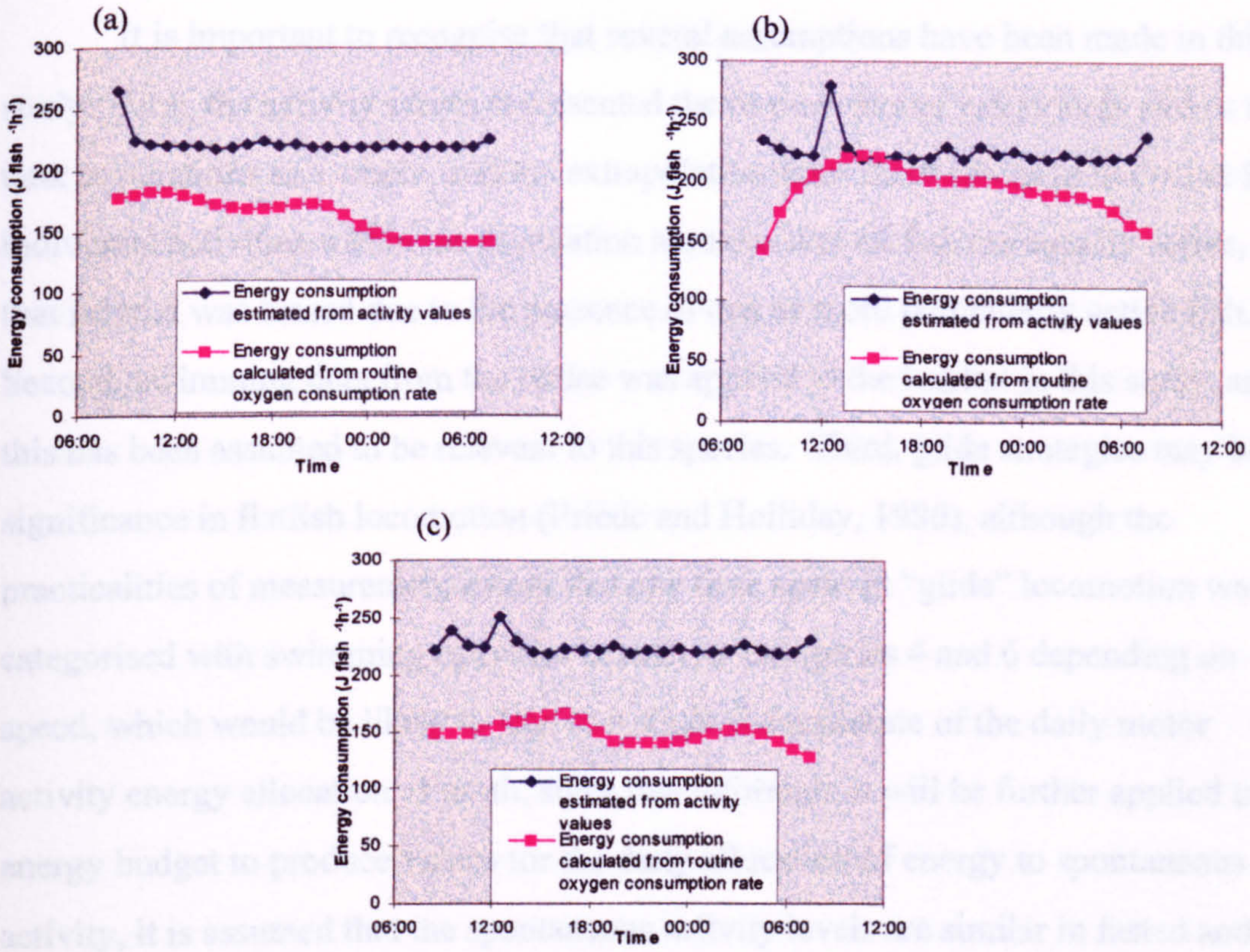

Fig. 5.7. (a-c) Comparison between energy consumption values (J fish^{-1} h^{-1}) calculated from recorded routine oxygen consumption rate and activity category analysis in 24 hour periods in small Atlantic halibut. Photoperiod 12L:12D (08.00h:20.00h).

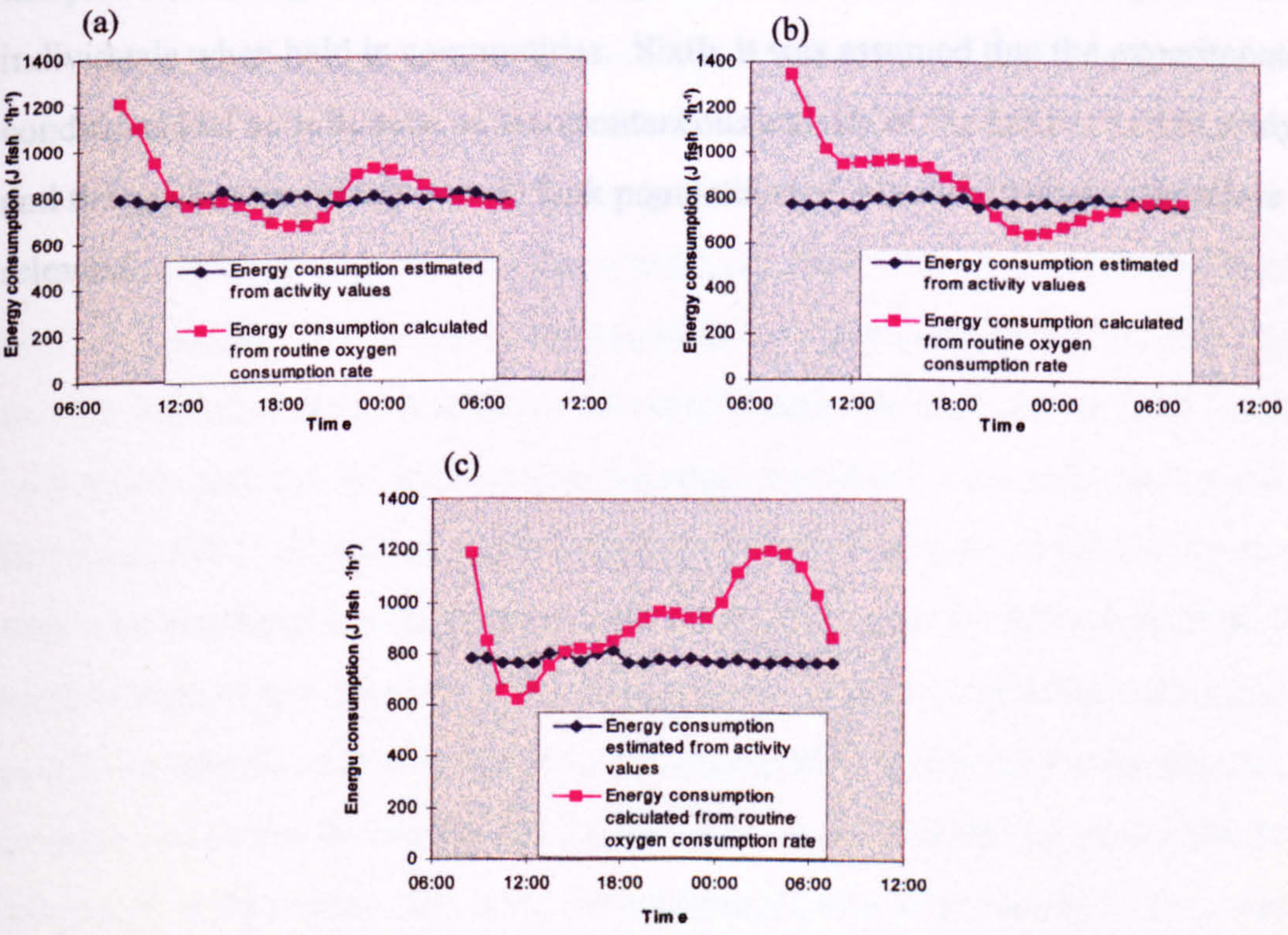

Fig. 5.8. (a-c) Comparison between energy consumption values (J fish^{-1} h^{-1}) calculated from recorded routine oxygen consumption rate and activity category analysis in 24 hour periods in large Atlantic halibut. Photoperiod 12L:12D (08.00h:20.00h).

It is important to recognise that several assumptions have been made in this study. First, the activity scores represented the movements of individuals within the tank populations as a whole, and the extrapolation from these scores to the value for individual activities within the population assumes that all fish are equally active, i.e. that no trial was biased due to the presence of one or more particularly active fish. Second, swimming data from the plaice was applied to the halibut in this study, and this has been assumed to be relevant to this species. Third, glide strategies may be of significance in flatfish locomotion (Priede and Holliday, 1980), although the practicalities of measurement meant that any time spent in "glide" locomotion was categorised with swimming activities in activity categories 4 and 6 depending on speed, which would be likely to lead to a slight overestimate of the daily motor activity energy allocation. Fourth, since this information will be further applied to an energy budget to produce values for the daily allocation of energy to spontaneous activity, it is assumed that the spontaneous activity levels are similar in fasted and fed fish. Fifth, fish at rest within the trial populations have been assumed to have a daily metabolic energy expenditure close to the values produced for the resting rate study of Chapter 3., although there may be a "population effect" on metabolic expenditure of individuals when held in communities. Sixth, it was assumed that the experimental conditions had no influence on the spontaneous activity of the halibut in this study, and the application of this data to tank populations of Atlantic halibut is therefore relevant.

5.4. Discussion

This study set out to determine the presence of any circadian periodicity in the activity pattern of the Atlantic halibut (*H. hippoglossus*) held in tank populations. No evidence for a diurnal, crepuscular or nocturnal activity pattern was obtained, and although the frequency of diurnal activity appeared to be slightly elevated over nocturnal activity there was no distinct pattern observed in these trials. Under the trial conditions, halibut appeared to be exhibiting a dualistic pattern of activity.

A dualistic pattern of activity has been observed in some species of fish such as the brown trout (Eriksson, 1978), the goldfish (Sánchez-Vázquez *et al.*, 1996) and the sea bass (Sánchez-Vázquez *et al.*, 1995) which is considered to reflect an adaptive behavioural response produced by an opportunistic feeder. Work on the brown trout by Heggenes *et al.* (1993) and on the Atlantic salmon by Gries *et al.* (1997) indicates the ability of these species to modify circadian activity rhythms according to season, with nocturnal patterns of activity seen in the winter in the brown trout in two streams of south-central Norway, and nocturnal activity patterns observed in 1+ salmon parr in rivers of eastern USA in late summer when ambient water temperature exceeded 13°C. Understanding of the reasons behind such behaviour remains relatively poorly understood, although Eriksson (1978) regards dualism as conferring an adaptive advantage on semi-opportunistic fish species, allowing flexibility in the "fulfilment of ecological needs." Feeding and motor activity in wild populations of the silver hake, another important predator of the north-west Atlantic, has been shown to be located nocturnally and may be linked to prey location or predator evasion (Bowman and Bowman, 1980). It is clear that the existence of such flexibility in life history strategy would be beneficial to a large open ocean piscivore such as the Atlantic halibut, the modification of any internal rhythm allowing the exploitation of mobile stocks of prey species to take place as and when the opportunity presents itself. The anatomical existence of a large stomach in the Atlantic halibut (see Chapter 1.) would also be an advantage in this respect, allowing the ingestion of very large meals at a time which are digested over a relatively long period as the fish remains on the ocean floor.

There are clear implications for the results of this work, and the above discussion on the potential of dualism, for both the ecology of wild Atlantic halibut and for the culture of this species in intensive systems. Locomotor rhythms in some species of fish have been found to exhibit daily cycles which may become entrained by the timing of feed presentation (Sánchez-Vázquez *et al.*, 1997). The test fish used in this study are among the first generation of farmed Atlantic halibut stock, and even with the diurnal presentation of food throughout the life of these fish, there is a clear indication that behavioural rhythms are not confined to this period. The results therefore suggest that daily activity pattern in the Atlantic halibut is not directly modified by a food-entrainable rhythm. Consequently, a synchronisation of the feeding pattern with any inherent activity rhythm may be the best way to optimise the intake of food in this species. This requires further research to clarify such activity patterns in terms of water temperature, photoperiod and size of fish. Particularly, it may be of benefit to monitor activity in this species over longer periods of time than the 24 hour period undertaken in this study. The large stomach of the halibut, the consumption of meals of relatively large size, and the long period for gut evacuation (Davenport *et al.*, 1990) suggest that feeding and activity rhythms in this species under natural conditions may be governed by a cycle with a periodicity greater than 24 hours. The metabolic cost of SDA is likely to restrict activity following the ingestion of a meal, as a significant portion of energy within the metabolic scope of the organism is allocated towards the M_F component. A suggestion has also been made for a link between SDA and the return of appetite in fish (Sims and Davies, 1994).

Kadri *et al.* (1991) studied feeding rhythms in the Atlantic salmon through the provision of a video monitoring system in commercial sea cages. These authors monitored feed response every two hours, and swimming speed every hour, and found a strong correlation between diurnal rhythms in swimming speed and feeding response, with peaks between 06.00h and 08.00h and troughs between 14.00h and 16.00h. Timing of feed presentation to this peak is likely to be beneficial in achieving greater efficiency of feed utilisation, and indicates how the elucidation of an inherent

feeding rhythm may be examined, evaluated, and the results applied to the successful production of this species.

The work of Sánchez-Vázquez *et al.* (1995a,b) on the sea bass approaches research into feeding rhythms of another intensively cultured fish species in a similar way. Fish feeding rhythms were recorded by the use of a demand feeding system in sea bass under experimental conditions where photoperiod was controlled. Sea bass were observed to feed in both the light and the dark phase, and the authors postulated the existence of dualism in the diel feeding pattern of this species. The results suggested that the timing of feed presentation itself is one of several zeitgebers in the sea bass, adding further complexity to the determination of rhythmicity in this species.

Clearly there is much to be achieved with the Atlantic halibut, a species only very recently introduced to intensive culture conditions. A full examination of the behavioural patterns operating in the halibut will allow the accurate simultaneous phasing of feeding with the natural rhythm, and is likely to optimise food intake in this species, having a beneficial effect on the production in intensive systems. Importantly, the timing of feeding of this organism in tanks is likely to be relevant in terms of maximising feed ingestion and utilisation, and the optimum routine may differ significantly from the current pattern of feeding once or twice a day between the hours of 09.00h and 18.00h. If time of feed presentation provides an entrainable rhythm in this species, this may overlap an inherent nocturnal rhythm, given the offering of feed to these animals only in periods of daylight in culture systems. Certainly the results for resting and routine oxygen consumption rate in Chapters 3 and 4 showed nocturnal peaks in many of the trials, which does raise questions over the presentation of feed only in daylight.

There is some documentary evidence suggesting that flatfish species in general show an increased level of activity nocturnally (Kruuk, 1963; Verheijen and de Groot, 1967; Edwards *et al.*, 1971). A circadian pattern of activity has also been identified in the plaice, with pattern of activity linked to tidal movements (Gibson, 1973). Any influence of tidal movements on the Atlantic halibut is likely to be found in juveniles

which tend to inhabit shallow nursery areas in the wild, as opposed to the adults which live at much greater depth. However, tidal effects were not investigated in this study and the influence of such an effect on the activity of tank held populations of fish is unclear. There is no documentation on the periodicity of Atlantic halibut activity or feeding patterns, although anecdotal evidence suggests that there may be a strong nocturnal component to activity rhythms in this species associated with the activity pattern of one of the major prey species, *Sebastes marinus*. It is clear that a nocturnal or a dualistic pattern of activity would benefit this species in the wild, corresponding to the movements and exploitation of prey species, and also as a strategy for predator evasion with the main predator of the Atlantic halibut being the seal (Scott and Scott, 1988), a visual hunter generally operating in the hours of daylight.

Although this study did not show the presence of any nocturnal rhythm, the results do indicate a dualistic rhythmicity which is of genuine interest for culture. The results are not distinct, and as noted by Eriksson (1978) data produced in fish behavioural studies seldom shows well defined patterns, however two important reasons may have modified the behaviour of the fish in this study: 1. These fish are the first generation of farmed Atlantic halibut stock, and have been presented with feed only in daylight throughout their rearing period. 2. The technique in this study involved the use of video under conditions of red light, which although at an extremely low intensity may not provide a true picture of nocturnal activity patterns. The second point may be remedied through the application of an infrared camera, unfortunately outwith the scope of this study. The first point demands further investigation into feeding patterns in this species, similar to the work which has been achieved with sea bass by Sánchez-Vázquez *et al.* (1995) in order that a more accurate picture of activity patterns in this species may be obtained and timing of feeding adjusted accordingly.

Since the Atlantic halibut is a species of fish which is found at high latitudes in the wild (see Chapter 1 for distribution), it is likely that the influence of photoperiod length on any activity pattern in this fish will be significant. The results

produced in this study, with activity observed both diurnally and nocturnally, may also be a reflection of an intermediate photoperiod at 12L:12D. Müller (1978) emphasises the importance of photoperiod as the dominant factor affecting the activity phase of organisms at high latitudes. The possibility therefore exists that any behavioural rhythm in the Atlantic halibut may be modified relative to the length of the daylight period, similar to that which has been observed in the brown trout (Eriksson, 1978; Heggenes, 1993). Length of photoperiod is therefore of great importance in future studies on activity pattern in the Atlantic halibut.

Studies on the activity of wild Atlantic halibut could also provide more relevant information into the activity rhythm of this species, with the possibility that the activity of reared fish is biased in some manner according to the history of timing of feed presentation. The technique of telemetry has been used successfully to monitor activity in many species of fish (Holliday *et al.*, 1974; Priede and Tytler, 1977; Priede and Young, 1977; Diana, 1980; Stewart *et al.*, 1983), and the Atlantic halibut would appear to be an ideal candidate for such a study, being large and very robust. Energy budgets have been calculated for fish species under natural conditions using the data produced from such studies (Priede, 1985), and with the potential for the Atlantic halibut to cover great distances, such work could prove to be illuminating in determining both the capacity for movement and timing of activity in this species under unrestricted conditions, providing information which will contribute to the determination of an energy budget for this species in the wild. The results of such a study would give an insight into the bioenergetics of this species under natural conditions and is a valuable tool in fisheries management (Lucas, 1996), and this information is also likely to be extremely relevant to the intensive culture of the Atlantic halibut.

The activity patterns of the Atlantic halibut as observed in this study showed periods of movement of short duration combined with much longer periods where there was no activity. A similar swimming activity pattern has been observed in other flatfish species, with short periods of movement interspersed with periods of rest (Priede and Holliday 1980; Duthie, 1982). These authors also noted the significance

of a "glide" strategy in flatfish locomotion. In a study of the relationship between body shapes and mode of locomotion in fish, Webb (1984) places a species of flatfish, the flounder, immediately below the pike as an acceleration specialist. The body shape of the Atlantic halibut shows a greater degree of streamlining than the flounder, and is likely to occupy a space intermediate between the flounder and the pike in the scheme of Webb (1984), with a mode of locomotion classified by Wootton (1990) as "body, caudal fin, (BCF) transient propulsion." Fish showing BCF transient propulsion have a variety of body shapes, and Wootton (1990) includes the sculpin (*Cottus*) and the pike (*Esox lucius*) in this group, listing the sculpin as a "thrust maximiser" and the pike as a "drag minimser" in their adaptation to this form of locomotion.

Elements of the various body pattern plans observed in fish showing BCF transient patterns of locomotion appear to be achieved in the Atlantic halibut, including minimised drag through relatively small non-muscle mass, and a shallow head silhouette. The anatomy of the Atlantic halibut appears closer to that of a "drag minimiser" than that of a "thrust maximiser". BCF transient propulsion is characteristic of ambush predators, typically occupying structurally complex habitats which afford cover, and where the local populations of prey species are reasonably numerous and the predator does not have to travel large distances to feed. As with many members of the *Pleuronectidae*, the upper surface of the Atlantic halibut confers an excellent camouflage ability which is likely to be associated with the capture of prey, as well as predator evasion in the wild. The patterns of locomotion identified in this study for the Atlantic halibut appear to be characteristic of a fish showing BCF transient propulsion.

The energy costs associated with locomotion in flatfish species have been recognised as being relatively high in comparison to roundfish species (Priede and Holliday, 1980; Duthie, 1982). This fact is reflected in the life history strategies of wild flatfish, where the fish swim close to their critical limit, taking opportunities to rest on the ocean floor as they occur. There is also some literature on the plaice relating to the use of tidal streams for movement over wide areas, a method for

reducing the costs of locomotion during migrations (Greer Walker *et al.*, 1978; Weihs, 1978; Harden Jones, 1979, 1980). Such life history strategies reflect the response of a highly evolved and well adapted group of species to their environment. However, any reduced capacity for work in terms of activity may reflect a relatively limited metabolic scope in flatfish species, another fact which will have implications for the culture of this species.

Historically, the metabolic scope of many species of fish has been defined through experiments on maximum aerobic capacity during sustained swimming experiments with the maximal costs of locomotion setting the upper limit (Brett and Groves, 1979). The metabolic scope sets the upper and lower limits within which the organism must regulate its activities if it is to survive, the lower limit set according to the energy costs associated with homeostasis (see Chapter 1.). Such an approach still has universal application in fish studies, with the index for metabolic scope (known as the metabolic power index) ranging from 0 to 1 (Lucas, 1996). A review of metabolic scope in fish by Priede (1985) notes the exception of the cod to this rule, where the maximum aerobic capacity is observed after the ingestion of a meal, a level in excess of the power output during swimming.

The lack of information on both the M_A and M_F components of metabolism in the Atlantic halibut means that little direct information on the metabolic scope in this species is available. However, interpretations of life history patterns, anatomy, and the results generated in this thesis suggest that it is likely that the metabolic scope of the Atlantic halibut may be similar to that observed in other flatfish species, and is relatively limited. (The possibility of a close similarity with metabolic scope in the cod also exists - see Chapter 4. for discussion). With this in mind, there are clear implications for the culture of this species, and husbandry practices involving the physical handling of Atlantic halibut are areas where an element of caution appears justified. The above discussion suggests that flatfish may exceed the levels of their metabolic scope with little difficulty, leading to conditions of anaerobiosis and the associated occurrence of an oxygen debt. This means that the normal routine husbandry practices associated with weighing and grading of stocks may exert a

serious metabolic cost in this species, associated with struggling when the population is crowded and struggling in nets. This metabolic cost is likely to be manifest as a large relative increase in oxygen consumption rate during and also following handling as any oxygen debt incurred is repaid. Precautionary measures to ensure that all stock are adequately starved before handling will reduce the impact of this stressor on stocks, through reduction of the energy allocation to the M_F component thus allowing the metabolic scope to be diverted to the M_A component. The importance of asserting that the stock is starved prior to such work so that the metabolic scope is maximised, should not be underestimated.

In this study an increased level of fish activity and associated increase in metabolic expenditure, was observed with decreasing stocking density in the small fish trials. This is in direct contrast to the work of Ross and DeRooi (unpublished results) who showed that motor activity in the tilapia, *Oreochromis niloticus*, increased with increasing stock density. An increase in daily motor energy expenditure with decreasing stock density could be due to the development of hierarchies within the test population. Aggression between individuals was only recorded in the medium density small fish trials, however a greater time was spent by halibut at the water/air interface in activity category 5 in the low density trial.

Hierarchies are known to develop at low stock densities, and to be associated with an increase in the level of aggressive behaviour as individuals defend a resource, with strong effects often observed in small populations numbering between five and ten individuals (Alanara, 1997). In a study of the European eel (*Anguilla anguilla*), Knights (1987) found dominant larger eels to spend less time swimming high in the water column, and to be less active than eels positioned lower in the social hierarchy in tank populations. Carter *et al.* (1996) observed feeding hierarchies in the greenback flounder (*Rhombosolea tapirina*) with inter-individual variation in feed consumption from 0% to 22% of the available food, suggesting that members of the *Pleuronectidae* may conform to generally held views on fish social behaviour.

A high level of activity close to the water surface as seen in the lowest stocking density in this work, may be the response of highly stressed subordinates within the population, and it would appear that some degree of hierarchy was present at least in the low density populations of halibut in this study. Clearly such activity involves a significant increase in metabolic expenditure, and with a greater portion of the available energy allocated in this way the effect is likely to be manifest as a reduction in growth in any individual, as less energy is available to this component in the energy budget. The behavioural results of this study are of general interest, but since stocking densities are likely to be maintained at a level close to or above the maximum achieved in this study, such behaviour is unlikely to be significant in culture.

In a comparison of the energetics of running, swimming and flying homeotherms, swimming poikilotherms, and running poikilotherms, Peters (1983) regards swimming as a slow but relatively metabolically inexpensive mode of locomotion. Specific energetic costs in terms of distance and time are reduced with increasing size of animal, a factor which is likely to be of relevance to an organism like the halibut where body size in adults covers a wide range. A mass specific reduction in the costs of locomotion has been observed in the sockeye salmon (Brett, 1965), although the relationship is complex with the weight exponent found to vary with activity level. Brett and Groves (1979) noted that the effect of size on the active metabolic rate is an area which has received little attention, and the same appears true at this point. A mass specific reduction in the costs of locomotion may be of evolutionary significance for an open ocean predator like the Atlantic halibut which appears to cover large distances in the natural environment, driving the importance of large body size in this species and influencing the intermediate place of this fish along the r-K strategy continuum (Chapter 1. details movements and life history patterns). Rowan and Rasmussen (1996) observed the activities of mature fish to be significantly higher than those of immature conspecifics, which could be a reflection of these reduced costs of locomotion in larger fish, or they could also be linked to an increase in aggression in mature fish associated with sexual behaviour. It is interesting to note that in this study the total time at rest for the large fish was less

than for the small fish at the two higher stock densities, and that the calculated energetic costs of motor activity in the large fish were reasonably low and close to those of the small fish at the highest stock density. However, experimental conditions make such comparisons unclear, and a more valid comparison may be made when large fish are held in a proportionately larger tank, and stock densities (expressed as kg m^{-2}) are at the same level.

A study of the activity pattern of the pike (*Esox lucius*) in the natural environment by Diana (1980) showed that this piscivore was inactive for approximately 80% of the total time of study. The above discussion draws similarities between the anatomy and swimming characteristics of the pike and the Atlantic halibut, and although comparisons between activities of tank held fish and fish in the wild are clearly subject to environmental influences, the value of Diana (1980) is within the range of results produced in this study for the Atlantic halibut.

There is a time lag effect in open system respirometry which is a consequence of the volume and flow rate of the respirometer used. As the fish respiration rate changes there is an effect on local dissolved oxygen levels in the immediate environment within the respirometer, and it will take some amount of time for this to register as a change in recorded dissolved oxygen levels in the effluent, depending on flow rate and respirometer volume. Taking a possible time lag into account, the results show reasonable agreement between routine oxygen consumption rate and activity levels in this study. These results are similar to those of Sims *et al.* (1993) for work on routine oxygen consumption rate and activity rhythms in the lesser spotted dogfish (*Scyliorhinus canicula*), a study which also involved the use of video recording to assess activity levels. In this work, routine oxygen consumption rate did not show the marked nocturnal increase observed in many of the community tank trials in Chapter 4 and individual respirometry trials in Chapter 3. This does raise questions over the applicability of the experimental conditions, and the employment of red light for filming, in particular. Sims *et al.* (1993) achieved results with a similar arrangement using red light to film in the "dark" phase, however there could be species differences in response to light of this wavelength.

One further point which may be of relevance to the culture of all species of flatfish in tanks is the nature of the material from which the tank is constructed. With the fish spending such long periods in close proximity to the tank floor, as have been identified in this study, it is important that the correct environment is provided. A smooth fibreglass sheet is easy to clean and provides a highly manageable system for the intensive culture of roundfish species such as salmonids, however such surfaces may not be ideally suited to flatfish culture. Throughout the course of the work for this thesis, halibut were observed daily in tanks of various sizes. General observations indicated that halibut had a tendency to slide on smooth tank floors, and fish were observed to readjust position in response to these movements. Occasionally fish exhibited epidermal lesions on the surface in contact with the tank floor, indicating a possible impact on fish health. Clearly such readjustment activity results in an energetic cost, and if daily motor activity could be reduced through the development of a more suitable system, there may be beneficial effects on the culture of this species in intensive systems.

At this juncture no approach to providing an intensive tank system solely adapted to the culture of flatfish has been developed in this country, however some halibut farms in Iceland operating at commercial densities utilise netting stretched over tank bottoms to provide a stabilising medium for the fish (E. Jonsson, pers. comm.). Such a system has trade-offs, and tank hygiene may be slightly compromised since any medium will allow the concentration of detritus, faeces and uneaten feed. However epidermal lesions and stress levels could be reduced in populations of halibut maintained in such systems. It appears that an opportunity exists for the development of tank systems targeted precisely at the culture of Atlantic halibut, given the unique characteristics of this species.

In summary, this work produced evidence for dualistic patterns of activity in the Atlantic halibut under the test conditions of a 12L:12D photoperiod. This activity correlated with routine oxygen consumption rate, although the results of this part of the work were relatively crude, reflecting the unsophisticated nature of the technique. Decreasing stocking densities produced increasing activity levels and daily motor

activity costs in the smaller sizes of fish examined, with aggressive episodes observed only in small fish at intermediate stocking densities. Large halibut were relatively less active at the more energetically costly levels of activity. Development and interpretation of the results allowed the determination of the time allocated to motor activity in tank held populations of Atlantic halibut and the energy costs associated with such activity, and this is valuable information which may be incorporated into an energy budget for this species in culture. A large percentage of time was spent at rest in the observation tank in the halibut under study, however the provision of a stabilising medium may increase the time at rest further, to the possible benefit of growth rate. This work provides an insight into activity patterns in the Atlantic halibut, and suggests that there are strong implications of such patterns for the intensive culture of this species, although further work is required to clarify activity patterns in the Atlantic halibut under conditions of varying photoperiod length, temperature, season and fish size.

Chapter 6

A bioenergetics study of juvenile Atlantic halibut (*Hippoglossus hippoglossus* L.).

6.1 Introduction

Component values in energy budget equations are generally expressed as joules (J) per unit time, and with the duration of experiments varying considerably (Lucas, 1996) it is important to note the experimental time scale involved in any research work. Experimental studies aim to quantify the individual components of the energy budget equation to provide information on the physiological transformations and energy allocation within the fish (Jobling, 1994). In a study of the energetics of the perch, *Perca fluviatilis*, Solomon and Brafield (1972) identified several important points in the undertaking of such work, namely that all energetic pathways should be measured concurrently; that metabolic measurements should be long term to reduce any effects of stress, and cover the full 24 hour period to include any diurnal fluctuations; that measurements should be recorded over a period when the fish are growing continuously and a significant change in weight is recorded.

The energy available to an organism for metabolism, growth, locomotion and reproduction, is determined by the quantity consumed, the nutrient content, and the proportion assimilated of the ingested food. The relationship governing energy allocation and food intake in fish is a complex interaction of a variety of biotic and abiotic factors including temperature, photoperiod, salinity, fish size, activity, behaviour, appetite, feeding regime amongst others (Talbot, 1985). These interactions all operate on the energy budget equation, influencing the allocation of energy within the various component parts. Laboratory experiments allowing direct observation of consumption evacuation and absorption of food are fundamental to an understanding of the interactions of these factors. In aquaculture, knowledge on the interactions of environmental factors and the effect on energy partitioning in a species will benefit production when optimal conditions for growth (the factor "P" in the energy budget) are identified, and such conditions may then be maintained in a culture system.

The quantification of the F component permits the calculation of the digestibilities of the nutrient classes, and in particular the Digestible energy (DE), which is the value of energy entering the system (the gross energy of food - energy lost in the faeces). Further subtraction of the energy associated with nitrogenous excretion in the U component from the digestible energy value produces the metabolisable energy (ME) fraction. Differentiation of the energy available to the organism for work and growth involves the subtraction of the M_A component and the energy costs associated with S.D.A. from the ME fraction, producing a value known as the net energy (NE) (Brody, 1945). A diagrammatic representation of the partitioning of energy within the fish is presented in Fig. 6.1.

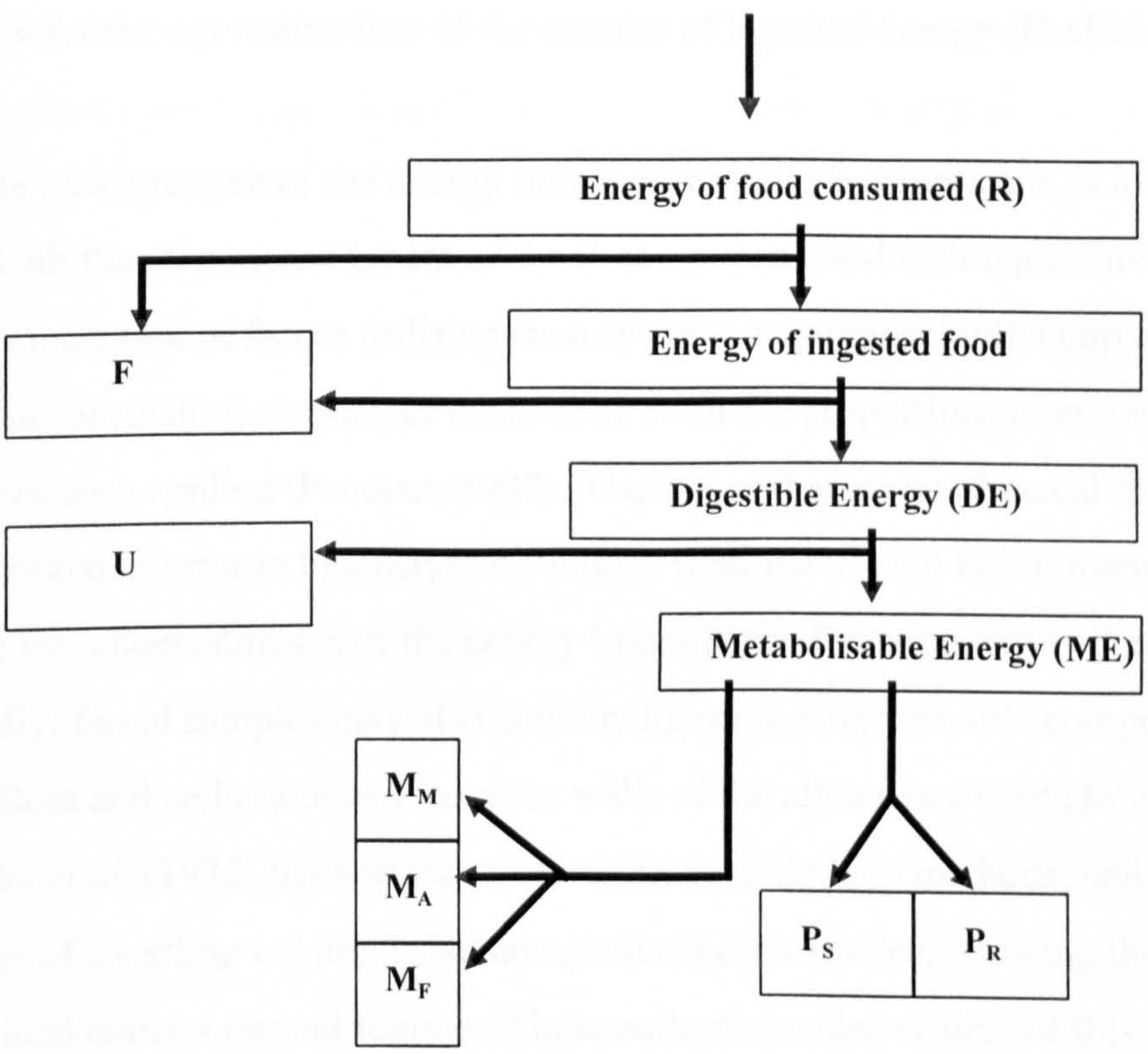

Fig. 6.1. Diagram illustrating the partitioning of energy within the fish. Components are as identified in the text. Net energy is the energy available to the M_M, M_A and P components.

Braaten (1979) reviews the many methodologies that have been employed in research into fish bioenergetics by a large number of authors. Many of the difficulties in measuring the metabolic components M_M, M_A and M_F, have already been discussed in Chapters 3., 4. and 5. Measurements of the other components of the energy budget equation also pose similar problems.

In experimental studies, the R component of the energy budget is relatively easily measured as the total amount of feed consumed, and a variety of techniques have been successfully employed for the determination of this factor including direct observation of feeding activity, examination of gut contents through serial slaughter and sampling of live fish, radiography, and demand feeders (Talbot, 1985). Processes such as regurgitation of a meal by fish, and the leaching of nutrients from pellets, as well as the more obvious lack of ingestion of an offered meal by test fish provide an opportunity for the overestimation of the amount of ingested energy (Brafield, 1985).

The measurement of the energy loss in faeces, the F component, is inherently more difficult than the measurement of the R component, and techniques involving either total recapture of faeces utilising such systems as purpose built sump collectors and mechanical rotating screens, or calculation from the proportions of an indigestible marker have been applied (Pandian, 1987). Capture and analysis of faecal matter is a potential source of error in bioenergetic studies. If all the voided faecal matter is not accounted for, underestimates of the energy losses in the F component will ensue. Additionally, faecal samples may also contain digestive enzymes, bile components, gut microflora and cells sloughed from the walls of the alimentary tract (Jobling, 1993). Cho *et al.* (1976) list a series of seven faecal collection methods, and argue that the use of a settling column is advantageous in such studies, allowing the fish to be maintained under a normal regime. These authors are also critical of this method in allowing the potential for leaching of the nutrients from the faecal matter when deposited in a settling column, although subsequent trials did not show significant differences between this method and other methods such as anal suction and dissection. Brett and Groves (1979) quote a 20% loss of ingested energy to faecal matter as a general figure for carnivorous fish.

The U component (termed nonfaecal loss or urinary energy by Braaten (1979)), involves the measurement of nitrogenous excretion through serial sampling of respirometer or tank effluent. Relative to energy losses in metabolism, loss of energy to this component is low although it is an important factor in aquaculture systems since elevated ammonia concentrations may affect metabolism, production and survival (Knights, 1985). The daily cyclical production of exogenous nitrogen excretion in fish held under intensive conditions (Brett and Zala, 1975; Kaushik, 1980) is an important factor in this regard, since ambient ammonia concentrations are likely to be constantly changing, and should be monitored over a 24 hour period if fish health and production are considerations. The loss of energy to the organism in the form of nitrogenous excretion is known to increase with temperature, fish size and ration size (Elliott, 1979; Jobling, 1981b). A figure of 4-15% of ingested energy was postulated by Jobling (1994) to account for energy losses to nitrogenous excretion, depending on test conditions, dietary constituents and feeding regime.

At its most simple calculation, the P component may be measured as the change in wet weight over a period long enough to record significant changes in weight (Braaten, 1979). Exponential transformations have been applied to determine specific growth rates, and are expressed according to the equation:

$$SGR = \frac{\ln W_1 - \ln W_0}{t_1 - t_0} \times 100 \qquad \text{(Braaten, 1979)}$$

The calculation of specific growth rates using wet weight changes assumes that moisture contents remain unchanged throughout the measurement period, and this is an assumption which may not necessarily always prove valid. Carcass moisture content was found to vary directly with lipid content in intensively reared Atlantic salmon by Aursand *et al.* (1994), while during smoltification moisture content has been found to vary inversely with lipid content in Atlantic salmon and steelhead trout (Fessler and Wagner, 1969; Farmer *et al.*, 1977). Wet weight change does also not take into account the costs of production of body tissues with a high energetic content,

such as gonadal tissue (Ursin, 1979), and the majority of fish bioenergetic studies are undertaken on sexually immature fish to avoid error due to this factor. Since lipid and protein deposition rates are known to vary with fish size and activity level (Jobling, . 1993), it is also of great importance to ensure that growth studies are performed on fish within a tight size range under the same conditions of activity. Growth rate may also be predicted if the level of feed intake is known, and this technique has been used to provide estimates of growth rate in Atlantic salmon and rainbow trout (Austreng *et al.*, 1987), and successfully to predict growth in the Atlantic halibut (Tuene and Nortvedt, 1995).

Bioenergetic studies related to aquaculture set out to identify optimal feeding regimes where consumption, absorption and production efficiencies may be maximised (Knights, 1985). The most important factors affecting the utilisation of feed by fish are temperature, salinity and ambient oxygen concentration (Kaushik, 1986). Temperature is considered to be the most important factor, affecting enzyme kinetics and thus a range of physiological processes such as maintenance requirements, metabolic rate, growth, food intake and maturation. Conducting bioenergetics trials as close to the optimum temperature for any given species and fish size, will help to fulfil one of Solomon and Brafield's (1975) criteria for such work - namely that there is a significant growth of fish recorded over the test period. Thermal growth optima in the Atlantic halibut have been determined by Björnsson and Tryggvadóttir (1996) for the size ranges 10g to 60g (14°C), 100g to 500g (11.4°C) and 3kg to 5kg (9.7°C) in growth experiments lasting between 99 and 216 days, for fish fed both dry and moist diets.

A wide range of literature is devoted to the effect on growth and body composition of differing ratios and sources of the major nutrient classes in feedstuffs, much of this work dedicated to finding the optimum ratio of protein to lipid in the diet. The efficiency of protein utilisation decreases above an optimum, with increasing ration in fish (Cho and Kaushik, 1985), and the aim of many of these studies is to replace the protein above the requirement for protein deposition, with lipid and carbohydrate which may be used preferentially as an energy source by the

fish - the so-called "protein-sparing" effect (Payne, 1979). Dietary protein in temperate marine aquaculture comes predominantly from a fishmeal source. High quality fish meal is extremely expensive, and feed costs may account for 40-60% of capital costs in intensive systems (Tacon and Jackson, 1985). There is therefore economic pressure to optimise protein levels in diets in response to growth rate, although only relatively recently have studies on some species of flatfish such as the turbot (Andersen and Alsted, 1991; Danielsen and Hjertnes, 1991; Nijhof, 1991) and the Atlantic halibut (Berge and Storebakken, 1991; Hjetrnes *et al.*, 1991) been conducted with this aim. Such work is a reflection of the perceived importance of the culture of flatfish to the fish farming industry in temperate regions.

Historically there have been several important studies which have set out to determine energy budgets for a variety of fish species including the perch (Solomon and Brafield, 1972), the largemouth bass (*Micropterus salmoides*) (Niimi and Beamish, 1974), the brown trout (Elliott, 1976), the rainbow trout (Cho *et al.*, 1976; Staples and Nomura, 1976), the minnow (*Phoxinus phoxinus*) (Cui and Wootton, 1988a,b) the grass carp (*Ctenopharyngodon idella*) (Carter and Brafield, 1991) and the common carp (Ivlev, 1939; Chakraborty *et al.*, 1995). The information gathered by these studies is significant both from an ecological and aquacultural perspective, enabling a more elaborate understanding of the ecological energetics for these species in their natural environment, and a greater knowledge of the requirements and production in culture systems.

The application of a bioenergetic approach to aquaculture principally allows for the prediction of growth (P component) through elucidation of the interactions involved in the energy budget equation. The prediction of growth in an aquaculture system is fundamental to the construction of aquaculture models, which in turn are necessary to ensure an element of economic stability. Schuur (1991) notes the economic importance of the interactions between management of culture space, feed, oxygen, water and thermal resources, in the development of a bioenergetic model for operation with intensive culture systems. The development of such predictive measures are of great importance in the highly intensive fish farming systems of

temperate region mariculture, where a balanced calculation of production targets may offset the high capital investment which generally characterises such systems. It is likely that the culture of the Atlantic halibut will fall into this category, and the evaluation of an energy budget for this species is thus of great importance.

Following on from the work in previous chapters, the aim of this study was to conduct an experiment within which energy transference through all the energy pathways could be measured in small populations of Atlantic halibut. In order to do this, specifically designed apparatus was employed, allowing the measurement of feed intake, growth, faecal production, oxygen consumption and nitrogen excretion of small populations of Atlantic halibut held within metabolic chambers. In addition, proximate analysis of fish was undertaken in order to investigate energy accumulation within individual halibut in trial populations, and to assess energy flow through the entire system.

6.2 Materials and Methods

6.2.1 Fish

1996 year class Atlantic halibut of Mannin Sea Farms (Isle of Man) stock, and 55 fish of 70g average weight were provided by the British Halibut Association in May 1997. These fish were of similar genetic composition to the Ardtoe stock, having derived from the original captive wild broodstock Atlantic halibut caught in Icelandic waters in the late 1980s. As a precautionary measure, the fish were monitored for signs of ill health or abnormal behaviour for some weeks after they had arrived on site. During this time they were routinely fed at 2% body weight d^{-1} with B.O.C.M. Paul's flatfish diet, and the stock tank was flushed daily and brushed weekly. No mortalities were observed over this period, and all stock responded well to feed and appeared healthy. At the commencement of the trial, the fish weighed 136.8g (± 28.4).

6.2.2. System Components

The metabolism unit was housed in the old lifeboat station at the Marine Environmental Research Laboratory, University of Stirling, Machrihanish. This area is quiet, and was considered to provide an undisturbed environment for the experiment.

6.2.2.1 Water Supply, Water Quality and Temperature Control

The sea water supply to the metabolic chambers was conditioned to remove suspended solids and maintain a constant temperature in the region of 12°C. This temperature is close to the temperature for optimum growth for Atlantic halibut of 100g to 500g body weight, as defined by Björnsson and Tryggvadóttir (1996). Chilling of the ambient supply was required in order that this temperature could be maintained over the trial period. The system was arranged in such a manner that ambient water was received into a 400 litre sump tank from the site header tank.

Attached to the sump tank was a closed loop system with an "Acry-Tec" (San Diego, California) refrigerated cooler through which sea water continuously circulated. The sump tank also contained two 15cm diameter airstones linked to an electrical air pump which was operated continuously, and this provided vigorous aeration and mixing in the sump.

Sea water was continually pumped to a 277 litre header tank through a sand filter (LaCron Ltd., England) and 3 in-line "By-Pass" cartridge filters in series, which provided a gravity feed to the metabolic chambers. All pipes connecting these components were 1.5" diameter PVC tubing. Water from the header tank continuously overflowed back to the sump, thus allowing constant turnover and exchange within the unit. A representation of this system is shown in Fig. 6.2.

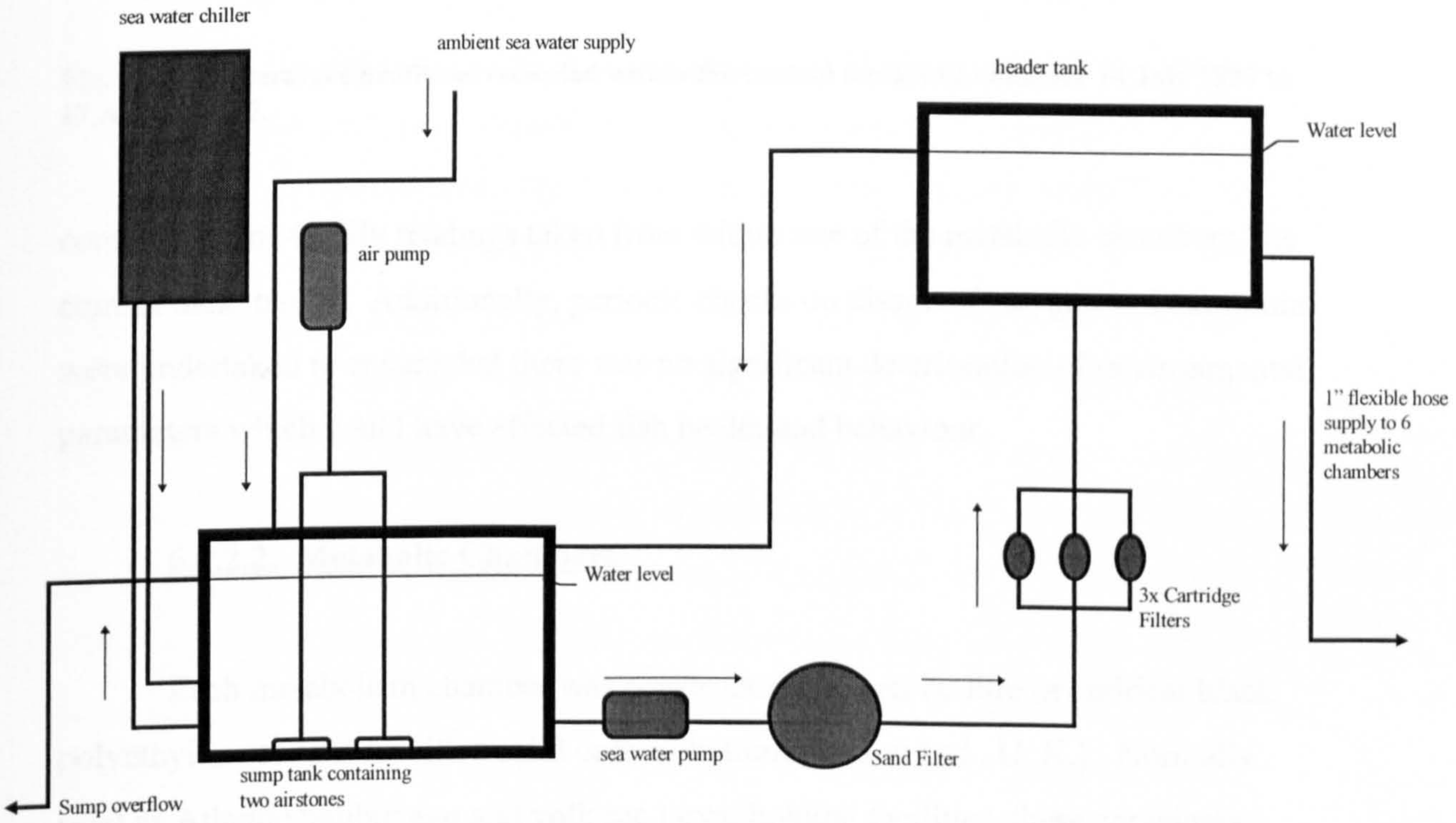

Fig. 6.2. Schematic representation for the water conditioning unit supply to the metabolic chambers. Water flow direction is indicated by blue arrows.

Although the sea water supply to the metabolic chambers was single pass, the water conditioning unit was a partial recirculation system, allowing the reduction of

ambient temperature, and an effective dampening of the variations in the temperature of the ambient site supply. Fig. 6.3 indicates the temperature profile of sea water,

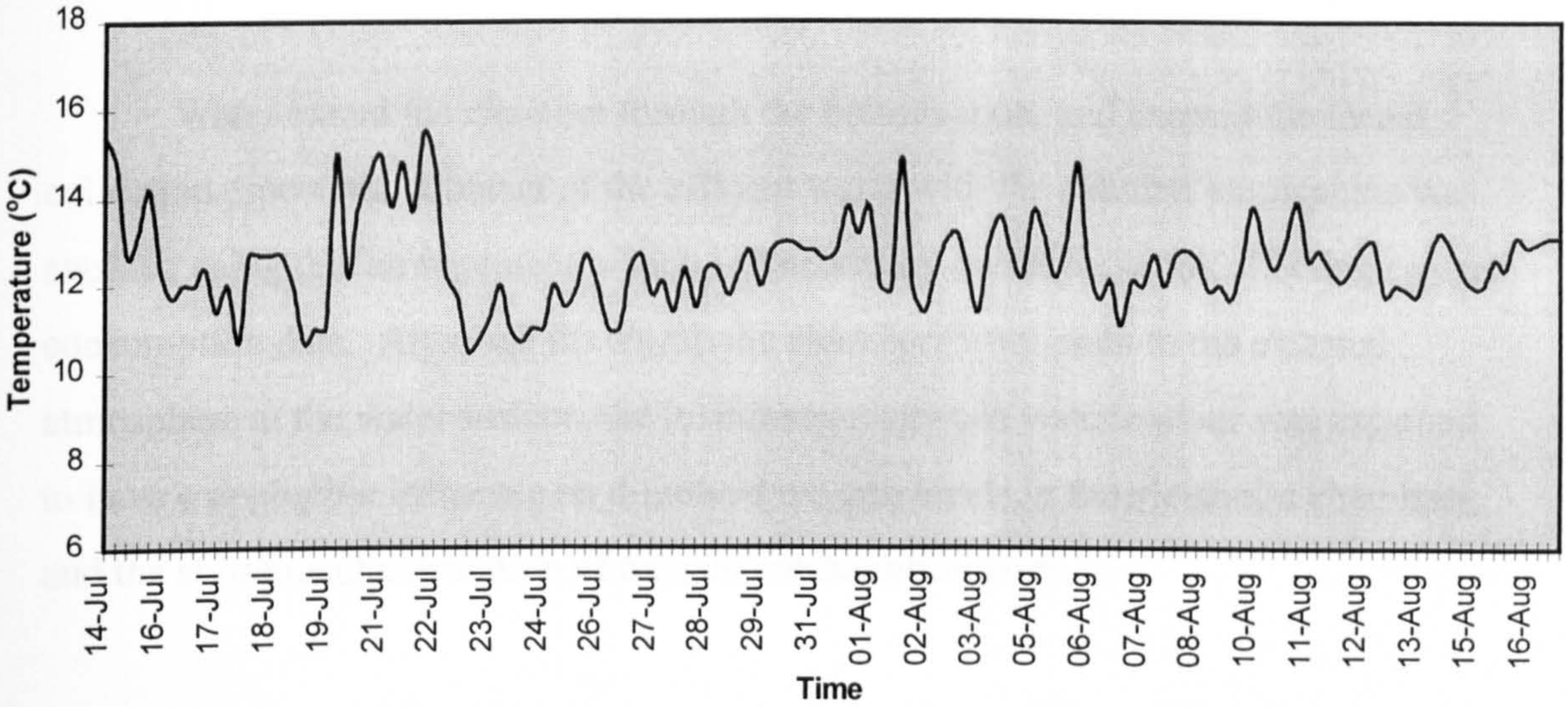

Fig. 6.3. Temperature profile as recorded within the control metabolic chamber 14 July 1997 to 17 August 1997.

compiled from 4-daily readings taken from within one of the metabolic chambers, the control tank, tank 4. Additionally, periodic checks on dissolved oxygen and ammonia were undertaken to ensure that there was no significant deterioration of environmental parameters which could have affected fish health and behaviour.

6.2.2.2. Metabolic Chambers

Each metabolism chamber was constructed from an 80 litre cylindrical black polyethylene container with a solid conical bottom (Paxton Ltd., U. K.). Normally used as Atlantic halibut egg and yolk sac larval holding facilities, these containers were modified through the introduction of 5mm slotted stainless steel screens situated horizontally above the conical base, and held in position by silicon glue. The screens were strong enough to provide an adequate base on which the fish could rest within the tanks, having the ability to support the fish weight without moving or distorting in shape. In principle the screens would allow the passage of faecal material to the lower

level of each tank, with feed pellets retained within the upper portion of the chamber. Six metabolic chambers were set up in the unit for the trial, with each individual chamber receiving sea water through a separate inlet. All fittings were 15mm polyethylene "Speedfit" pipes and connectors.

Water exited the chamber through the bottom cone, and entered the faecal collection pipework. Contact of the effluent water with the external atmosphere was avoided using this arrangement, which is a necessary prerequisite to collecting oxygen consumption data. Although the metabolic chambers were open to the external atmosphere at the water surface, the interchange between water and air was assumed to have a negligible influence on dissolved oxygen levels in the metabolic chambers, and the subsequent calculation of oxygen consumption rates.

The faecal collection pipes were also manufactured from "Speedfit" polyethylene. In essence, a short piece of 15mm pipe exited the chamber vertically for 5cm, entered a 90^0 bend, from whence it ran the radius of the chamber to the vertical faecal collection pipe. The faecal collection pipe was constructed from 22mm "Speedfit" pipework, which would allow the reduction in flow rate of effluent water, and a consequent deposition of faecal matter in a lower container tube. Below the container tube, a system of 2 valves in parallel allowed collection of faeces with a minimum quantity of water, similar to the system employed by Cho *et al.* (1976). The vertical collection pipe rose to the water level externally to the chamber, and then through a series of reducers into 10mm elastic polyethylene tubing, which fed the metabolic measurements unit. In order that fish escapes could be avoided, a fitted panel of black 10mm netting was stretched tightly and held in place by elastic over the top of each chamber. Each individual chamber also contained an airstone as a precautionary measure. A representation of a metabolic chamber is shown in Fig. 6.4. The 6 metabolic chambers were arranged in a parallel series of 3 pairs, all supported on a framework stand which allowed easy access to the faecal collectors. Individual chambers were identified by number, and chamber 4 was chosen arbitrarily for the control.

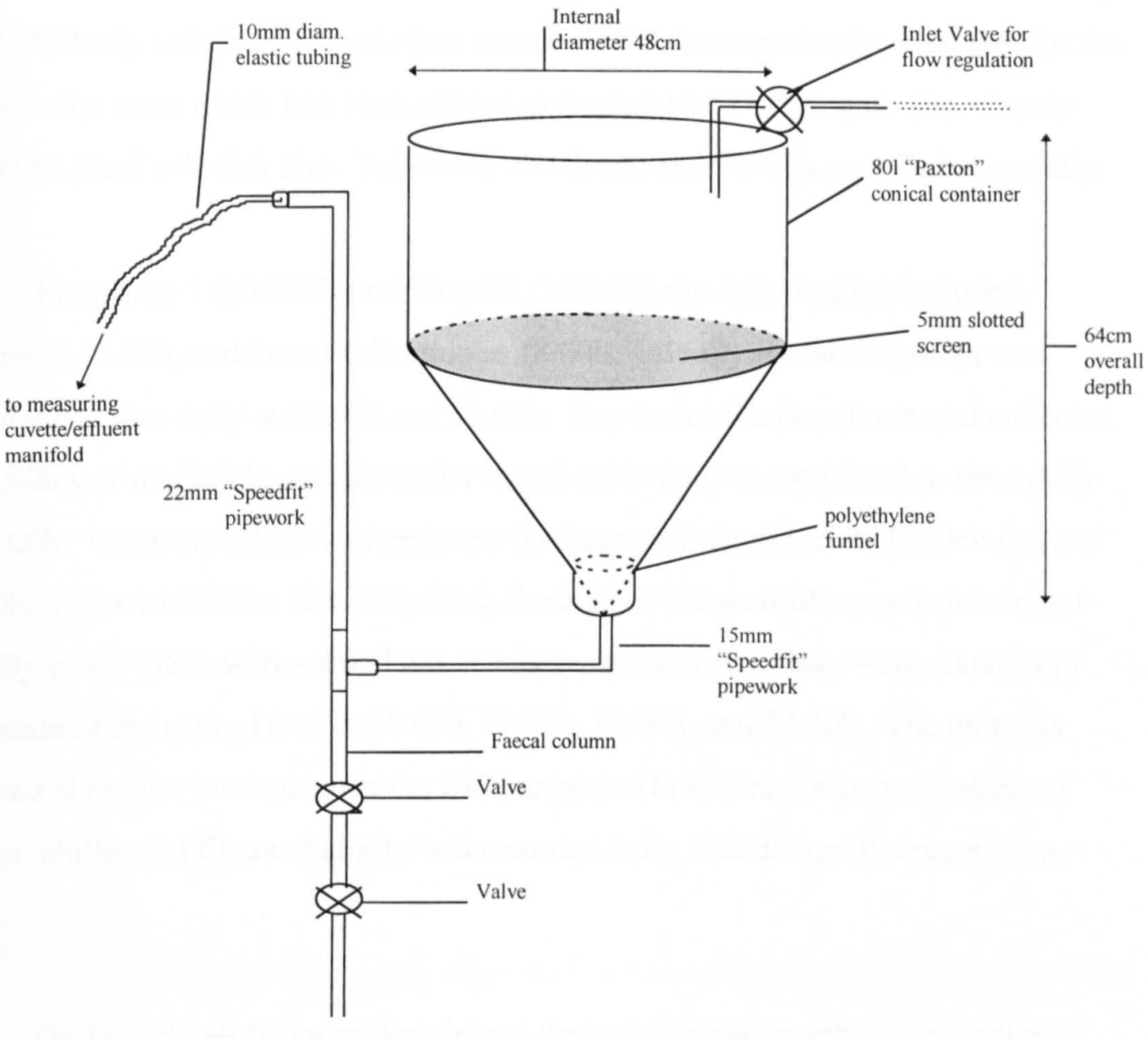

Fig. 6.4. Details of metabolism chamber.

6.2.3 Procedure

Following the quarantine period, and once it was established that general behaviour and activity was normal, 30 fish were chosen at random from the population, and divided between 5 metabolic chambers within the bioenergetic unit on 15 July 1997. A settlement period of 4 days within the system was allowed, during which halibut in all chambers were offered feed at 2% body weight d^{-1}. On 19 July 1997 (taken as Day 0), all fish were wet weighed under 2-phenoxy ethanol anaesthesia, giving a start point for growth data. Additionally, one fish from each population was sacrificed (termination by 2-phenoxy ethanol overdose), dried at 105°C, re-weighed, and then frozen for later proximate analysis. Fish in one tank were starved for the full 28 day period (tank 3), with the remaining 4 tanks forming 2

pairs of tanks: at feeding rates of 0.5% body weight d^{-1} (metabolism chambers 5 & 6), and 1.0% body weight d^{-1} (metabolism chambers 1 & 2) respectively. The diet for the trial was the same which had been offered to the fish since arriving on site, namely B.O.C.M. Paul's flatfish diet. Pellet size was 8mm, and the fish were fed once daily.

From Day 1 (20/7/97) until Day 28 (16/8/97) the daily routine included feeding at 12.00h, and faecal collection at 18.00h. Initially faecal collection was undertaken twice daily at 08.00h and 18.00h. In practice the faecal material collected at 08.00h was negligible, and thereafter faecal collection was restricted to once daily at 18.00h. Uneaten pellets were removed (and counted) from metabolic chambers at 14.00h. After 14.00h the necessary back-flushing of the sand filter, and cleaning of the "By-pass" filters were carried out. Daily temperature readings were taken from the inside of the control tank at 08.00h, 12.00h, 18.00h, and 22.00h. The unit was checked at regular intervals over the 24 hour period to ensure adequate working of pumps, chiller and filters. Salinity was recorded daily, and did not fluctuate from 34‰.

On Day 29, all fish were terminated through 2-phenoxy ethanol anaesthetic overdose, and wet weights recorded. The fish were then dried for 24 hours at 105°C, and dry weights recorded. All halibut were kept separate throughout, and through the provision of identification numbers, individual chamber populations and fish within those populations, could be identified. After drying, all fish were stored at -20°C for later proximate analysis.

6.2.4. Feed

Although the diet used in this trial was a commercial 8mm flatfish feed, the pellets often varied in size and morphology. Pellets were assessed by eye daily prior to weighing the allocation of feed to ensure that only pellets of a regular size were eaten by fish. (This was necessary in order that pellets small enough to squeeze through the slots in the internal screens were not offered to fish). Counting of pellets,

and recording of weights ensured that the weight of eaten feed could be calculated by the deduction of uneaten pellets from the offered weight.

6.2.5. Faeces

Faecal matter was collected daily at 18.00h. A quantity of approximately 500ml of seawater and faeces was obtained through the double valve system at the base of the recovery tubes, into 600ml glass beakers. The resulting matter was filtered on previously dried and weighed papers (Whatman GF/C), and then dried for 3-4 hours at 105°C. The dry weight was recorded at the end of this time, and the papers were then stored at -20°C. The resulting dry matter was pooled for each individual metabolic chamber, and kept for subsequent proximate analysis. No faeces were collected from tank 3.

6.2.6. Metabolic Measurements

All 10mm flexible tubing exiting the metabolic chambers was directed through a 3-way valve system, allowing the switching of chamber effluent between a manifold running to waste or a measuring cuvette housing a YSI 5739 oxygen probe. The cuvette was situated on top of a magnetic stirrer, and water passing through the system exited through a flow meter before running to waste, also through the manifold system. (The manifold system comprised a series of holes of equal size cut in a frame, which allowed the accurate horizontal location of effluent tubes at identical heights.)

Calculations of oxygen consumption and ammonia excretion rates were carried out from data generated by a 24 hour sampling regime on days 12, 16, 19, 22 and 26. A series of recordings of dissolved oxygen, temperature and flow rate were made for each individual chamber on a 2 hourly basis over the sample period. Water samples were obtained from the chamber effluent by collection in 250ml B.O.D. bottles from the tubing exiting the flow meter, as each chamber was connected through the measuring cuvette in turn. These were immediately filtered (Whatman GF/C papers),

and frozen at -20°C, for subsequent analysis of ammonia levels (see Chapter 2 for method of ammonia analysis and calculation of oxygen consumption and ammonia excretion rates).

6.2.7. Growth

Growth was measured as the change in wet weight over the study period. Initial weights and final weights were recorded for all fish on day 0 and day 29, and the growth rate determined according to the equation:

$$SGR = \frac{\ln W_1 - \ln W_0}{t_1 - t_0} \times 100 \qquad \text{(Braaten, 1979)}$$

where W_1 = final weight of fish; W_0 = initial weight of fish; t_1 = day 29; t_0 = day 0.

Feed conversion ratios were determined according to the formula:

$$FCR = (R \times t) / (W_1 - W_0)$$

where R = total food intake (g).

Protein efficiency ratio (PER) was determined according to the formula:

$$PER = (W_1 - W_0) / P$$

where P = crude protein ingested (g)

6.2.8. Energy Determination and Proximate Analysis

Individual fish and feed samples were homogenised by coffee grinder. Faecal samples were prepared by physical removal from filter paper, taking care not to include filter paper fibre, and homogenisation with mortar and pestle. Proximate analysis was carried out in triplicate for all materials except where stated otherwise.

6.2.8.1. Ash

Ash samples were determined through combustion of 1 gram of sample in a porcelain crucible at 600°C in a muffle furnace for 12 hours, and recording the

difference between weights. Only one analysis of faecal ash was determined due to the reduced quantity of this material.

6.2.8.2. Crude Protein

Samples were analysed by the micro-Kjeldahl method (Tecator, Kjeltec system, 1003 Distilling unit; Digestion system 40, 1016 Digestor) to determine the total nitrogen content, which was converted to percentage protein by applying the empirical factor 6.25 (Kjeldahl factor for animal protein). Faecal crude protein values were determined through the values for nitrogen produced from the CHN analyser.

6.2.8.3. Crude Lipid

Fish and feed samples were analysed using the Soxhlet apparatus (Tecator, Soxtec system HT, 1043 Extraction Unit). Crude lipid from a known quantity of sample is extracted with analytical grade petroleum ether (B.P. 40-60°C), and the final weight of extracted lipid is obtained after drying of residue at 105°C for one hour. The small quantity of faecal matter collected precluded crude lipid analysis according to this technique, and these samples were analysed using the Folch-Lees method (modified Bligh and Dyer, 1959).

6.2.8.4. Bomb Calorimetry

Gross energy content of fish feed and fish carcass samples was determined by the method of bomb calorimetry, using a Gallenkamp Autobomb Adiabatic Bomb Calorimeter. Samples were analysed in triplicate. A dried homogenised sample was pelleted with a known quantity of analytical grade benzoic acid. (The known energy content of the benzoic acid is 26.45kJ g^{-1} and is subtracted from the final result). The weight of this pellet provided the weight of sample (between 0.4g and 0.6g) for calorimetry. The pellet was then combusted within the bomb calorimeter in the presence of oxygen at approximately 30 bar, and the rise in temperature following combustion recorded by the temperature difference in the water jacket surrounding the

bomb before and after firing. The energy content was calculated according to the formula:

$$\textbf{ENERGY CONTENT (kJ g}^{-1}\textbf{)} = \frac{\textbf{[(Final temp. - Initial temp.) x 10.82] - 0.0896}}{\textbf{sample weight (g)}}$$

(The figure 0.0896 is the combined energy value of the nickel wire (~ 0.014g x 1.4 kJ g^{-1}, = 0.0196) and the cotton (~ 0.004g x 12.5 kJ g^{-1}, = 0.0700, which are used to support the pellet within the bomb calorimeter).

6.2.8.5. CHN Analysis

Recovered faecal matter provided extremely small quantities for analysis, which necessitated a micro analytical procedure. A Perkin Elmer Series II 2400 CHNO/S analyser was used to determine total carbon and total nitrogen in ashed and non-ashed faecal samples. The organic carbon content was used to determine gross energy (multiplication by the experimental coefficient 42.515 kJ g^{-1} organic carbon), and the total nitrogen content to determine crude protein (multiplication of total nitrogen value by 6.25). All carcass and feed samples were also processed through the CHN analyser, providing a verification of crude protein and energy content results.

6.2.9. Data Analysis

All oxygen consumption and ammonia excretion rate data were analysed according to the equations and protocols of Chapters 2., 3. and 4. Oxygen consumption and ammonia excretion rate differences between individual metabolism chambers were analysed through two-way Analysis of Variance, and proximate analysis of carcass samples were compared by one-way Analysis of Variance (Zar, 1984). Regression analysis was used to compare ration size with peak and mean oxygen consumption rate; ration size and peak and mean ammonia excretion rate; quantity of energy ingested and change in wet weight; quantity of energy ingested and change in dry weight.

6.3. Results

6.3.1. Ingested Energy, (Energy budget component "C").

The quantity of food (percentage body weight) and the value of energy ingested (kJ d^{-1}) by each individual fish group over the experimental period is shown in Table 6.1. The results show a high variability in feed intake over the course of the study for fish groups 1, 2, 5 and 6. As a percentage body weight, mean feed intakes of 0.38 (±0.15), 0.45 (±0.22), 0.28 (±0.14) and 0.27 (±0.14) were recorded for fish groups 1,2 5 and 6 respectively. The greatest feed intake variation was recorded in metabolism chamber 2, and varied between 0 and 0.94% body weight ingested in a day.

6.3.2. Metabolism (Energy budget component "M").

All fish groups, including the unfed group, showed a daily cyclical variation in oxygen consumption rate, with peaks generally occurring in the hours of darkness, coinciding with a point approximately 12 hours after feeding. Generally, oxygen consumption rate reached a level close to that recorded prior to feeding within the 24 hour period of measurement. Raw and smoothed oxygen consumption curves for fish groups 1, 2, 3, 5 and 6 over the five individual 24 hour periods of measurement, are presented in Figs. 6.5., 6.6., 6.7., 6.8. and 6.9. There was no significant relationship between peak post-prandial oxygen consumption rate and daily quantity of feed ingested (percentage body weight) in the fed metabolism chambers (Fig. 6. 10.). Mean oxygen consumption rate and the calculated energy cost of metabolism in the chambers 1, 2, 3, 5 and 6, is summarised in Table 6.2. One-way analysis of variance showed that oxygen consumption rate was significantly increased in fed chambers relative to the starved chamber ($F_{1,58} = 5.91$; $p = 0.018$). Mean oxygen consumption rate increased significantly with ration size ($r^2 = 0.35$, d.f. = 18; $p = 0.002$), and this relationship is shown in Fig. 6.11.

Table 6.1. Food consumption and quantity of energy ingested by small populations of *Hippoglossus hippoglossus* held in metabolism chambers, over the experimental period.

	Food ingested (% body weight) by fish group					Food energy ingested (kJ d^{-1}) by fish group				
Day	1	2	3	4	5	1	2	3	4	5
1	0.23	0	0	0.15	0.05	31.59	0	0	17.31	6.06
2	0.09	0.22	0	0.05	0.14	12.60	31.18	0	5.63	18.36
3	0.24	0.35	0	0.24	0.50	32.27	49.82	0	29.11	66.90
4	0.28	0.45	0	0.21	0.33	38.52	63.02	0	25.20	44.02
5	0.19	0.27	0	0.11	0.28	25.34	37.65	0	12.91	37.41
6	0.23	0.35	0	0.27	0.19	31.84	50.27	0	32.10	25.27
7	0.41	0.53	0	0.25	0.34	57.17	75.61	0	30.13	45.48
8	0.50	0.27	0	0.21	0.33	69.68	38.27	0	25.13	45.12
9	0.43	0.45	0	0.38	0.15	59.79	65.49	0	46.26	20.03
10	0.13	0.41	0	0.22	0.25	18.90	59.94	0	26.57	33.64
11	0.33	0.45	0	0	0.10	46.50	65.40	0	0	13.15
12	0.38	0.32	0	0.16	0.34	53.83	46.44	0	20.13	46.38
13	0.28	0.23	0	0.16	0.15	39.75	33.17	0	20.13	19.88
14	0.54	0.68	0	0.43	0.19	76.27	100.54	0	53.42	26.50
15	0.09	0.14	0	0.17	0.10	13.46	20.24	0	20.60	13.46
16	0.24	0.19	0	0.43	0.24	33.47	27.53	0	53.83	33.00
17	0.52	0.64	0	0.42	0.44	74.96	94.64	0	52.73	60.86
18	0.51	0.60	0	0.32	0.40	72.88	89.14	0	39.55	54.72
19	0.46	0.55	0	0.16	0.10	66.26	81.67	0	19.88	13.47
20	0.52	0.32	0	0.38	0.15	75.52	47.40	0	47.70	20.34
21	0.50	0.75	0	0.48	0.39	72.88	113.80	0	73.07	53.28
22	0.36	0.53	0	0.32	0.24	52.73	80.33	0	39.65	33.64
23	0.48	0.70	0	0.49	0.35	70.25	106.83	0	74.96	48.06
24	0.50	0.52	0	0.45	0.15	73.07	79.92	0	69.34	20.65
25	0.54	0.78	0	0.43	0.49	79.48	119.26	0	80.42	68.40
26	0.54	0.30	0	0.16	0.34	80.04	46.68	0	19.92	46.74
27	0.53	0.94	0	0.43	0.57	77.59	144.45	0	68.11	80.02
28	0.54	0.55	0	0.42	0.39	79.48	85.36	0	79.10	54.44
Mean	**0.38**	**0.45**	**0**	**0.28**	**0.27**	**54.15**	**66.22**	**0**	**38.67**	**37.48**
s.d.	**0.15**	**0.22**		**0.14**	**0.14**	**22.37**	**33.36**		**23.27**	**19.31**

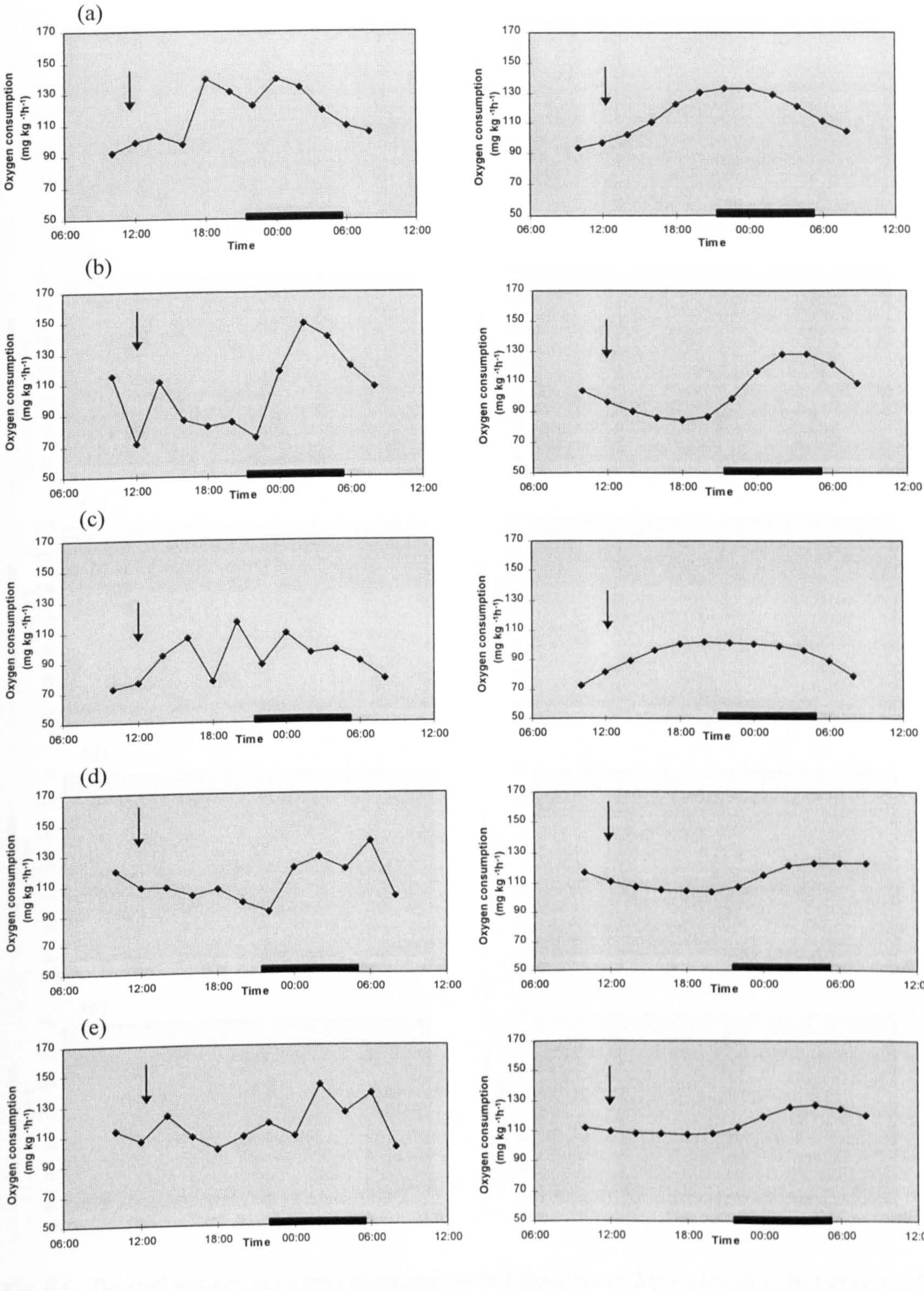

Fig. 6.5. Diurnal pattern of oxygen consumption in *Hippoglossus hippoglossus* in fish group 1, fed at 1% body weight d^{-1}. Trial dates are (a) 31 July 1997; (b) 4 August 1997; (c) 7 August 1997; (d) 10 August 1997; (e) 14 August 1997. Point of feeding is represented by vertical black arrow. Photoperiod is represented by black bar. Raw data curves are shown on the left hand side, smoothed data curves on the right hand side. Quantity of feed ingested was: (a) 0.38; (b) 0.24; (c) 0.46; (d) 0.36; (e) 0.54 (% body weight).

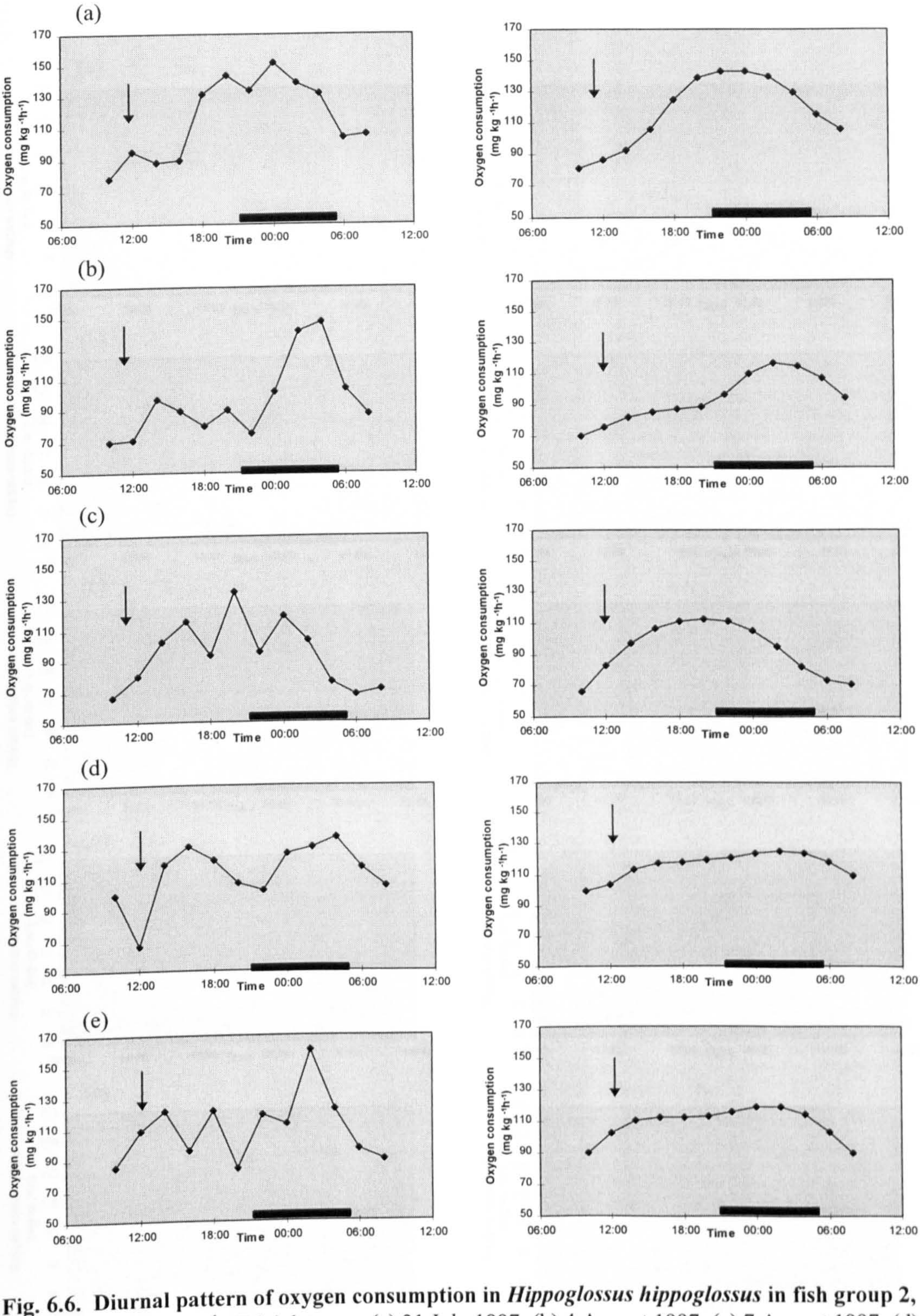

Fig. 6.6. Diurnal pattern of oxygen consumption in *Hippoglossus hippoglossus* in fish group 2, fed at 1% body weight d^{-1}. Trial dates are (a) 31 July 1997; (b) 4 August 1997; (c) 7 August 1997; (d) 10 August 1997; (e) 14 August 1997. Point of feeding is represented by vertical black arrow. Photoperiod is represented by black bar. Raw data curves are shown on the left hand side, smoothed data curves on the right hand side. Quantity of feed ingested was: (a) 0.32; (b) 0.19; (c) 0.55; (d) 0.53; (e) 0.30 (% body weight).

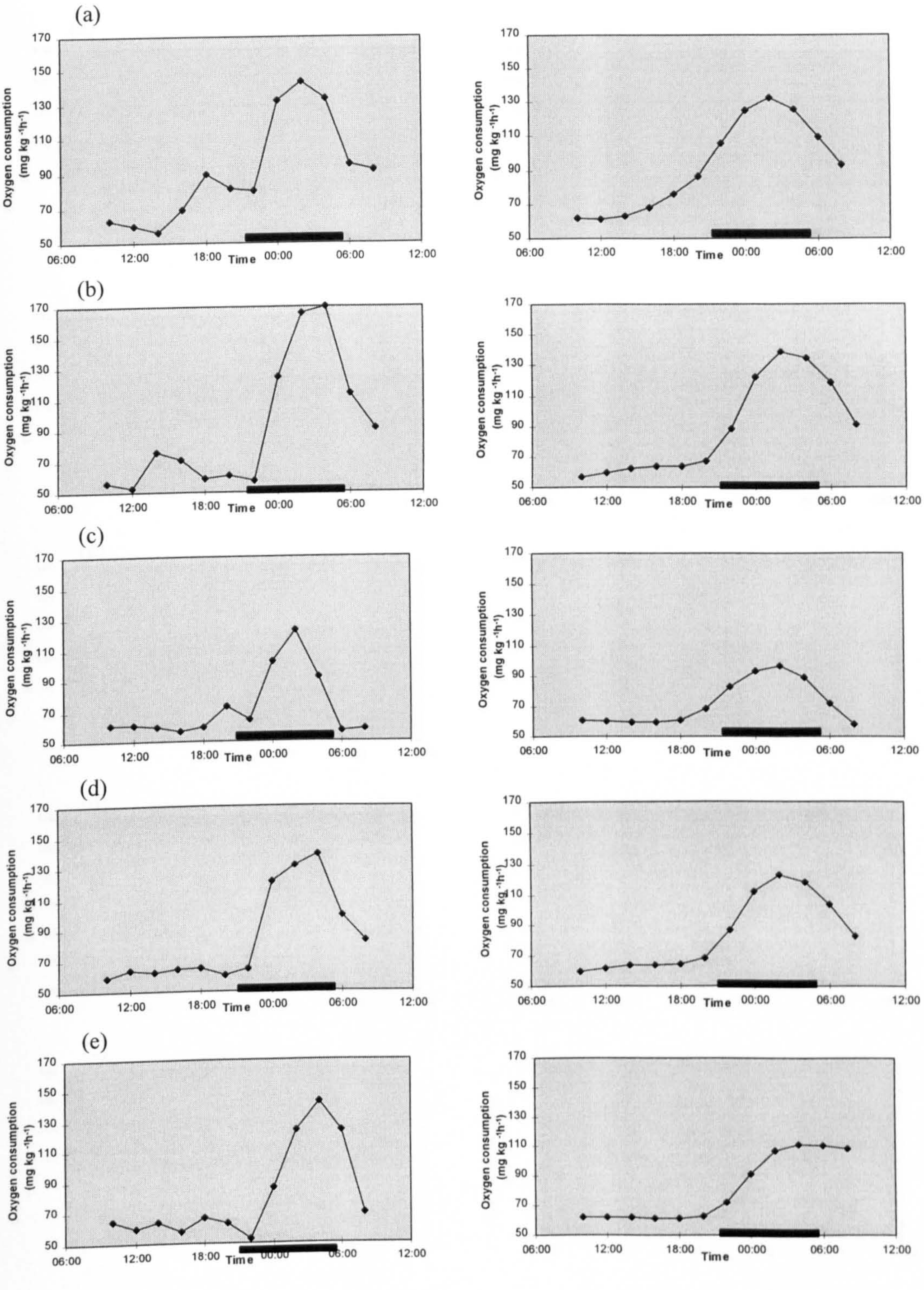

Fig. 6.7. Diurnal pattern of oxygen consumption in *Hippoglossus hippoglossus* in fish group 3, starved fish. Trial dates are (a) 31 July 1997; (b) 4 August 1997; (c) 7 August 1997; (d) 10 August 1997; (e) 14 August 1997. Fish were not fed over the experimental period. Photoperiod is represented by black bar. Raw data curves are shown on the left hand side, smoothed data curves on the right hand side.

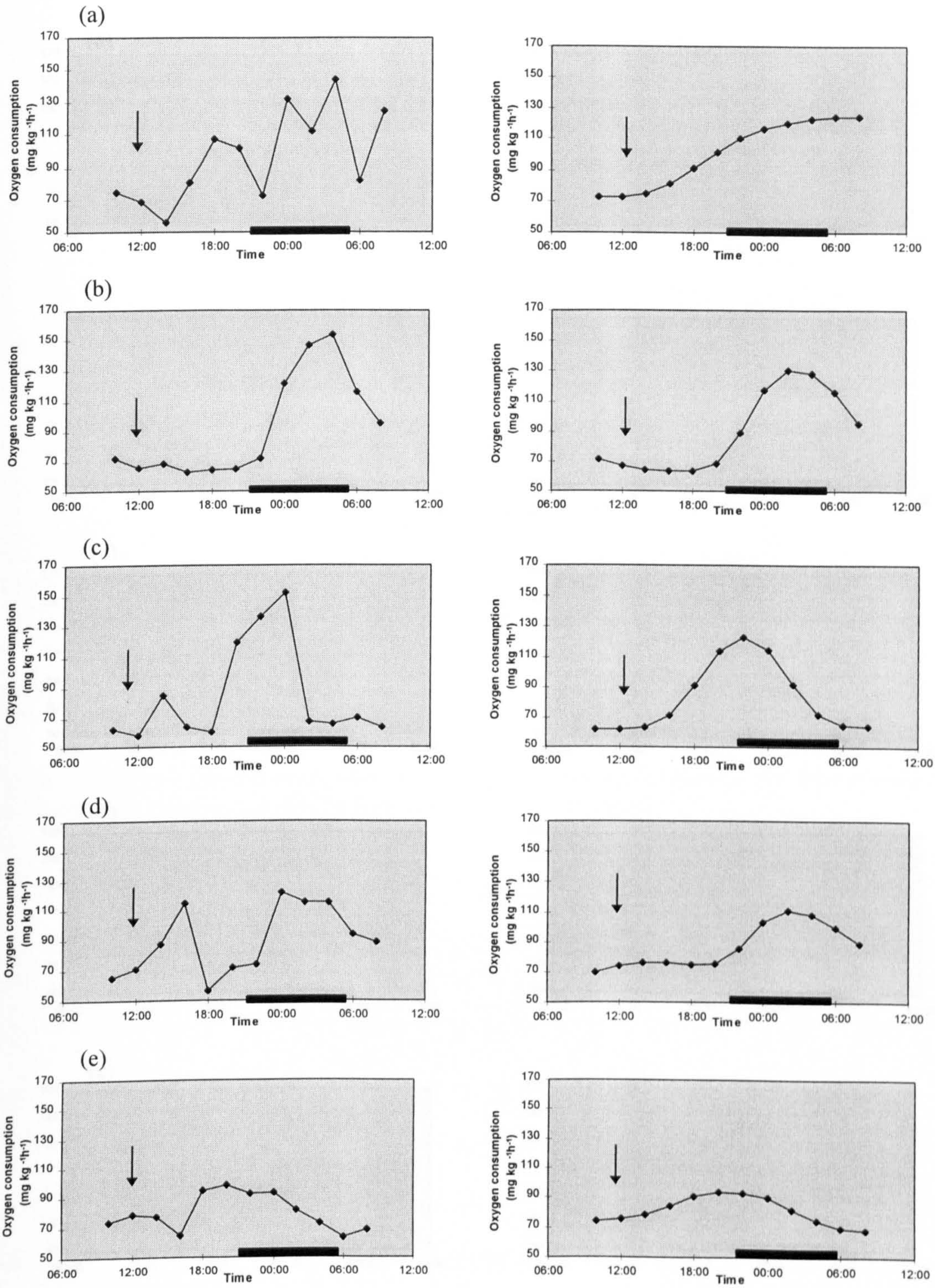

Fig. 6.8. Diurnal pattern of oxygen consumption in *Hippoglossus hippoglossus* in fish group 5, fed at 0.5% body weight d^{-1}. Trial dates are (a) 31 July 1997; (b) 4 August 1997; (c) 7 August 1997; (d) 10 August 1997; (e) 14 August 1997. Point of feeding is represented by vertical black arrow. Photoperiod is represented by black bar. Raw data curves are shown on the left hand side, smoothed data curves on the right hand side. Quantity of feed ingested was: (a) 0.16; (b) 0.43; (c) 0.16; (d) 0.32; (e) 0.16 (% body weight).

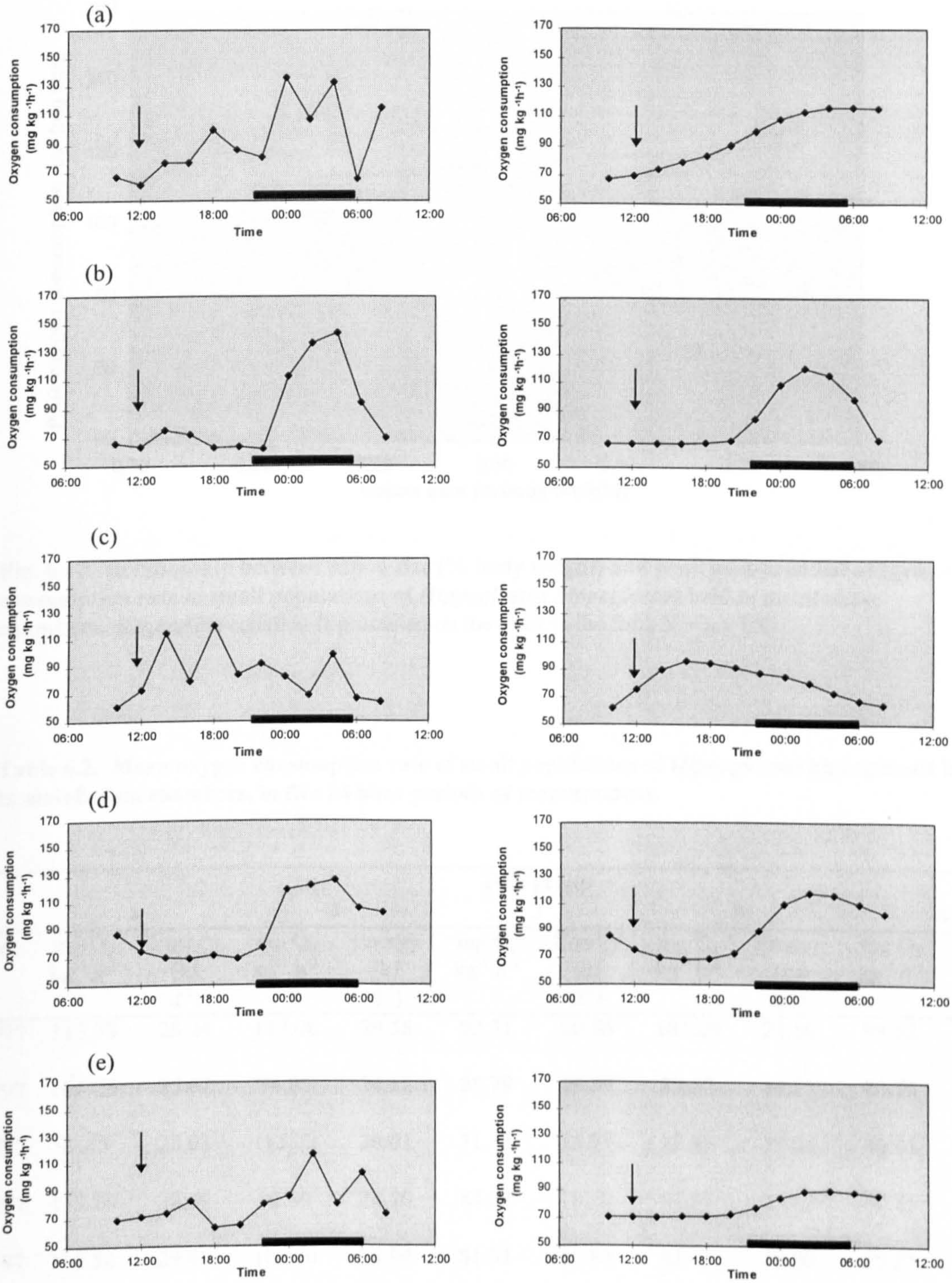

Fig. 6.9. Diurnal pattern of oxygen consumption in *Hippoglossus hippoglossus* in fish group 6, fed at 0.5% body weight d^{-1}. Trial dates are (a) 31 July 1997; (b) 4 August 1997; (c) 7 August 1997; (d) 10 August 1997; (e) 14 August 1997. Point of feeding is represented by vertical black arrow. Photoperiod is represented by black bar. Raw data curves are shown on the left hand side, smoothed data curves on the right hand side. Quantity of feed ingested was: (a) 0.34; (b) 0.24; (c) 0.10; (d) 0.24; (e) 0.34 (% body weight).

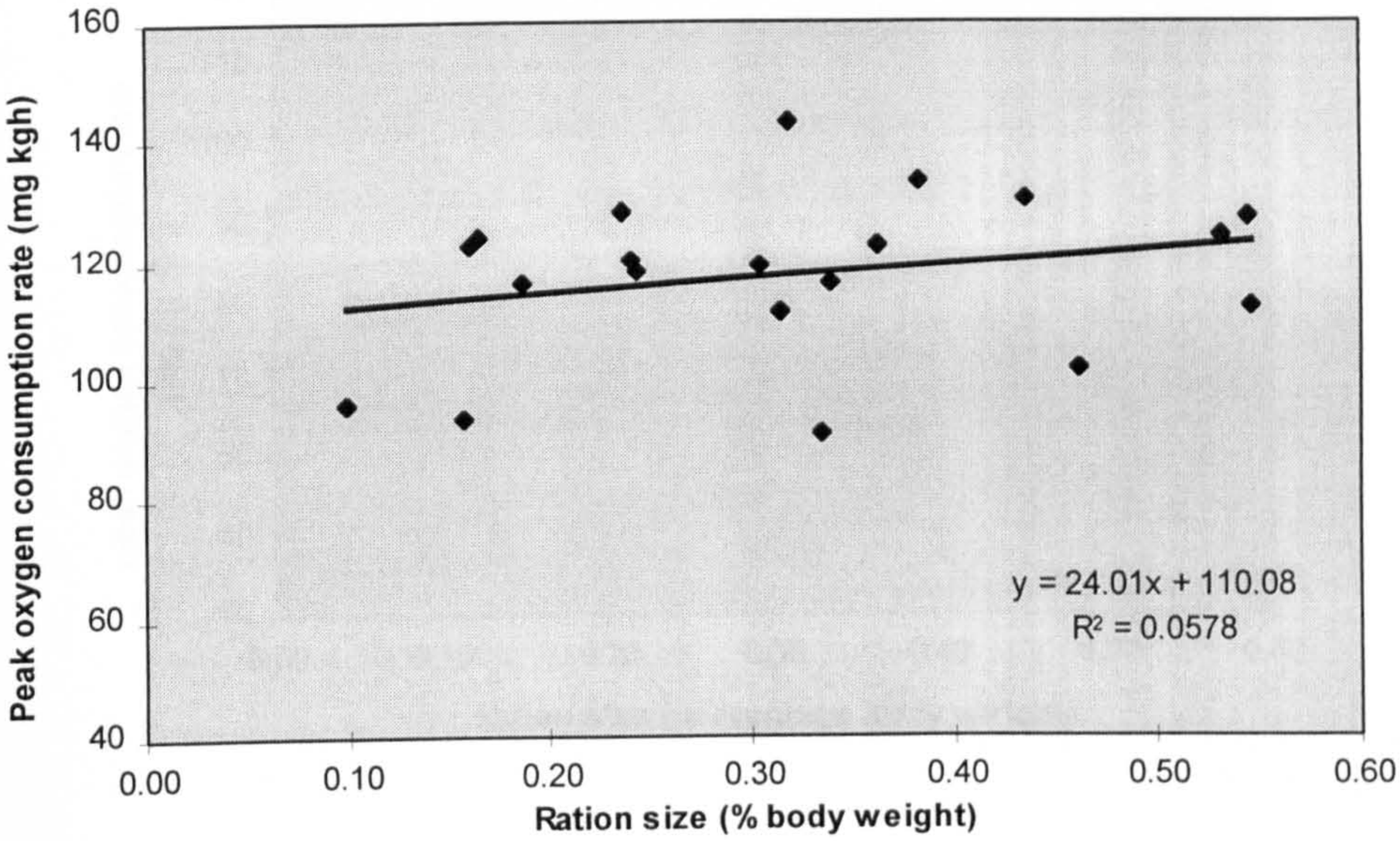

Fig. 6.10. Relationship between ration size (% body weight) and peak post-prandial oxygen consumption rate in small populations of *Hippoglossus hippoglossus* held in metabolism chambers. Regression equation is presented on the chart in the form Y = a + bX.

Table 6.2. Mean oxygen consumption rate of small populations of *Hippoglossus hippoglossus* held in metabolism chambers, in five 24 hour periods of measurement.

	Fish group									
	1		2		3		5		6	
Date	mg O_2 kg^{-1} h^{-1}	Energy (kJ d^{-1})	mg O_2 kg^{-1} h^{-1}	Energy (kJ d^{-1})	mg O_2 kg^{-1} h^{-1}	Energy (kJ d^{-1})	mg O_2 kg^{-1} h^{-1}	Energy (kJ d^{-1})	mg O_2 kg^{-1} h^{-1}	Energy (kJ d^{-1})
31/07/97	115.98	28.24	117.06	29.58	92.31	20.86	101.29	21.50	94.52	22.33
04/08/97	104.05	25.67	94.03	24.12	88.79	19.89	89.57	19.23	83.70	19.90
07/08/97	92.45	23.03	115.72	30.01	71.73	15.97	82.95	17.96	80.45	19.22
10/08/97	113.26	28.49	92.89	24.36	84.04	18.60	87.09	19.02	90.85	21.81
14/08/97	115.56	29.45	108.70	28.94	81.31	17.85	81.45	18.00	79.25	19.15
Mean	108.26	26.98	105.68	27.40	83.64	18.63	88.47	19.14	85.75	20.48
±s.d.	10.07	2.61	11.60	2.91	7.90	1.89	7.86	1.44	6.66	1.49

Energy values for the fed fish (metabolism chambers 1, 2, 5 and 6) were calculated from the oxycalorific coefficients of 14.76, 13.72 and 13.36 13.56 J $mg^{-1}O_2$ for carbohydrate, protein and lipid metabolism (Brafield and Llewellyn, 1982). Energetic values for the unfed fish (metabolism chamber 3) were calculated from the oxycalorific equivalent of 13.56 J $mg^{-1}O_2$ for fish respiring their own tissue (Brett and Groves, 1979).

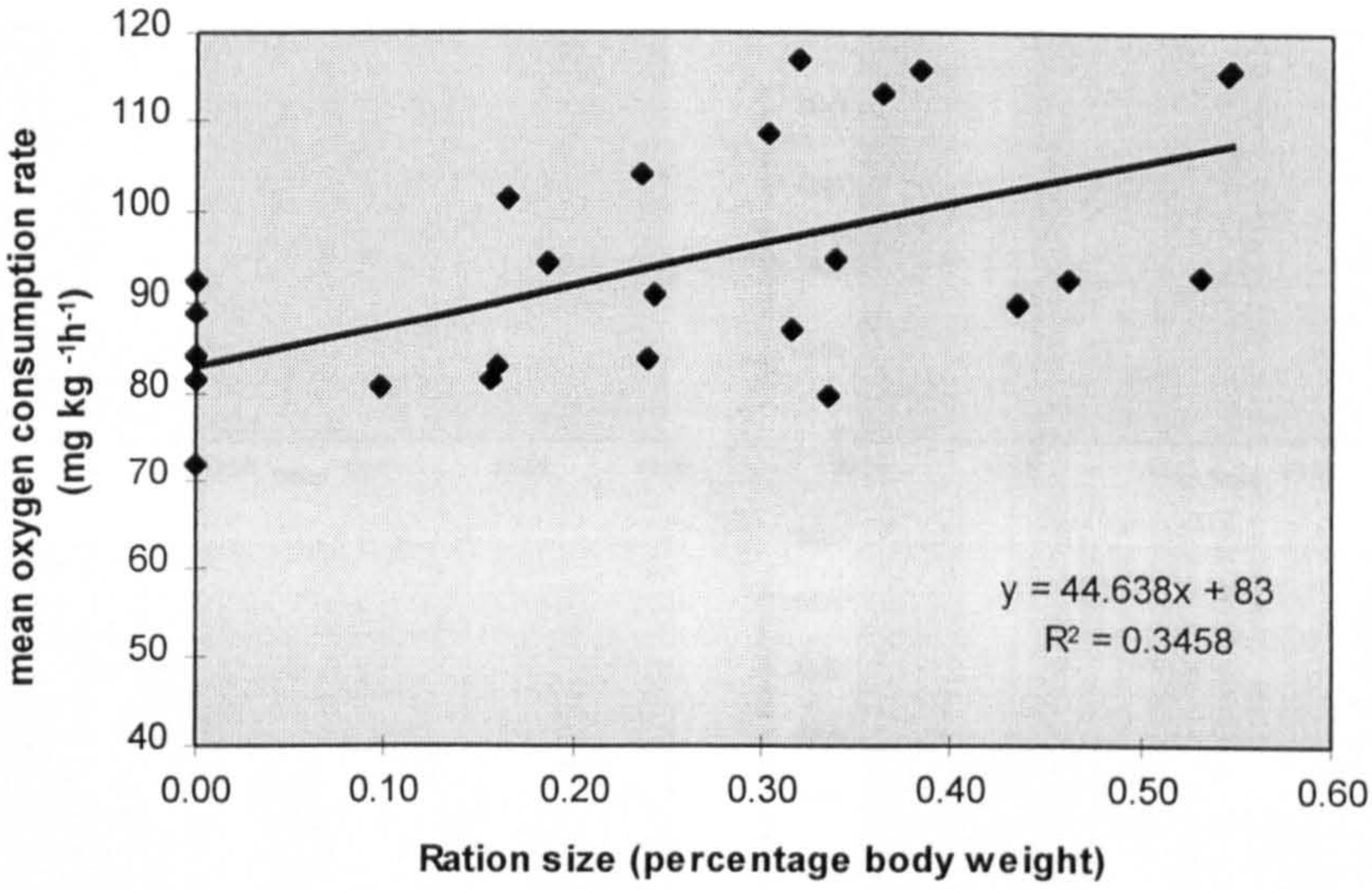

Fig.6.11. Relationship between Ration size and mean Oxygen consumption rate in small populations of *Hippoglossus hippoglossus* held in metabolism chambers. Regression equation is presented on the chart in the form Y = a + bX

6.3.3. Ammonia Excretion (Energy Budget component "U")

In common with the results for the oxygen consumption trials, daily pattern of ammonia excretion exhibited a cyclical nature in the fed fish groups, although peak ammonia excretion rate generally preceded peak oxygen consumption rate. Raw and smoothed data ammonia excretion curves for fish groups 1, 2, 3, 5 and 6 over the five individual 24 hour periods of measurement, are presented in Figs. 6.12., 6.13., 6.14., 6.15. and 6.16. Peak ammonia excretion rate was recorded at a point between three and twelve hours after feeding. Some daily variation in endogenous ammonia excretion in the starved fish in fish group 3 was also observed, although levels of excretion in these fish were much reduced over fed fish.

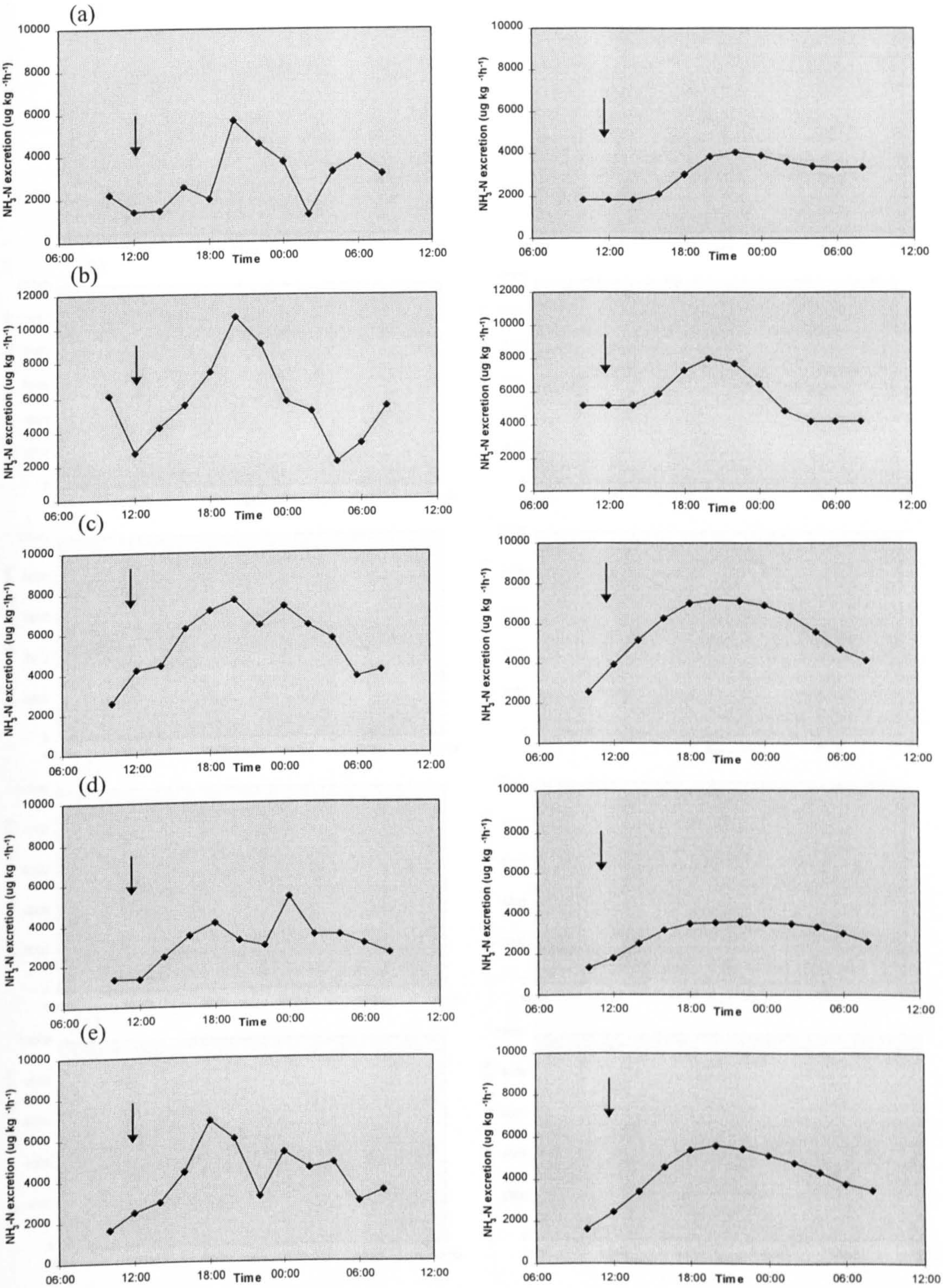

Fig. 6.12. Diurnal pattern of ammonia excretion in *Hippoglossus hippoglossus* in fish group 1, fed at 1% body weight d^{-1}. Trial dates are (a) 31 July 1997; (b) 4 August 1997; (c) 7 August 1997; (d) 10 August 1997; (e) 14 August 1997. Point of feeding is represented by vertical black arrow. Raw data curves are shown on the left hand side, smoothed data curves on the right hand side. Quantity of feed ingested was: (a) 0.38; (b) 0.24; (c) 0.46; (d) 0.36; (e) 0.54 (% body weight).

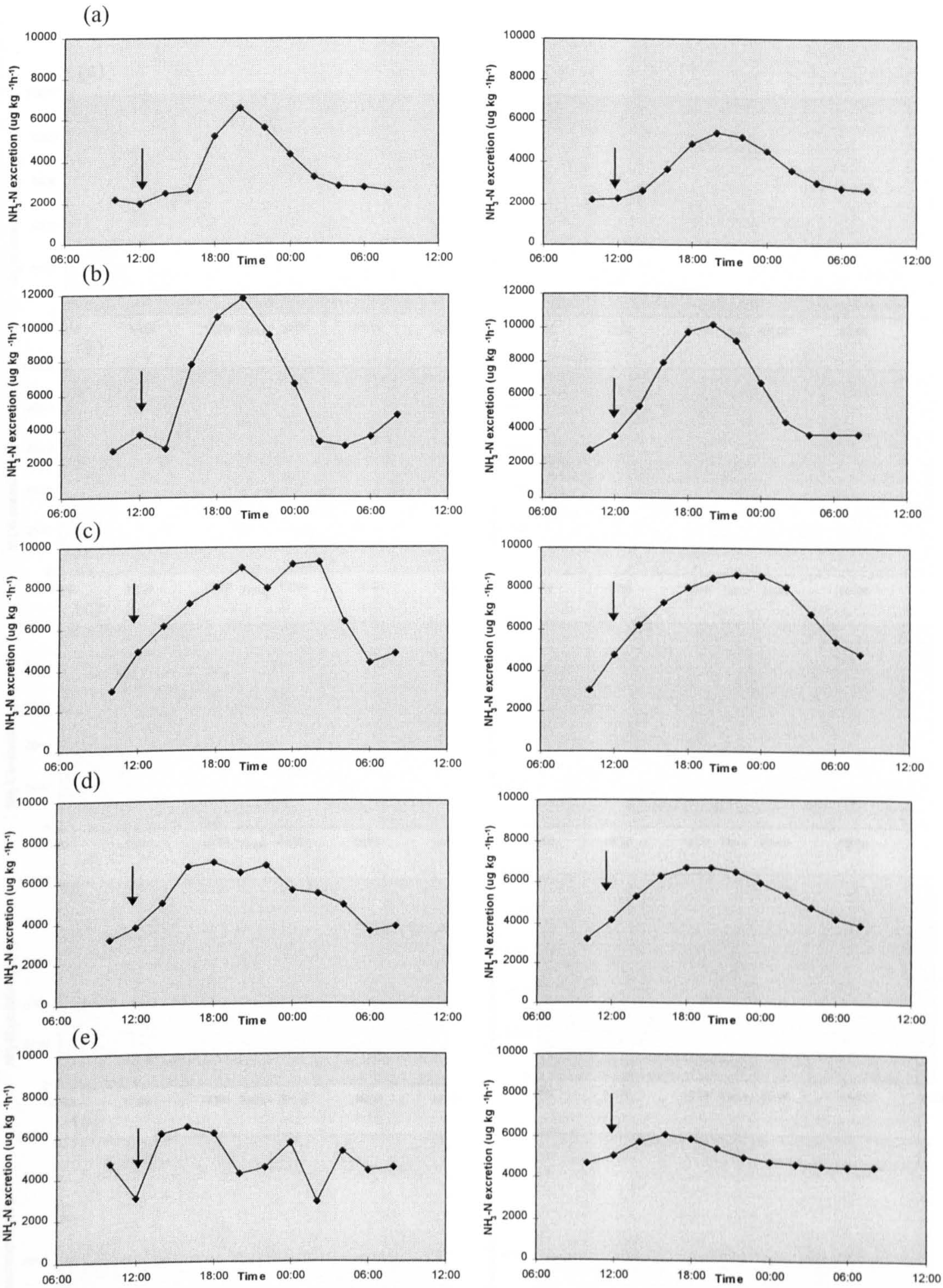

Fig. 6.13. Diurnal pattern of ammonia excretion in *Hippoglossus hippoglossus* in fish group 2, fed at 1% body weight d^{-1}. Trial dates are (a) 31 July 1997; (b) 4 August 1997; (c) 7 August 1997; (d) 10 August 1997; (e) 14 August 1997. Point of feeding is represented by vertical black arrow. Raw data curves are shown on the left hand side, smoothed data curves on the right hand side. Quantity of feed ingested was: (a) 0.32; (b) 0.19; (c) 0.55; (d) 0.53; (e) 0.30 (% body weight).

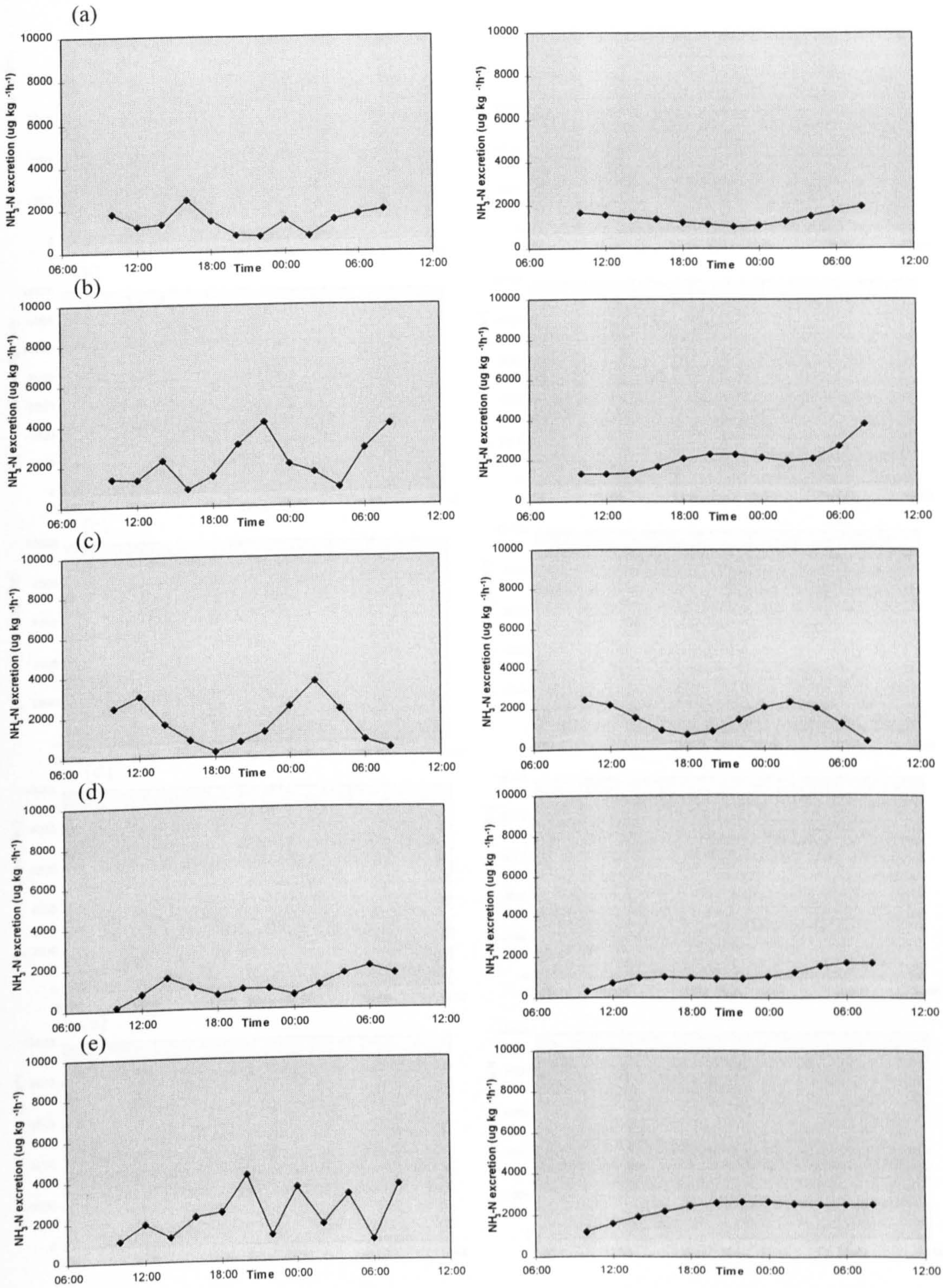

Fig. 6.14. Diurnal pattern of ammonia excretion in *Hippoglossus hippoglossus* in fish group 3, starved fish. Trial dates are (a) 31 July 1997; (b) 4 August 1997; (c) 7 August 1997; (d) 10 August 1997; (e) 14 August 1997. Fish were not fed over the experimental period. Raw data curves are shown on the left hand side, smoothed data curves on the right hand side.

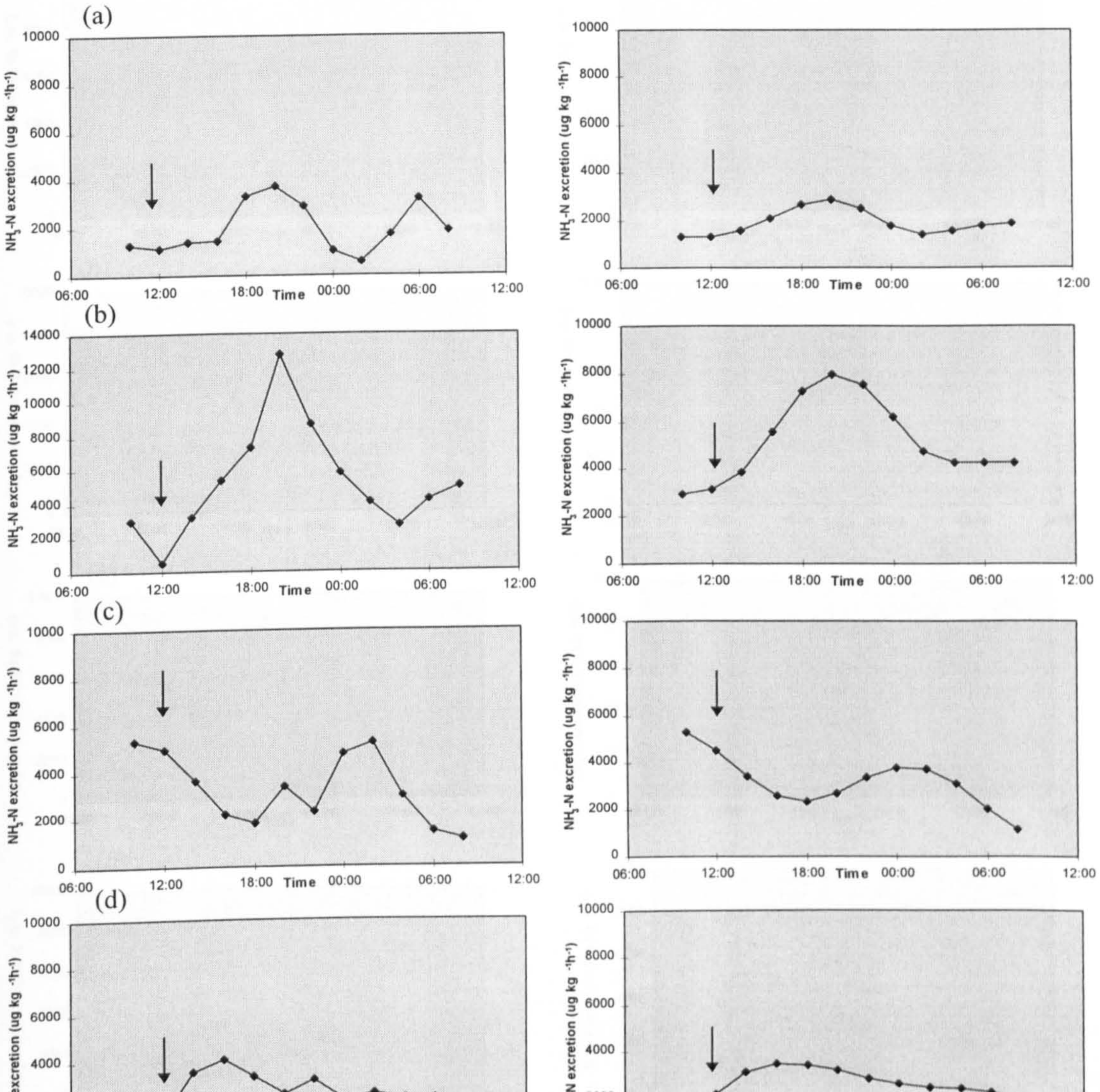

Fig. 6.15. Diurnal pattern of ammonia excretion in *Hippoglossus hippoglossus* in fish group 5, fed at 0.5% body weight d^{-1}. Trial dates are (a) 31 July 1997; (b) 4 August 1997; (c) 7 August 1997; (d) 10 August 1997; (e) 14 August 1997. Point of feeding is represented by vertical black arrow. Raw data curves are shown on the left hand side, smoothed data curves on the right hand side. Quantity of feed ingested was: (a) 0.16; (b) 0.43; (c) 0.16; (d) 0.32; (e) 0.16 (% body weight).

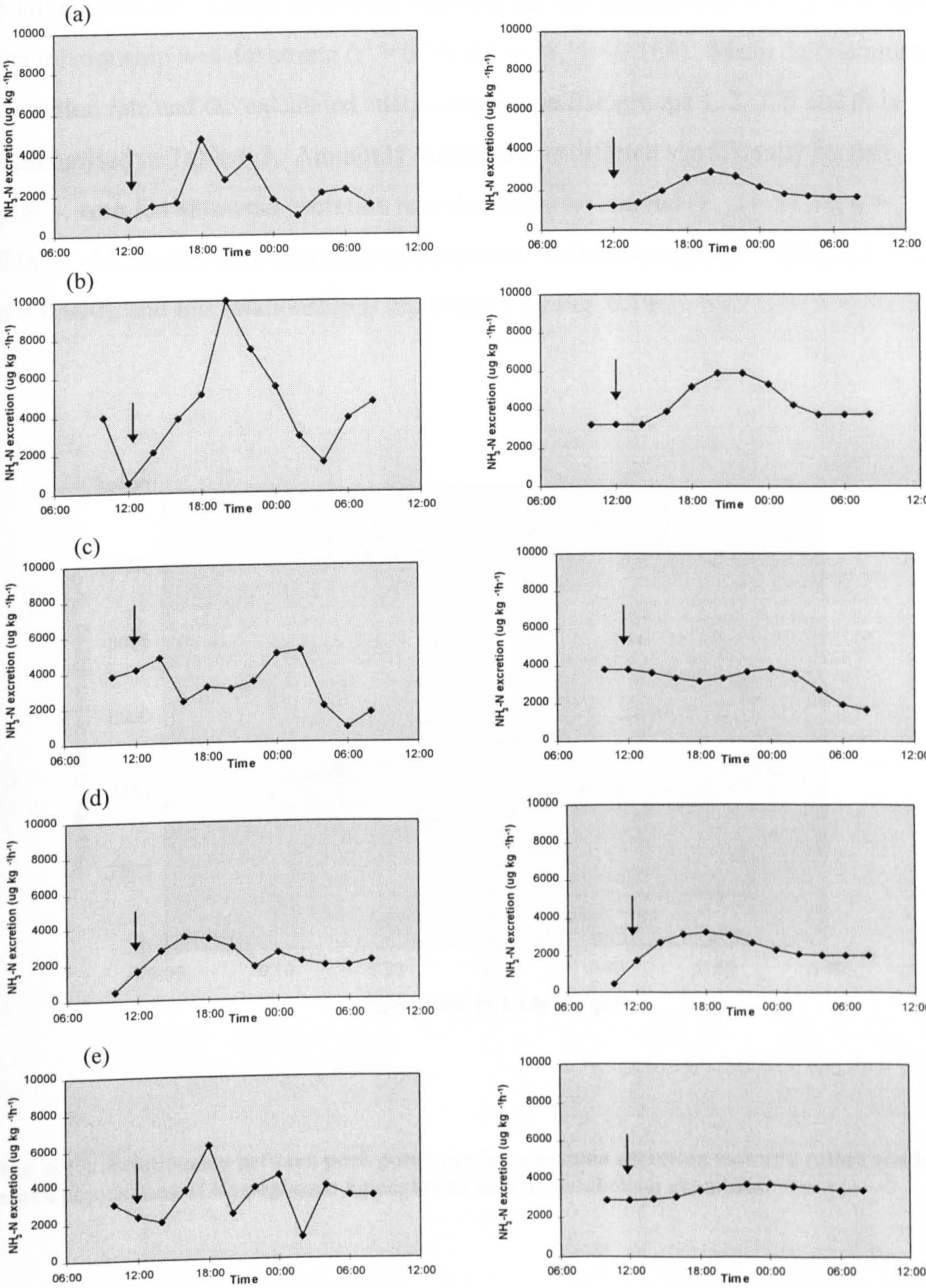

Fig. 6.16. Diurnal pattern of ammonia excretion in *Hippoglossus hippoglossus* in fish group 6, fed at 0.5% body weight d^{-1}. Trial dates are (a) 31 July 1997; (b) 4 August 1997; (c) 7 August 1997; (d) 10 August 1997; (e) 14 August 1997. Point of feeding is represented by vertical black arrow. Raw data curves are shown on the left hand side, smoothed data curves on the right hand side. Quantity of feed ingested was: (a) 0.34; (b) 0.24; (c) 0.10; (d) 0.24; (e) 0.34 (% body weight).

Peak post-prandial ammonia excretion rate increased with the daily quantity of feed ingested (percentage body weight) in the fed fish groups (Fig. 6.17.), although the relationship was not strong ($r^2 = 0.10$, d.f. = 18, $p = 0.164$). Mean daily ammonia excretion rate and the calculated energy cost in the fish groups 1, 2, 3, 5 and 6, is summarised in Table 6.3. Ammonia excretion rate differed significantly by fish group, with fed ammonia excretion rate elevated over starved ($F_{1,59} = 37.32$; $p = 0.000$). Mean ammonia excretion rate increased with ration size ($r^2 = 0.42$, d.f. = 18; $p = 0.000$), and this relationship is represented by Fig. 6.18.

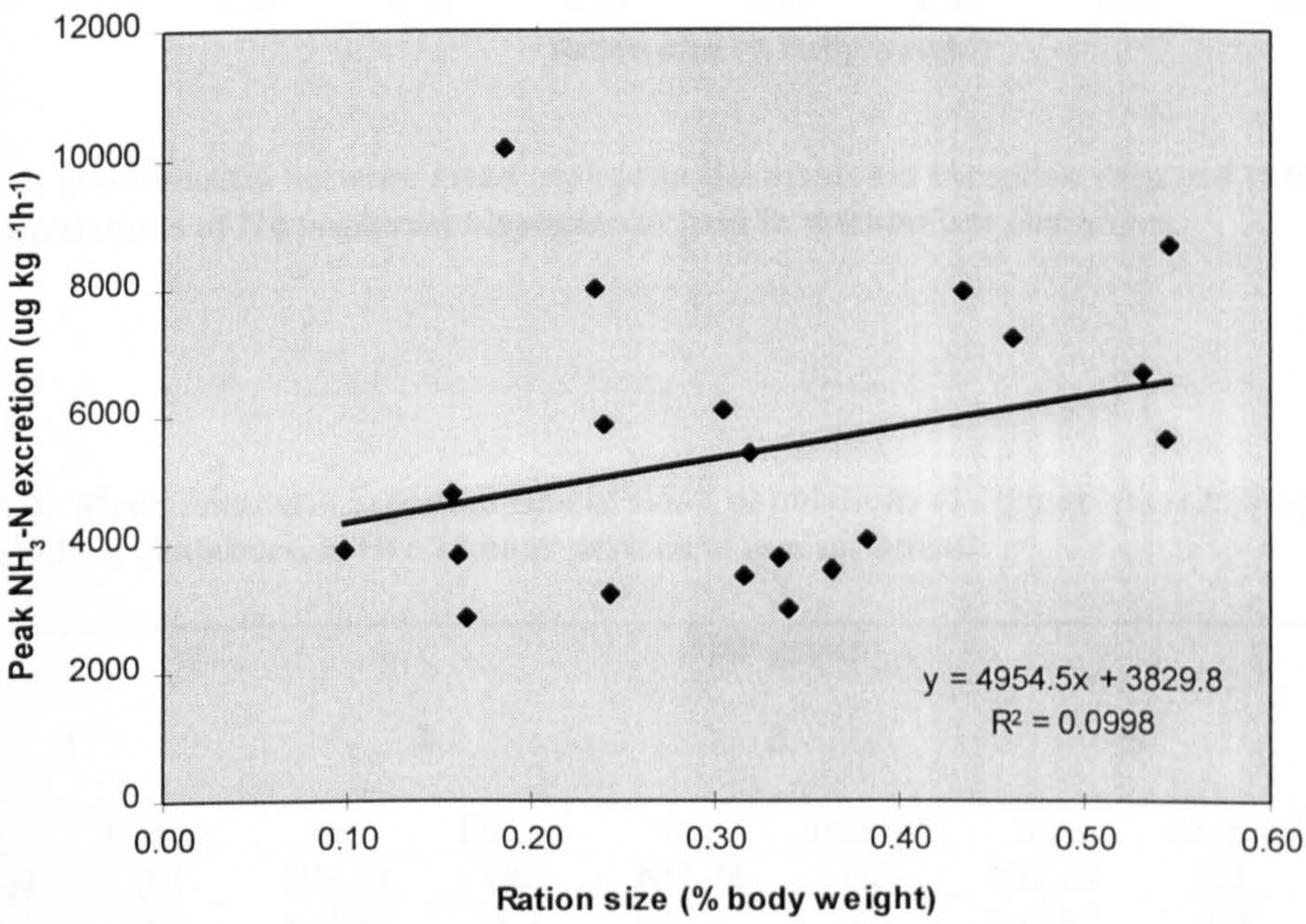

Fig. 6.17. Relationship between peak post-prandial ammonia excretion rate and ration size in small populations of *Hippoglossus hippoglossus* held in metabolism chambers.

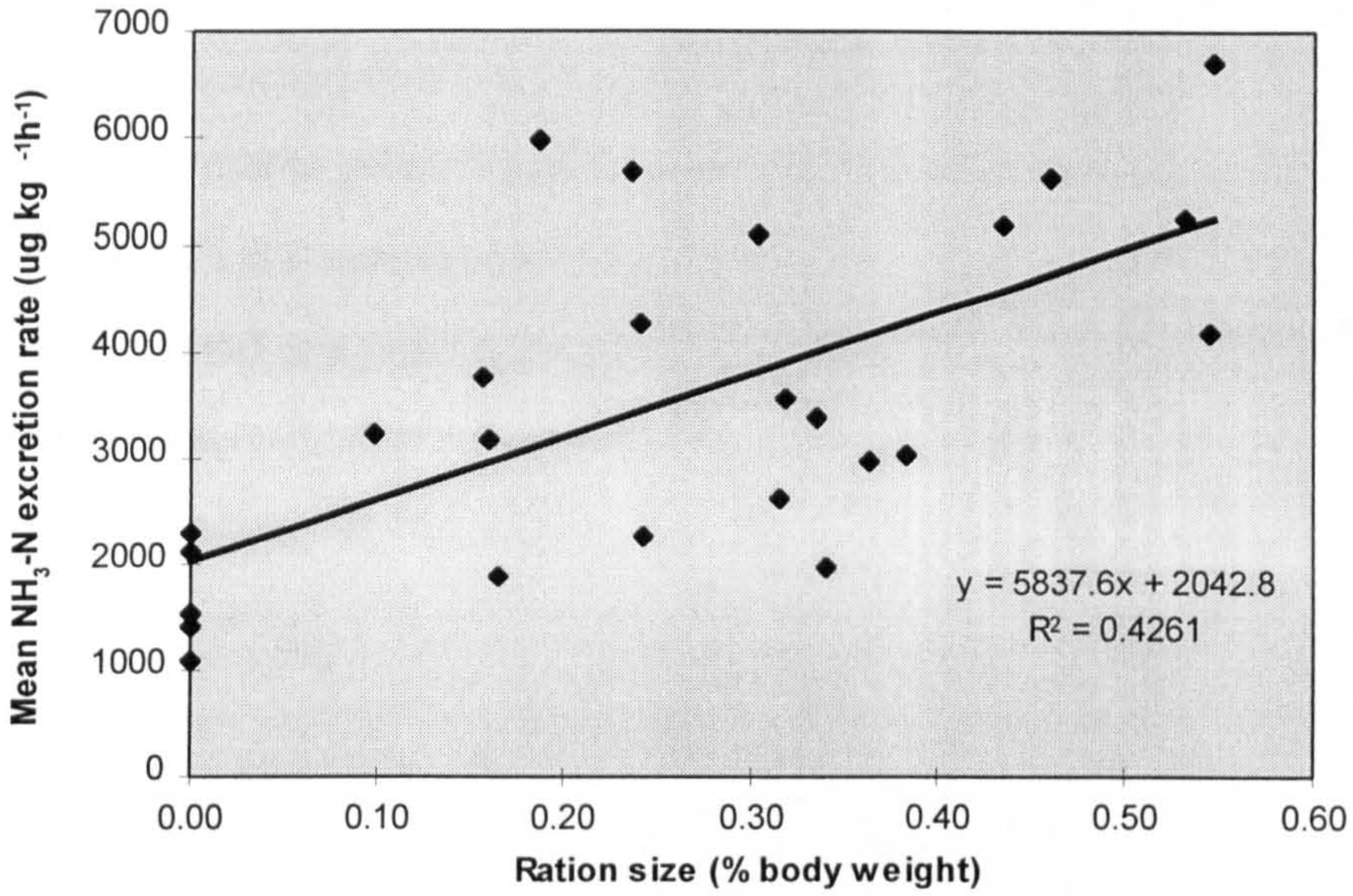

Fig. 6.18. Relationship between mean post-prandial ammonia excretion rate and ration size in small populations of *Hippoglossus hippoglossus* held in metabolism chambers.

Table 6.3. Mean ammonia excretion rate of small populations of *Hippoglossus hippoglossus* held in metabolism chambers, in five 24 hour periods of measurement.

	Fish group									
	1		2		3		5		6	
Date	mg NH_3-N kg^{-1} h^{-1}	Energy (kJ d^{-1})	mg NH_3-N kg^{-1} h^{-1}	Energy (kJ d^{-1})	mg NH_3-N kg^{-1} h^{-1}	Energy (kJ d^{-1})	mg NH_3-N kg^{-1} h^{-1}	Energy (kJ d^{-1})	mg NH_3-N kg^{-1} h^{-1}	Energy (kJ d^{-1})
31/07/97	3.02	0.74	3.57	0.91	1.42	0.32	1.89	0.40	1.96	0.47
04/08/97	5.69	1.42	5.96	1.54	2.12	0.48	5.17	1.12	4.28	1.03
07/08/97	5.61	1.41	6.70	1.75	1.53	0.34	3.18	0.70	3.24	0.78
10/08/97	2.97	0.75	5.25	1.39	1.09	0.24	2.62	0.58	2.27	0.55
14/08/97	4.17	1.07	5.08	1.37	2.30	0.51	3.75	0.84	3.38	0.82
Mean	4.29	1.08	5.31	1.39	1.69	0.38	3.32	0.73	3.03	0.73
±s.d.	1.33	0.33	1.17	0.31	0.51	0.11	1.24	0.27	0.93	0.22

Energy values were calculated from the energetic equivalent of 0.62 cal mg^{-1} (= 13.73J mg^{-1} NH_3-N) for carnivorous ammoneotelic fish provided by Elliott and Davison (1975).

The location of daily peak oxygen consumption rate and ammonia excretion rate was examined using the data recorded from all fish groups in which the fish were fed. Mean values recorded over the five 24 hour periods of measurement show peak ammonia excretion rate to precede peak oxygen consumption rate by approximately six hours (Fig. 6.19.) at a temperature of 12.7 (±0.6) °C. Mean peak ammonia excretion rate occurred at a point approximately 8 hours after feeding, and mean peak oxygen consumption rate occurred approximately 14 hours after feeding and coincided with the nocturnal mid-phase.

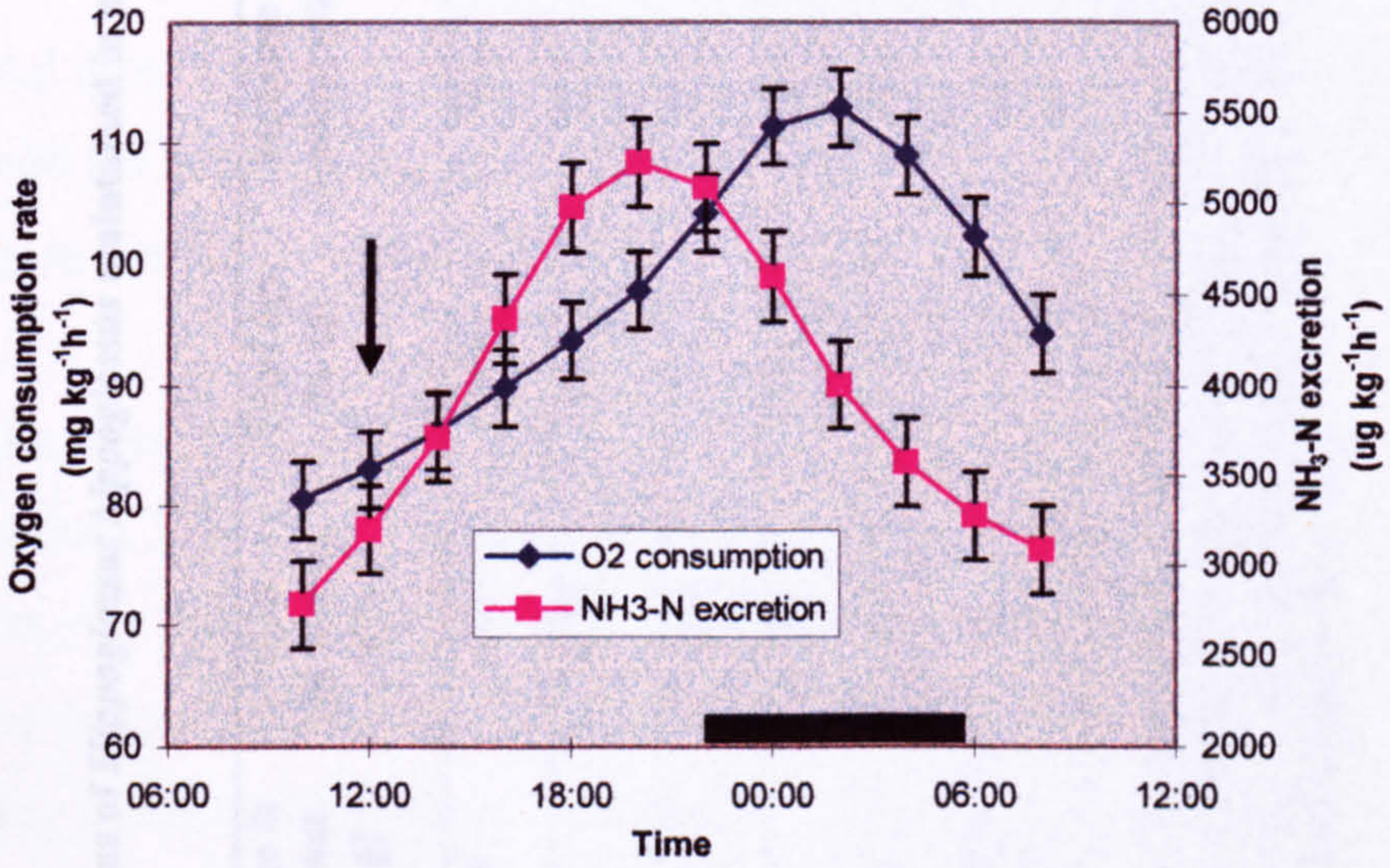

Fig. 6.19. Location of peak post-prandial oxygen consumption and ammonia excretion rates in small populations of *Hippoglossus hippoglossus* maintained in metabolism chambers at a mean temperature of 12.7°C. Average weight of fish was predicted at 148g from growth data, at the time of the experimental period. Values are means from data collected in a total of twenty 24 hour periods. Bars represent standard error measurements. Photoperiod is represented by the horizontal black bar, and feeding time is represented by the vertical black arrow.

6.3.4. Growth (Energy Budget Component "P").

A summary of the growth performance of halibut within the metabolism chambers is presented in Table 6.4. The results clearly show an increase in biomass wet weight in the fed fish groups 1, 2, 5 and 6, over the 28 day experimental period. Specific growth rates of 0.16 to 0.37 % body weight d^{-1} are calculated from the change in wet weight. The unfed fish group, chamber 3, showed a decrease in wet weight,

Table 6.4. Summary of the growth performance of small populations of *Hippoglossus hippoglossus* maintained in metabolism chambers over a 29 day experimental period.

Metabolism chamber	"R" ingested energy (kJ d^{-1})	Initial total wet wt. (g)	Final total wet wt. (g)	Change in total wet wt. (g)	SGR (% wet wt. d^{-1})	Total dry wt. (g)	Moisture content (%)	Change in dry wt. (g)	Carcass Energy content (kJ g^{-1})	Total growth "P" (kJ)
Initial sample	n/a	n/a	591	n/a	n/a	174.7	70.4	n/a	20.05	n/a
1	54.15 (±22.37)	720	791	71	0.32	203.1	74.3	-2.87	18.64	-53.50
2	66.22 (±33.36)	743	828	85	0.37	215.7	73.9	3.22	19.86	63.95
3	0	708	667	-41	-0.21	165.0	75.3	-37.49	17.56	-658.32
5	38.67 (±23.27)	630	685	55	0.29	170.9	72.9	-9.29	17.92	-166.48
6	37.48 (±19.31)	711	745	34	0.16	187.2	74.9	-16.19	19.88	-321.86

All figures are expressed as biomass totals for each metabolism chamber, containing a total of 5 halibut. Carcass gross energy content was calculated from mean energy values recorded for individual halibut carcasses within each metabolism chamber, and for the initial sample (n = 5; one halibut removed from each tank on day 0). Change in dry weight is calculated by applying the results for moisture content recorded from the initial sample of halibut taken at the start of the experiment to the wet weights recorded for the metabolism chambers at this point. Ingested energy values are daily mean (±s.d.).

with a specific growth rate of -0.21% body weight d^{-1}. However, wet weight comparisons in this instance are subject to a degree of variability in moisture content of the experimental halibut, which increased over the 28 day period from 70.4% in the initial sample to 72.9-74.9% in the halibut held in the fed metabolism chambers at the end of the study.

If the change in dry weight is considered, the results presented in Table 6.4. present a different scenario to that observed with change in wet weight. An increase in dry weight of the group was only recorded in the halibut maintained in metabolism chamber 2, with an overall increase in dry weight of 3.22g over the experimental period. All other fish groups showed a decrease in dry weight over the experimental period, with the largest change in dry weight recorded for the biomass of the unfed fish group in metabolism chamber 3.

The mean daily quantity of energy ingested (kJ d^{-1}) varied by fish group in the fed chambers 1,2, 5 and 6. Regression analysis showed that change in both wet and dry weight over the experimental period increased with the quantity of ingested energy per day per metabolism chamber (Figs. 6.20. and 6.21.). These relationships were highly significant for both wet weight ($r^2 = 0.96$, d.f. = 3, $p = 0.003$ for energy ingested as kJ d^{-1}; $r^2 = 0.98$, d.f. = 3, $p = 0.000$ for energy ingested as kJ kg^{-1} d^{-1}) and dry weight ($r^2 = 0.98$, d.f. = 3, $p = 0.001$ for energy ingested as kJ d^{-1}; $r^2 = 0.98$, d.f. = 3, $p = 0.001$ for energy ingested as kJ kg^{-1} d^{-1}). In addition, carcass energy content (expressed as kJ g^{-1}) showed a variation with metabolism chamber, the highest value of 20.05 kJ g^{-1} recorded in the initial sample, and the lowest value of 17.56 kJ g^{-1} recorded in halibut from the unfed metabolism chamber 3. Halibut from the fed metabolism chambers showed carcass energy values intermediate between these figures.

The regression equation in Fig. 6.21 allows the calculation of a maintenance ration for Atlantic halibut of approximately 150g at 13°C at 51.20kJ d^{-1}.

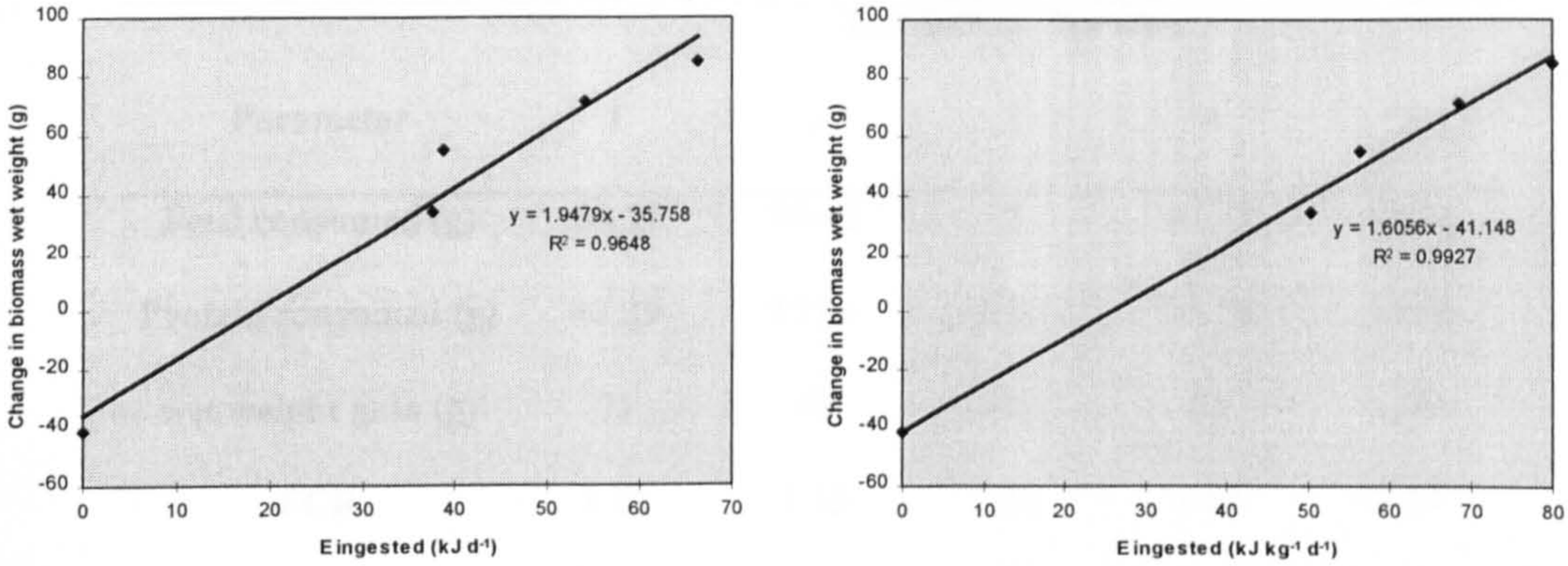

Fig. 6.20. Relationship between Energy ingested (kJ d^{-1} and kJ kg^{-1} d^{-1}) and change in wet weight for small populations of *Hippoglossus hippoglossus* maintained in metabolism chambers over a 28 day period.

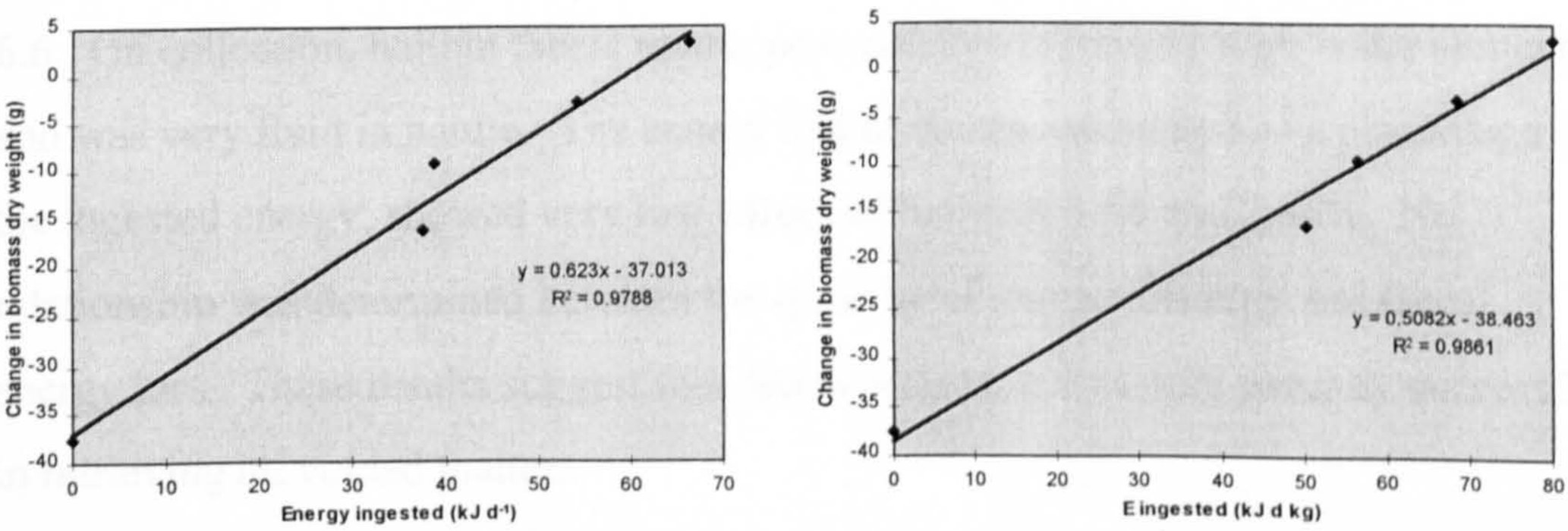

Fig. 6.21. Relationship between Energy ingested (kJ d^{-1} and kJ kg^{-1} d^{-1}) and change in dry weight for small populations of *Hippoglossus hippoglossus* maintained in metabolism chambers over a 28 day period.

Calculated feed conversion ratios (FCR) and protein efficiency ratios (PER) based on change in wet weight, are presented in Table 6.5. No significant relationship between either FCR or PER with feed rate (% body weight d^{-1}) was determined.

Table 6.5. Feed conversion ratio (FCR) and protein efficiency ratio (PER) calculated for small populations of Atlantic halibut maintained in metabolism chambers.

	Metabolism chamber				
Parameter	**1**	**2**	**3**	**4**	**5**
Feed consumed (g)	80.51	98.45	0	57.51	55.74
Protein consumed (g)	47.29	57.83	0.00	33.78	32.74
wet weight gain (g)	71	85	-41	55	34
FCR	1.13	1.16	n/a	1.05	1.64
PER	1.50	1.47	n/a	1.63	1.04

6.3.5. Faeces (Energy Budget Component "F").

A summary of faecal production by metabolism chamber is presented in Table 6.6. On collection, halibut faecal matter possessed an extremely high water content, and was very fluid in nature. The energy lost as faeces, calculated as a percentage of the ingested energy, showed very low values of between 1.44 and 2.46%. No relationship was determined between the quantity of ingested energy and faecal energy loss. These results suggest that faecal collection was only partially successful in retrieving all voided matter.

From the manufacturer's figures of 16.81MJ kg^{-1} digestible energy in the flatfish diet (P. Morris, pers. comm.), a value for faecal energy loss may be calculated. This figure is based on faecal recovery experiments with Atlantic salmon, and so the assumption that a similar figure is realistic for Atlantic halibut must be made. Application of this figure to the manufacturer's feed energy value of 20.4 kJ g^{-1} feed provides an estimated faecal energy loss value of 17.6%, indicating that the actual quantity of collected halibut faecal matter was only in the region of 12% of that voided. Clearly the lack of success in faecal recovery in this experiment is associated with the specific characteristics of halibut faeces, i.e. the very high water content. The use of faecal trap systems such as have been previously applied to salmonid faecal recovery do not appear to be applicable to the Atlantic halibut.

Table 6.6. Summary of faecal production by small populations of *Hippoglossus hippoglossus* maintained in metabolism chambers.

Metabolism Chamber	Daily feed ingested (% body weight)	Total Ingested Energy (kJ) "R"	Total dry wt. of faeces recaptured (g)	Faecal energy value (kJ g^{-1})	"F" as % of "R"
1	0.38	1516.1	1.405	16.55	1.53
2	0.45	1854.0	1.999	17.51	1.89
3	0	0	0	n/a	n/a
5	0.28	1082.9	0.972	17.00	1.53
6	0.27	1049.3	1.543	17.70	2.60

6.3.6. Proximate composition

The proximate composition of halibut carcasses, feed and faecal matter is presented in Table 6.7.

The percentage moisture content of the initial carcass samples was lower at 70.4% than the values obtained for metabolism chamber fish carcass samples at the end of the experiment ($F_{1,28} = 13.72$; $p = 0.001$). The mean moisture content of the carcass samples in the unfed fish group 3 was slightly higher at 75.3% than that of the carcass samples in the fed fish groups 1, 2, 5 and 6, although the statistical significance of this difference was low ($F_{1,23} = 2.24$; $p = 0.148$). The moisture content of carcass samples from the fed fish groups showed a low degree of variation between metabolic chamber ($F_{3,16} = 1.12$; $p = 0.370$). A 5.6% moisture content was recorded for the feed.

Crude protein, lipid, ash and total energy levels recorded for the initial carcass samples also differed in comparison to the results for analysis of halibut carcasses at the end of the experimental period. Initial carcass samples showed a reduced percentage crude protein ($F_{1,88} = 22.33$; $p = 0.000$), percentage lipid ($F_{1,88} = 16.18$; $p = 0.000$) and percentage ash ($F_{1,88} = 23.22$; $p = 0.000$) as percentage dry matter, in comparison to the carcass samples of halibut removed from metabolism chambers at the end of the trial. Carcass analysis of halibut from the unfed chamber 3 showed a reduced percentage lipid ($F_{1,73} = 16.45$; $p = 0.000$) and an increased percentage crude protein ($F_{1,73} = 21.39$; $p = 0.000$) and percentage ash ($F_{1,73} = 42.33$; $p = 0.000$) as percentage dry matter, over the fed fish groups. Within the fed fish groups some variability was observed in percentage crude protein content ($F_{3,56} = 2.35$; $p = 0.082$), percentage crude lipid content ($F_{3,56} = 2.94$; $p = 0.041$) and percentage ash content ($F_{3,56} = 2.46$; $p = 0.072$) as percentage dry matter, although at a much lower level than observed between the fed chambers and the unfed chamber. A significant relationship between ration level and carcass percentage crude protein ($r^2 = 0.98$; d.f. = 3; $p = 0.001$) was observed, with percentage carcass crude protein decreasing with increasing ration size (Fig. 6.22.). A similar relationship between ration level and

Table 6.7. Proximate analysis of *Hippoglossus hippoglossus* carcasses, feed and faecal matter.

Sample	Ration level (% body wt. d^{-1})	Moisture (%)	Dry matter (%)	Crude protein (%)	Crude lipid (%)	Ash (%)	Energy (kJ g^{-1})
Initial carcass	2	70.4	29.6	57.4 (±6.2)	22.8 (±0.7)	9.5 (±1.1)	20.05 (±0.91)
Metabolism Chamber 1 carcass	0.38	74.3	25.7	60.7 (±1.0)	25.7 (±1.3)	10.4 (0.6)	18.64 (±1.39)
Metabolism Chamber 2 carcass	0.45	73.9	26.1	60.4 (±1.7)	26.8 (±2.0)	10.2 (0.8)	19.86 (±2.99)
Metabolism Chamber 3 carcass	0	75.3	24.7	63.7 (±2.5)	23.3 (±3.2)	12.1 (1.0)	17.56 (±1.35)
Metabolism Chamber 5 carcass	0.28	72.9	27.1	61.6 (±2.4)	25.3 (±2.2)	10.8 (0.9)	17.92 (±1.78)
Metabolism Chamber 6 carcass	0.27	74.9	25.1	61.8 (±1.3)	25.2 (±0.9)	10.9 (0.6)	19.88 (±1.62)
Feed	n/a	5.6	94.4	58.7 (±0.3)	14.7 (±0.2)	11.7 (±0.1)	19.95 (±0.06)
Metabolism Chamber 1 faeces	n/a	n/a	n/a	50.0 (±0.2)	25.9 (±4.1)	16.4	16.6 (±0.14)
Metabolism Chamber 2 faeces	n/a	n/a	n/a	54.0 (±0.6)	13.6 (±4.1)	12.7	17.5 (±0.02)
Metabolism Chamber 5 faeces	n/a	n/a	n/a	52.7 (±1.8)	16.6 (±7.3)	14.0	17.0 (±0.34)
Metabolism Chamber 6 faeces	n/a	n/a	n/a	53.7 (±1.9)	23.3 (±2.0)	13.4	17.7 (±0.09)

carcass percentage ash was observed ($r^2 = 0.98$; d.f. = 3; $p = 0.001$), (Fig. 6.23.). In contrast to percentage crude protein and ash, carcass percentage lipid was observed to increase with increasing ration size ($r^2 = 0.96$; d.f. = 3; $p = 0.003$), and this relationship is presented in Fig. 6.24.

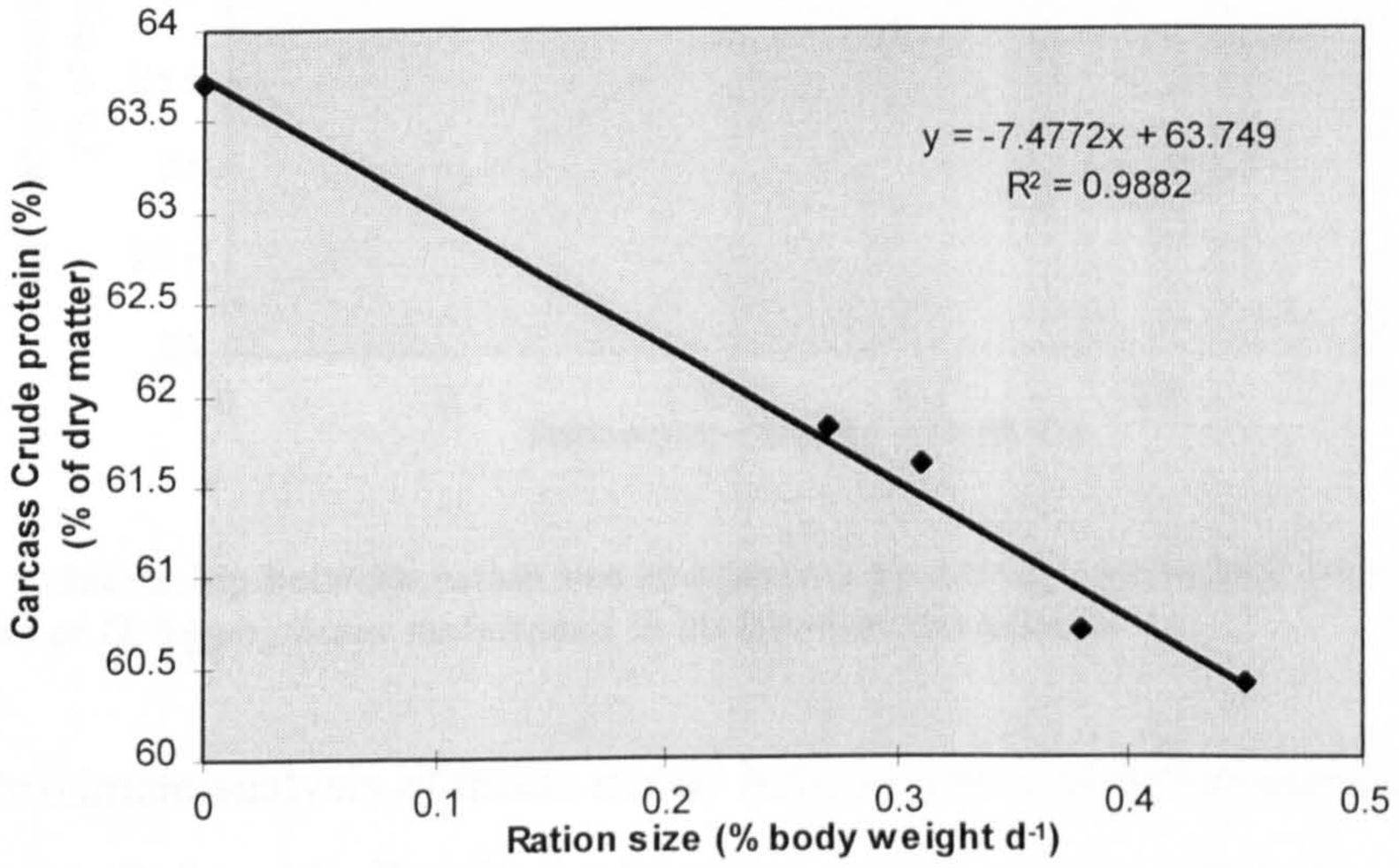

Fig. 6.22. Relationship between ration size and carcass percentage crude protein level for small populations of *H. hippoglossus* maintained in metabolism chambers.

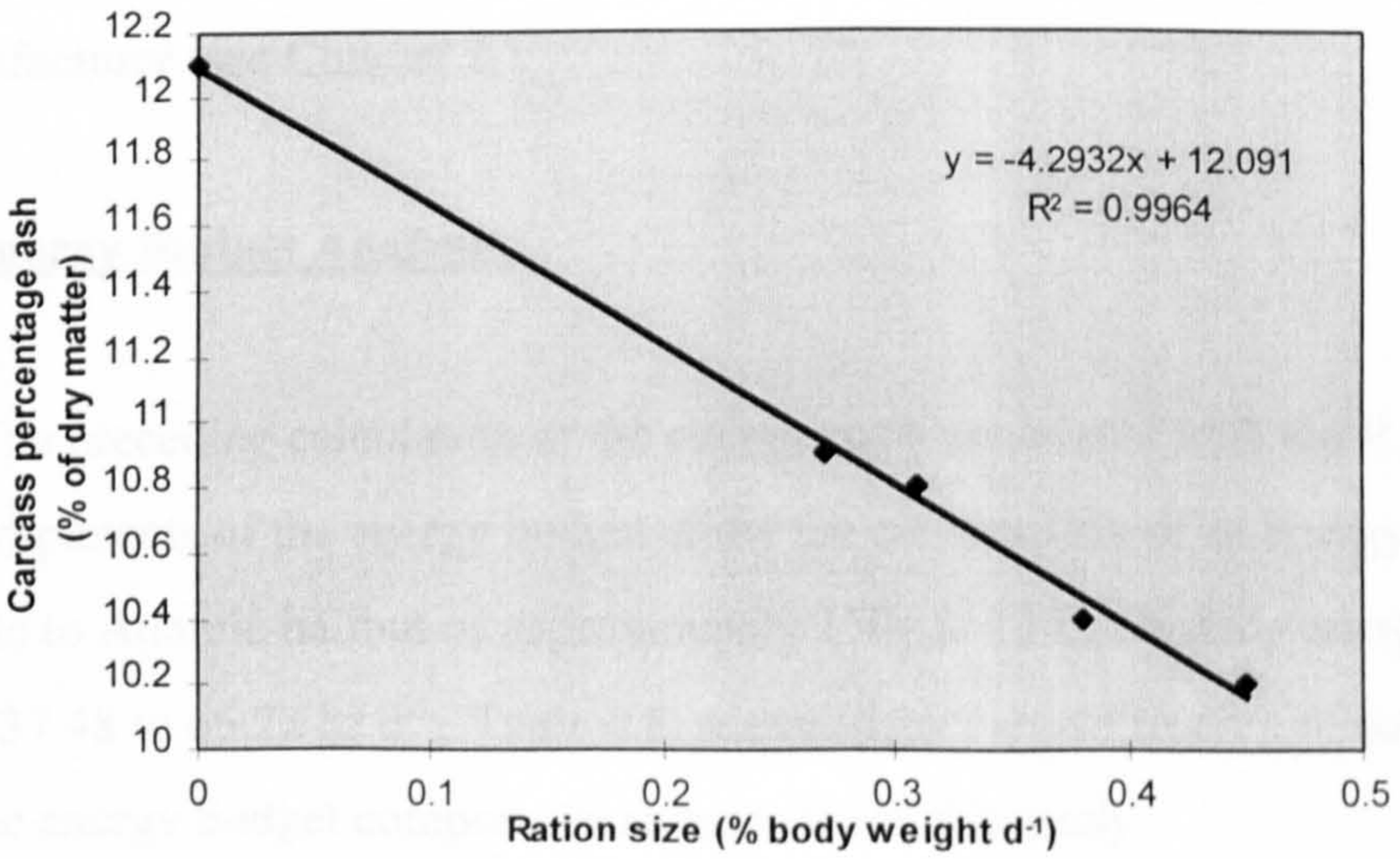

Fig. 6.23. Relationship between ration size and carcass percentage ash level for small populations of *H. hippoglossus* maintained in metabolism chambers.

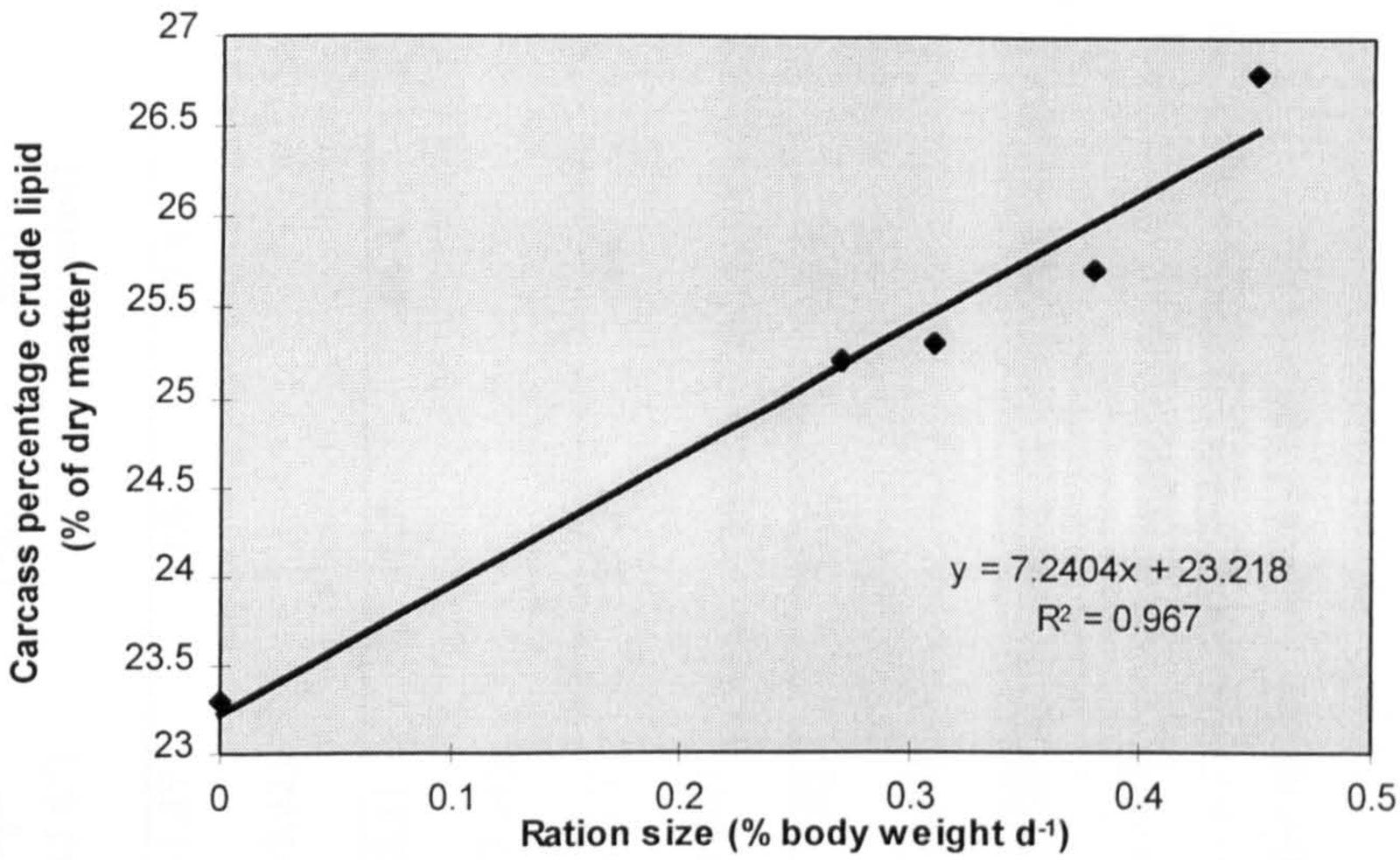

Fig. 6.24. Relationship between ration size and carcass percentage crude lipid level for small populations of *H. hippoglossus* maintained in metabolism chambers.

Proximate analysis of faecal matter indicated several differences between the fish groups 1, 2, 5 and 6. Results for faecal percentage crude protein ($F_{3,7} = 3.58$; $p = 0.125$), faecal percentage crude lipid ($F_{3,7} = 4.26$; $p = 0.045$), faecal percentage ash and faecal energy values ($F_{3,7} = 14.85$; $p = 0.012$), all showed a variation between fish group, although no distinct pattern was established.

Proximate analysis of food showed results corresponding closely to those of the manufacturer (see Chapter 2.).

6.3.7. Energy Budget Analysis.

The preceding calculation of the energy costs associated with the R, F, U, M and P components of the energy budget allow the construction of an energy budget applicable to Atlantic halibut of approximately 150g at 13°C, on daily energy intakes of 0 and 37.48 to 66.22 kJ d^{-1}. Table 6.8. summarises the partitioning of energy within the energy budget components as recorded in this study.

The calculated loss of dry weight (negative value for the P component) in three of the four fed fish groups is unexpected, and adds a degree of complexity to the

Table 6.8. Energy partitioning within *Hippoglossus hippoglossus* as defined from a bioenergetic study on small populations of 5 individuals held within metabolism chambers.

Metabolism Chamber	Feed rate (% body weight d^{-1})	Energy ingested (kJ d^{-1})	Recorded Faecal energy "F" (kJ d^{-1})	Calculated Faecal Energy "F" (kJ d^{-1})	Excretion "U" (kJ d^{-1})	Metabolism "M" (kJ d^{-1})	Growth "P" (kJ d^{-1})	Balance (%)	Balance (%) (corrected)
1	0.38	54.15 (±22.37)	0.83		1.08	26.98	-1.85	49.9	67.5
		(100)	(1.53)	(17.6)	(1.99)	(49.82)	(-3.42)		
2	0.45	66.22 (±33.66)	1.25		1.39	27.40	2.21	48.7	66.3
		(100)	(1.89)	(17.6)	(2.10)	(41.38)	(3.34)		
3	0	0	0	*	0.38	18.63	-22.7	83.7	n/a
					(1.67)	(82.07)	(100)		
5	0.28	38.67 (±23.27)	0.59		0.73	19.14	-0.32	52.1	69.7
		(100)	(1.53)	(17.6)	(1.89)	(49.50)	(-0.83)		
6	0.27	37.48 (±19.31)	0.98		0.73	20.48	-0.56	57.7	75.3
		(100)	(2.61)	(17.6)	(1.95)	(54.64)	(-1.49)		

* not measurable according to the method employed in this study see text
Values in brackets are energy allocation as a percentage of ingested energy. Estimated faecal energy values are predicted from the manufacturer's digestible energy value of 82.4% for the flatfish diet. Corrected balance figures incorporate this faecal energy value.

results. It appears that the halibut in these metabolism chambers were utilising their own body tissues as an energy source to a degree. The level to which tissue was utilised as an energy source (as expressed by the negative value of P) increased with decreasing size of ration. As expected, by far the largest contribution of energy from body tissue was observed in the unfed fish group. It is less easy to account for the calculated loss in dry weight of the fed fish groups, however these results suggest that the halibut were ingesting energy at a level below that of maintenance requirements. Overall the results allow the prediction of maintenance energy requirements, which can be deduced from the regression analysis presented in Fig.6.21. The equation $Y = 0.6231X - 37.013$ predicts the change in dry weight in a fish group (Y) according to the energy value of the feed ingested (X). From this equation, in order that body weight (as dry weight) is maintained, a gross feed energy value of 59.4kJ d^{-1} is required for a fish group, equivalent to approximately 79.92kJ kg^{-1} d^{-1}. For a 150g Atlantic halibut this is approximately 0.6g feed d^{-1}, equivalent to a feeding rate of 0.4% body weight d^{-1}.

Faecal energy values recorded in this study were extremely low at 1.53-2.61% of ingested energy, raising doubts over the efficacy of the faecal traps employed in the metabolism chambers. An estimated figure of 17.6% of ingested energy was calculated for faecal energy loss (see above). Energy allocated to excretion (as measured by ammonia excretion) was within the range 1.89-2.10% of the ingested energy in the fed fish groups, and 1.67% of utilised energy in halibut respiring their own tissues (chamber 3). The greatest allocation of energy was to the metabolism component (M), with calculated values in the range 41.38-54.64% of ingested energy. In the starved fish, a figure of 82.07% of utilised energy was calculated.

Overall, the calculated energy allocation to the F, U, M and P components falls short of the total energy ingested for the five fish groups, and the balance is less than 100%. The highest energy balance percentage was recorded in the starved fish group at 83.7% of utilised energy, and this figure probably indicates the potential source of error associated with feeding and faecal collection. Prior to the application of the estimated figure for faecal energy loss, balance figures in the fed metabolism

chambers were in the range 48.7-57.7%, with the corrected values in the range 66.3-75.3%.

Potential sources of error in the calculation of these energy budgets which could have contributed to these low values are summarised below:

1. Faecal collection was incomplete possibly owing to the physical nature of the faeces and incompatibility with the faecal trap arrangement.
2. Energy loss to the "U" component was calculated from ammonia excretion rates, although excretion of both trimethylamine oxide and urea may also have contributed to this component.
3. These energy balances are based on the premise that all feed offered to the fish and unaccounted for during retrieval was fully ingested and metabolised. In practice fish were occasionally observed to masticate pellets, providing an opportunity for partial loss of feed from the mouth, and subsequent overestimation of the ingested energy value. Aside from this fact, regurgitation may also contribute to an overestimation of ingested energy, and the necessary maintenance of the fish populations on screens precluded any direct observation of feed particles within the chambers leading to the assumption that any feed unaccounted for was ingested and utilised by the fish.
4. Overall, feed intake values were extremely low, showing a great deal of daily variation with the test feed ration very infrequently ingested (or never in the case of the 1% body weight d^{-1}). Unfortunately this resulted in the ingestion of a sub-maintenance ration in three of the four fed metabolism chambers.
5. The metabolism chambers each contained 5 halibut, and feed ingestion was assumed to be evenly distributed throughout the members of each trial population. Chambers containing small quantities of fish were chosen for this work following the lack of success in training individuals to feed in respirometers (see Chapter 4). Possible inter-individual differences in feed intake, with the potential presence of one or more dominant fish in a small group, are likely to affect the proximate composition of such individuals

relative to others within the group, and thus affect the overall energy calculation for the group as a whole.

6.4 Discussion

This bioenergetic study of the Atlantic halibut provides some of the first information on energy partitioning in this species. In addition, it is among the very few studies of flatfish which attempt to elucidate the total energy allocation within the organism, and as such also provides interesting comparative data with the energetics of roundfish species.

Ingested Energy ("C" component)

One of the characteristics of this study is the large variation in daily feed intake by the test fish, as represented in Table 6.1. Occasionally the halibut in metabolic chambers entirely refused food, and a zero intake was recorded. In addition to such obvious daily variation, the total daily quantity of feed ingested (here represented as percentage body weight) falls short of the two target test feed rates of 0.5 and 1.0% body weight by a good margin, with mean daily feed intakes of only 0.27-0.45% body weight d^{-1}. The test feed rates are based on the figures of 0.5-0.6% body weight d^{-1} most recently provided by the manufacturer for halibut of 40g to 500g body weight at 12 to 15°C, and industry feed rates commonly of 2-3% body weight d^{-1} for this size of fish. Specifically the feed rates of 0.5 and 1.0% body weight d^{-1} were chosen to reflect the partitioning of energy at an intermediate and a high energy intake, in order that the experimental design may elucidate any differences between such intakes. As far as this aspect of the study is concerned the results are disappointing, however there is much relevant information on energy allocation in this species to be gleaned from the results. Importantly, this study provides an energy value for a maintenance diet for Atlantic halibut of approximately 150g at 13°C of 79.92kJ kg^{-1} d^{-1}.

Atlantic halibut were fed at the rate of 5% body weight d^{-1}(commercial dry pelleted diet) in a growth study of fish from 20g to 90g in weight by Hallaråker *et al.* (1995), although there is no reference to the actual feed ingestion rate by individual fish. Björnsson and Tryggvadóttir (1996) fed groups of 140g Atlantic halibut once

per day on a dry marine diet in a study on optimal growth temperature, although unfortunately the feed rate is not presented by these authors. In a study of protein synthesis in Atlantic halibut of a mean body weight of 77g, Fraser *et al.* (1998) measured feed consumption using a diet prepared with Ballotini glass beads and subsequent X-radiography of the fish after a meal. A mean feed consumption of 41.21 g kg^{-1} wet weight of fish d^{-1} was recorded, although a wide variation in the proportion of ingested meal within the population was observed, between 2.85% and 12.01%. For a 77g halibut, this equates to a feed intake of 4.12% body weight d^{-1}. A gross energy value of 23.24kJ g^{-1} dry weight is presented for the test diet by Fraser *et al.* (1998), equating to an energy intake of 73.73kJ $fish^{-1}$ d^{-1} or 957.5 kJ kg^{-1} d^{-1}. The results of this study show values ranging from 37.48 to 66.22kJ ingested by each population of 5 halibut each day (approximately 50.4-89.1kJ kg^{-1} d^{-1}), considerably lower than the values of Fraser *et al.* (1998). The temperature ranges of the two studies are similar, and even allowing for size differences between the experimental animals, it is clear that the halibut in this study had a reduced feed and energy intake over what may be regarded as normal in this species. These results raise questions over the applicability of the Atlantic halibut to confinement in studies where optimal feed ingestion and growth are to be examined.

Questions also remain over the quality of the test fish as discussed in Chapter 2. It is unclear whether the early crops of first generation cultured Atlantic halibut are entirely representative of this species as a whole. Once production techniques have developed sufficiently to provide a consistent supply of good quality halibut, it may be that halibut of a smaller size may be trained to feed in confinement, and future studies may be even more successful in monitoring the pattern of energy flow within this organism.

Metabolism "M" component.

Several important points are made clear from the results of the oxygen consumption rate data of this work. Firstly, in common with the results for individual and community respirometers presented in Chapters 3 and 4, there was a clear

rhythmic pattern in daily oxygen consumption rate exhibited by the Atlantic halibut in all fed and starved metabolic chambers. Peak oxygen consumption rates were most frequently located within the period of darkness, suggesting a greater metabolic demand at this time. Mean oxygen consumption rate in fed fish exceeded that of the starved fish, similar to the results for fed and starved populations in the experimental work of Chapter 4. In the starved fish group, the daytime oxygen consumption rate was often close to the value predicted from the global equation presented in Chapter 3 for resting oxygen consumption rate, of 63.52 mg kg^{-1} h^{-1} for a halibut of 147.7g body weight.

Of interest are the frequent large nocturnal peaks in oxygen consumption rate seen in the unfed population, which rose from the baseline value to a point which was often close to that of the fed halibut in magnitude. It is tempting to relate such a pattern in oxygen consumption rate to differences in activity pattern over the 24 hour period, with a daytime period of rest or little activity and a nocturnal period associated with a higher level activity. Mean oxygen consumption rate was clearly related to ration size, increasing with increased quantity of food ingested. This is in agreement with the generally held theory of S.D.A. in which an increase in metabolic rate follows the ingestion of a meal (Rubner, 1902; Brody, 1945; Winberg, 1956; Kleiber, 1961; Brett and Groves, 1979; Jobling, 1981, 1983, 1994), and a similar relationship between ration size and energy cost associated with metabolism has been observed by other authors such as Hamada and Ida (1973), Jobling and Davies (1980), Cho (1982), and Chakraborty *et al.* (1992c, 1995). With a range of values between 80.45 and 117.06 mg kg^{-1} h^{-1}, the mean daily oxygen consumption rates recorded in this study are somewhat lower than the value of 126 mg kg^{-1} h^{-1} recorded by Hallaråker *et al.* (1995) in fed Atlantic halibut of 90g body weight at 13°C. This difference is most likely a reflection of both the greater body weight of fish in this study, and a likely reduced level of ingested feed. Although Hallaråker *et al.* do not present information on the quantity of feed ingested, the halibut were fed at the rate of 5% body weight d^{-1} on a diet of very similar composition to the diet used in this trial. When these factors are taken into account, there would appear to be reasonable agreement between the two figures.

As a percentage of ingested energy, the energy allocated to metabolism was relatively high, with values ranging from 41.38 to 54.64% depending on the quantity of ingested feed. In the starved fish, a value of 82.07% of utilised energy was attributed to the M component. In their generalised predictive bioenergetic equation for carnivorous fish, Brett and Groves (1979) suggest a figure of 29(±6)% allocated to the M component, somewhat lower than the values obtained in this study.

Chakraborty (1992) determined values of 21.38 to 47.12% of ingested energy allocated to metabolism in a study of the common carp fed diets of varying percentage protein. In perch (*Perca fluviatilis*) fed a restricted above maintenance diet, Solomon and Brafield (1972) showed that between 54.40 and 59.04% of the ingested energy was used in metabolism. It is interesting to compare these slightly higher figures produced from fish fed on a relatively low ration, since the halibut in this study ingested feed only slightly above maintenance level in one metabolic chamber (chamber 2), and at a sub-maintenance level in the other fed metabolic chambers (chambers 1, 5 and 6). In their study of the bioenergetics of the grass carp (*Ctenophryngodon idella*), Carter and Brafield (1991) recorded values for metabolism varying between 32.9 to 109.9% of the ingested energy, with the higher values observed at low ration levels. However, the grass carp is a herbivore and such a dietary nature difference is likely to introduce further differences into the energy budget equation. In a generalised energy budget for herbivores, Brett and Groves (1979) calculate a figure of 37% of ingested energy allocated to metabolism, somewhat higher than the figure these authors calculated for carnivorous fish. In a study of the carnivorous brown trout (*Salmo trutta*), Elliott (1976) estimated that between 35 to 70% of the ingested energy was allocated towards metabolic rate, over a range of ration levels from maintenance to maximum intake, and over a range of temperatures covering the thermal range of this species. Higher values of 69 to 70% were estimated for fish fed maintenance rations in this species. These values are slightly higher than the figures produced in this study, which may be a reflection of the higher metabolic rate of roundfish in comparison to flatfish (Chapter 3.). Overall, the above literature values are in agreement with those calculated for other species of fish fed at ration levels close to maintenance.

Excretion "U" component

In this study only ammonia was measured as the excretion component of the energy budget equation. Ammonia excretion by halibut in the fed metabolism chambers was elevated above that of starved halibut, and a direct relationship between ration size and mean ammonia excretion rate was observed. The pattern of ammonia excretion in fed halibut showed a 24 hour cycle, with peak excretion occurring at a point between 3 and 12 hours post feeding. No rhythmicity in ammonia excretion was determined in starved halibut.

Elevated post-prandial ammonia excretion rates relative to starved, or pre-feeding levels have been shown in sockeye salmon (Brett and Zala, 1975), in rainbow trout by Paulson (1980), in rainbow trout and carp by Kaushik (1980), in the Atlantic cod by Lied and Braaten (1987) and Ramnarine *et al.*, (1987), in the eel by Heinsbroek *et al.* (1993), and in brown trout, rainbow trout, sea bass, sea bream and turbot (Dosdat *et al.*, 1995, 1996). In a study of the Atlantic halibut, Fraser *et al.* (1998) observed a rapid increase in ammonia excretion rate in fish of 77g following ingestion of a single meal. Peak ammonia excretion rates were found at a point between six and twelve hours after feeding by these authors at a temperature of 9-13°C, in close agreement with the results of this study (Fig. 6.18.). Ammonia excretion rates varied between 3.3 and 5.2 $\mu g\ g^{-1}\ h^{-1}$ in the study of Fraser *et al.* (1998), and these values are in very close agreement to the results of this study. Urea excretion was not measured in this work, however it was measured by Fraser *et al.* (1998), who determined a value of 0.85 ±.09 $\mu g\ g^{-1}\ h^{-1}$ urea-N. Urea excretion was not influenced by feeding, and remained similar throughout the 24 hour period in the work of these authors. A figure of 17% of nitrogen excretion in the urea form was calculated, falling within the 11-23% value for urea-N excretion recorded by Dosdat *et al.* (1996) for five species of marine and freshwater teleosts.

Chakraborty *et al.* (1992b) observed ammonia excretion rate to increase with increasing dietary protein content in the common carp. Burel *et al.* (1996) found

increasing average daily total ammonia excretion over the temperature range 8°C to 20°C in a study of juvenile turbot, and hypothesised that this was solely the result of an increasing meal size. Similarly, Dosdat *et al.* (1995, 1996) found a strong correlation between ammonia excretion rate and the quantity of ingested nitrogen in a variety of freshwater and marine fish species including the turbot. The results of this study conform to this pattern, with mean post-prandial ammonia excretion rate increasing with increasing meal size in fed halibut.

As a percentage of ingested energy the values allocated to ammonia excretion are relatively low, ranging from 1.89 to 2.10% in fed halibut. Ammonia is regarded as metabolically inexpensive (Sayer and Davenport, 1987), thus such low values may be expected. The values produced in this study relate well to the results of the tank community studies (Chapter 4) where the energy allocated to ammonia excretion was 1.2 to 2.3% of ingested energy. Brett and Groves (1979) apportioned 7% of ingested energy to excretion in a generalised model for carnivorous fish. Figures calculated from the results of Elliott (1976) for brown trout show values between 4.3 and 11.3%, and from the results of Solomon and Brafield (1972) for the perch show values between 7.6 and 37.5% of ingested energy allocated to excretion. Chakraborty (1992) observed values of 4.19 to 8.74% of ingested energy allocated to the "U" component in common carp fed a diet of varying protein level. In a study of the energy budget of six freshwater species of teleost, Cui and Liu (1990) determined the percentage allocation to the U component between 6.7 and 39.5%. Clearly all these figures are somewhat in excess of that achieved in this study, even after allowing an additional 17% for nitrogen excretion as urea-N (Fraser *et al.*, 1998). However, the ammonia excretion rates determined in this study are very close to other published values for the Atlantic halibut and may be a reflection of comparatively low rates of protein synthesis, degradation and deposition in this species. This is also the hypothesis of Fraser *et al.* (1998) who measured rate of nitrogen flux within small (77g) Atlantic halibut using a ^{15}N isotope tracer.

In their study of the daily pattern of nitrogen excretion in the sockeye salmon (*Oncorhynchus nerka*), Brett and Zala (1975) set out to elucidate the diurnal changes

in metabolic parameters in populations of intensively cultured salmonids. Specifically these authors measured ammonia excretion and oxygen consumption rates, providing good comparative information with the results of this study. In starved sockeye salmon oxygen consumption rate fluctuated from a baseline of approximately 1.75 times the resting rate to a peak of approximately 3 times standard in the middle part of the daylight period. In this study, starved Atlantic halibut showed a daylight oxygen consumption rate close to the resting oxygen consumption rate, rising to a peak in the middle of the nocturnal period of approximately 2-3 times this level. In fed sockeye salmon, oxygen consumption rate was found to be at the maximum at a point immediately before, or during feeding, occurring at about 09.00h or 2 to 3 hours after dawn. In this study peak oxygen consumption rate in Atlantic halibut did not coincide with feeding, but occurred at night approximately 12 hours after feeding. Peak ammonia excretion rate in sockeye salmon was found 4 to 4.5 hours after feeding, although in this study peak ammonia excretion rate was at a point approximately 8 hours after feeding in the Atlantic halibut.

The results of this work are important from an aquaculture perspective, and will give an indication of timing of peak metabolic demand within culture systems containing Atlantic halibut. Importantly it appears that in contrast to the culture of salmonids peak oxygen demand in tank systems containing Atlantic halibut is likely to occur within the hours of darkness. This has implications for the maintenance of such systems, since it is likely that this will also coincide with a period when there are no husbandry staff on site.

Faecal Energy Loss "F" Component

Faecal energy values recorded in this study were extremely low, raising doubts over the efficacy of the faecal traps employed in the metabolism chambers. Ultimately faecal energy loss was estimated from digestible energy values in order to produce a viable energy budget in this study. Since a similar design of faecal trap was employed successfully by Cho *et al.* (1976), it seems that such lack of success may be rather a function of the physical characteristics of halibut faeces rather than equipment

design. Direct observation of recently voided faecal matter showed it to contain an extremely high water content, leading to a lack of aggregation and a tendency for disintegration of the faecal material. The method of total faecal recapture was chosen in this study in order to provide a more accurate assessment of the energy loss to this component. Many such studies have been performed with some success by previous authors including Solomon and Brafield (1972) with the perch, Elliott (1976) with the brown trout, Musisi (1984) with tilapia, Carter and Brafield (1991) with grass carp, and Chakraborty *et al.* (1995) with common carp. The results of this study suggest that a dedicated faecal collection system will have to be designed to ensure the success of future work of this type with the Atlantic halibut.

Growth "P" Component

The results of this study show a positive increase in fish dry matter in only one of the four fed metabolism chambers, chamber 2, fed at the higher test ration. This is disappointing in that the original intention of this work was to compare the pattern of energy allocation at starvation, intermediate feed levels, and high feed levels. On a wet weight basis, the specific growth rates of between 0.16 to 0.37% body weight d^{-1} recorded in this study are slightly low, but within the range of values recorded by Tuene and Nortvedt (1995) of 0.217 to 0.406% body wet weight d^{-1}, although these halibut averaged 422g in weight. The results of Björnsson and Tryggvadóttir (1996) for a study of optimal growth temperature in the Atlantic halibut show maximum SGR close to 0.6% body weight d^{-1} at a temperature of 11.4°C for 100g to 500g fish, although this was observed to decline slowly with increasing temperature. The halibut of Fraser *et al.* (1998) fed an experimental diet of 32% moisture content showed a mean wet weight SGR of 1.31% d^{-1} over a 28 day period. Hallaråker *et al.* (1995) observed the highest SGR in Atlantic halibut of 20 to 90g weight at 13°C, with values between 1.8 and 2.0% wet body weight d^{-1}, in fish fed a commercial formulated feed. Hjertnes *et al.* (1991) observed SGRs of 0.68 to 0.76% wet body weight d^{-1} in Atlantic halibut averaging 34g body weight fed a commercial dry diet at 7-12°C.

Clearly the Atlantic halibut of this study showed SGR values which are low in comparison to what may be predicted from other published values in this species. It is most likely that the low specific growth rates were a result of the low level of ingested food in this work given the consumption-growth relationship in fish (Brett, 1979). The results of Tuene and Nortvedt (1995) showed a linear consumption growth relationship in 422g Atlantic halibut up to a SGR value of 1.2, and a linear consumption-growth relationship has also been observed in the closely related Pacific halibut (*H. stenolepis*) by Paul *et al.* (1994). As discussed above, the low food ingestion rate seen in the halibut held in metabolism chambers may be a behavioural response to confinement. Attempts to train individual halibut held under more extreme conditions of confinement to feed proved unsuccessful in earlier work of this thesis (Chapter 4), and it would appear that these fish do not respond readily to such conditions. This may be a function of the only recent domestication of the Atlantic halibut, with all the fish used in this work only first generation farmed stock. In contrast, other fish which have been successfully maintained in restrictive metabolic chambers such as the carp and the tilapia have been cultured for generations and respond well to crowded conditions.

As a percentage of ingested energy, the allocation towards the "P" component was extremely low with a value of 3.34% in the halibut which showed a positive increase in dry weight (chamber 2). As well as the low quantity of ingested feed, this also reflects the low priority of the "P" component in energy allocation terms relative to metabolism. The energy costs associated with the M_M component have prior claim on ingested energy as the costs of homeostasis must always be met in order that the form and function of the animal may be secured.

Many studies which have involved the measurement of energy flow within fish have been carried out on fish of a small size. The Atlantic halibut used in this study were relatively small in comparison to much of the other experimental work carried out in this thesis, although they were much larger than some of the fish involved in other bioenergetic studies. The restricted availability of Atlantic halibut juveniles at the time of this work necessitated the use of relatively large fish. Further studies

which incorporate halibut of a smaller body weight, as they become available, could well be more successful in achieving good rates of feed ingestion and growth.

Energy Budget

With the estimated faecal energy loss figures applied to the results for the other measured components in the fed metabolism chambers, the balance for the energy budget varies between 66.3 and 83.7%, with the highest figure recorded for the starved metabolism chamber. Since the energy budget is a "balance sheet of energy income set against energy expenditure" (Brafield, 1985), an exact balance of 100% would be expected and the values of this study suggest an element of error associated with the calculation of the energy budget parameters. Such a result implies either a loss of energy to the system or an unmeasured or inaccurately measured component in the energy budget equation. However, it is unusual in fish bioenergetic studies to record balances of 100%. Many experimental studies have calculated balances showing considerable variation above and below the 100% figure. Flowerdew and Grove (1980) produced values of -35 to 47% of ingested energy for energy costs in the thick-lipped mullet, *Crenimudil labrosus*. In the southern catfish (*Silurus meridionalis*), Xie and Sun (1993) estimated energy output to the F, U, M and P components at 85.3 to 150.5% of the energy ingested. In their study of the energetics of the grass carp (*Ctenopharyngodon idella*), Carter and Brafield (1991) attained balances of 74.7 to 102.8% but found that the energy budgets of fish losing energy were less accurate. In this study only the fish held within one metabolism chamber (chamber 2) retained energy over the study period, thus the low balance values observed in this study may also be associated with the utilisation of body tissue as an energy source by the test halibut, similar to the results of Carter and Brafield (1991). Chakraborty *et al.* (1995) achieved balances of 66.64 to 81.96% in the common carp at different dietary protein and ration levels. In an examination of potential sources of error in the calculation of the balance of the energy budget, these authors discuss the potential for nitrogen excretion in the form of urea as an unmeasured energy loss to the system. The conclusion was that other studies of carp quote urea excretion as only between 6 to 7% in fed carp and 25% in starved carp, and the associated energy loss

in this form is not likely to account for the imbalance in their study. Similarly in this study although nitrogen excretion was only measured in the form of ammonia, Fraser *et al.* (1998) quote a figure of nitrogen excretion in the Atlantic halibut as 17% urea, thus energy loss in this form can be assumed to be relatively low in terms of the imbalance of this study. Chakraborty *et al.* (1995) also discuss the potential for regurgitation as a source of error, leading to an underestimate of the ingested ration. In this work the halibut were carefully monitored at feeding, the screens in the metabolism chambers were designed to prevent the loss of pellets through the system, and uneaten pellets were removed 2 hours after feed was offered. These precautions were taken in order to achieve the most accurate determination of the C component. However, careful observation of feeding halibut showed that occasionally pellets were ingested and violently masticated within the buccal cavity. The pellets disintegrated immediately sometimes with the loss of small particles of feed in the water of the metabolism chamber. Such particles of feed may represent an underestimation of the quantity of ingested energy, and lead to energy budget balances lower than 100%. Given the observation of Carter and Brafield (1991) that lower energy balances were obtained with fish utilising their own body tissue as an energy source, and the fact that the starved halibut in this study actually possessed the highest balance value at 83.7%, the accurate determination of ingested energy is a potential source of error in the determination of energy budget balance in the fed metabolism chambers of this study, and may account for the imbalance.

Solomon and Brafield (1972) observed more accurate balances in perch fed a maintenance ration in comparison to higher feed rates, recording overall balances of between 84.1 and 248.8%. These authors discuss the contribution of metabolised body tissue to the energy budget, and question the assumption that the energy value of the fish tissue remains the same throughout the experimental period. Such an assumption had to be made in these experiments because the work was carried out on individual fish. In this study, an initial sample of one fish from each metabolism chamber was taken in order that information on proximate composition and energy content could be obtained on the fish at the start of the experiment. Since these were conspecifics of the test population, subjected to the same pre-experimental conditions

it has been assumed that these results provide relevant information on the experimental population at day 0. Such an approach circumvents the assumption that the proximate composition of the fish remains the same throughout the experiment, and in this work it was clearly proven that there was a change in proximate composition, moisture content and energy value of the fish over the experimental period. These results provided important information on dry weight gain in the test fish, which showed a negative value in three of the metabolism chambers despite a recorded increase in wet weight. Such an approach reduces any error linked to the assumption that fish tissue composition is unchanging over the experimental period, but assumes that the initial sample of fish is representative of the population at time zero.

A further assumption in this study is that there was no significant gaseous exchange between the water of the metabolism chambers and the atmosphere, which could have influenced the oxygen consumption data. As discussed by Brafield (1985), practically the only way to ensure that indirect calorimetry provides realistic energetic values of metabolism is to run direct calorimetry experiments concurrently. So far this has only been achieved on very few occasions, and Brafield notes that there has been good agreement in the results of such studies between the two techniques. In this study, there is good agreement between the oxygen consumption rate values as measured by the techniques presented in Chapters 3 and 4 of this thesis, thus it appears reasonable to assume that the open metabolism chambers were not a significant source of error in the determination of oxygen consumption rate and the calculation of the energy costs of the M component.

This work presents the first important information on the energetics of the Atlantic halibut under controlled conditions, and is one of very few studies on the pattern of energy allocation in a species of flatfish. Several key areas have been identified for focus in future studies. Faecal energy loss investigation in this study was unsuccessful, and it is clear that equipment which has been utilised as faecal trap systems in studies of some species of roundfish are not applicable to the Atlantic halibut, probably due to the physical characteristics of faecal matter in this species.

This experimental study was successful in monitoring the pattern of energy allocation in Atlantic halibut fed a diet at close to maintenance requirement. This was not the original goal of this study, and it appears that this species responds to confinement by reducing the quantity of ingested feed. Such a response would negate the use of such a system in monitoring pattern of energy allocation and maximal feed consumption and growth rates. Unfortunately this is information which would be of direct relevance to aquaculture, where consumption and growth are theoretically maximised. Houlihan *et al.* (1987, 1989) with the Atlantic cod, and Fraser *et al.* (1998) with the Atlantic halibut, measured rates of protein turnover through the application of tritiated phenylalanine and a ^{15}N isotope tracer. Such an approach provides an alternative laboratory method which could also provide information which could be incorporated into the energy budget equation, and most importantly under conditions where the fish are feeding and growing under conditions approaching normality.

Chapter 7. General Discussion

7.1. General

Since its inception and development as a discrete branch of biological science, the study of bioenergetics has become fundamental to the construction of predictive yield models in both fisheries and aquaculture. Much of the early work on fish bioenergetics was driven by a desire for knowledge on the growth performance of wild stocks of fish, resulting in the development of predictive models in fisheries research and early aquaculture. The growth in aquaculture over the latter half of the twentieth century and a concurrent depletion of wild fish stocks, has resulted in an increasingly greater economic importance allocated to this field. The study of "Metabolic Rate and Food Requirements of Fishes" by G. G. Winberg (1956) is regarded as a work of great importance in the field of fish energetics and the starting point for modern studies. Drawing from a wide range of comparative historical fish metabolic data this thesis transforms the subject area and develops many of the theories which are so crucial to this science. In recognising the development of fish bioenergetic studies, it is also important to acknowledge the contribution of Ivlev (1939) who is regarded by Brett and Groves (1979) as a pioneer in this field, influencing the work of Winberg.

A bioenergetics approach has been applied with some success to the field of aquaculture, where a greater element of control over many environmental factors as well as a large depth of research knowledge in fish nutrition, physiology and behaviour, are factors which combine to ensure that the element of error is reduced in the formulation of such predictive models. Reduction of the energy budget equation to its component parts, and further elaboration of the energy allocation to these component parts over a wide range of environmental conditions, ultimately allows the prediction of the growth component "P". In this manner, the necessary economic viability of a fish farm or the sustainability of a fishery may be achieved through knowledge and understanding of the processes involved, and the influence of abiotic and biotic factors on the growth dynamics of the stock may be more fully understood.

As noted by Alexander (1967), partitioning of energy within the energy budget is likely to be influenced by natural selection. Calow (1985) expands further, hypothesising that gene expression must exert an effect on the enzymes associated with metabolism, and the fitness of the organism will depend on how such enzymes interact to affect form and function, reproductive timing and effort, and survivorship. The Atlantic halibut is a relatively highly evolved species of fish, with a unique life history pattern bordering between an r- and K- selection strategy (Haug, 1990b). The attainment of a large size with the associated longevity, combined with a reproductive investment into large numbers of small eggs initially appears as something of a paradox from an evolutionary biology perspective. However, it is clear that such a strategy has been a success for this species with the fitness of this organism assuring the survivability of the species over a long time scale, and it is only with the recent advent of human beings as efficient predators of the Atlantic halibut, and the development of fishing technology, that this species has been threatened.

The most obvious morphological characteristic of the Atlantic halibut is its extremely large adult size relative to other aquatic poikilotherms. As discussed by Peters (1983), "Cope's Law" holds that large species tend to occur at later points in the evolutionary history of a taxonomic group, although there are occasional exceptions to this rule. Large size in animals is also generally correlated with a high degree of specialisation, manifest as greater mobility, greater visual power, higher fecundity, larger individual offspring and an increased capacity to learn. The Atlantic halibut would appear to fulfil the requirements of these general attributes with the possible exception of the last two. Clearly, the Atlantic halibut is an extremely specialised organism, and well adapted to its natural environment, suggesting that this species is at a point well along the evolutionary tree. Peters (1983) also points out that such a high degree of specialisation is something of an evolutionary "cul-de-sac", and such species are poor potential ancestors. From the perspective of culturing this organism, this is an important point since this is a fish species which is extremely well adapted to a marine environment of low temperature (2-8°C range of wild-caught fish) and depths of 200 to 300m with associated high hydrostatic pressure and low light

intensity. It may be argued that the physiological mechanisms which have evolved in response to life in this environment will not be operating at optimum levels in culture situations where the organism is maintained at depths of 0.2 to 1.0m, within a temperature range of 6 to 15°C, and at ambient light conditions at sea level. Importantly, ongrowing of the Atlantic halibut is currently practised under conditions where the environmental parameters of temperature, photoperiod and light intensity, are unlikely to be wholly suitable for this organism.

Temperature is the most important abiotic factor in governing fish metabolic rate - one of the "Controlling Factors" of Fry (1971). The ongrowing of Atlantic halibut has been carried out successfully on a small scale on seawater sites in Scotland, however the characteristics of the physical environment selected by this fish in the wild indicates that some element of environmental control within a culture unit will benefit production. A further important point is the stability of the physical parameters of the deep ocean environment within which the wild fish live. In contrast to this many seawater sites in Scotland show daily fluctuations in parameters such as temperature and salinity, due to relatively shallow water intakes to sites. A lack of stability in such environmental parameters is likely to influence the energetics of the organism, and fluctuations are unlikely to provide conditions favourable for optimal growth. A possible means of circumventing such drawbacks, and enhancing production, would be the application of recirculation technology to the ongrowing of the Atlantic halibut, providing a more stable physical environment for the organism.

Atlantic halibut in the wild tend to be solitary in behaviour (see Chapter 1). Generally, these fish only congregate in groups during spawning, and spend the rest of the year as isolated individuals. Although the latter years of the 1990s is still a very early stage in the development of an Atlantic halibut farming industry, farms in Norway, Iceland, Canada and Scotland do have experience of rearing small quantities of halibut under conditions approaching those which will be encountered by these fish in full-scale commercial production sites. All reports suggest that these fish cope with the large groupings that such systems entail. This may be the case, and under conditions of culture the Atlantic halibut have been observed to grow at rates in

excess of conspecifics in the natural environment (Björnsson, 1994), however two important points regarding the effect of grouping on this species have emerged from this study:

1. There are clear patterns of inter-individual aggression, observed in the filming study in fish of approximately 300g in weight (Chapter 5), and also observed in fish of 300 to 1400g in the tank population study (Chapter 4).
2. The increase in routine oxygen consumption rate in small populations of halibut over the resting oxygen consumption rate of individuals (a calculated 36-81% in 100g fish, and 20-24% in fish of 1000g - see Chapter 4 discussion) suggests the lack of a "group" effect on metabolic rate in this species. In common carp, Korowin-Kossakowski *et al.* (1981 - cited by Steffens, 1989) observed a 25% reduction in routine oxygen consumption rate in fish held in groups, over individuals. Such a result immediately raises questions over differences in the methodology between resting and routine rate measurements, further emphasising the importance of the protocol argument. However, it is clear that the presence or absence of conspecifics is important in the measurement of routine metabolic rate for some fish species, and in the ayu (*Plecoglossus altivelis*), Umezawa *et al.*, (1983) showed by measurement of oxygen consumption rate that the metabolic rate could either increase or decrease in individuals held within populations, depending on the animal's social history.

These two points suggest that the Atlantic halibut may not be as ideally suited to intensive culture conditions as other species such as the Atlantic salmon, although since these experimental fish are the first generation of farmed stock, there is a clear potential for further domestication. Such a potential may also influence growth rate in subsequent generations of farmed stock, if any elevation in metabolic rate due to a group response may be reduced through selection and breeding of individuals showing better suited traits. Any reduction in metabolic costs associated with the group effect could allow a proportionate increase in energy allocation to growth, the P component, with a consequent influence on production.

In terms of the proposed culture of this species, it is clear that there is likely to be a good market for any farm produced Atlantic halibut, based on the reduced landings of wild stock and the high market price for this species (see Chapter 1). The results of this thesis provide information on the response of this organism to intensive culture conditions, and are of direct relevance to a halibut farming industry. The focus of research up to the present time, has been towards the areas of egg production, broodstock nutrition and larval rearing, and there is little published work relevant to the ongrowing of this species. Bioenergetic models relative to the ongrowing of this species in intensive culture conditions will enhance an embryonic halibut farming industry. This thesis contains some of the first results of such work.

7.2. Experimental Protocol

The aim of this study was to provide information on energy partitioning and bioenergetics of the Atlantic halibut (*Hippoglossus hippoglossus*). Respirometery and the accompanying indirect calorimetry provided most of the information on the energy costs associated with daily activities of this fish through measurements of oxygen consumption and ammonia excretion rate. An approach combining information on life history strategies in Atlantic halibut in the wild together with information gathered on the energy transformations within the organism, may be used to predict the likely performance of this species under conditions of culture. The results of this thesis and their application to a farm environment are based on the assumption that the response of the halibut under experimental conditions did not differ significantly from the response of this organism to the farm environment.

The works of Winberg (1956) and Fry (1957) are an historical starting point for the development of modern studies, following the examination of the relationships between many abiotic and biotic factors with metabolic rate and clarification of these relationships through the development of models to describe the effects. These works continue to be cited in many papers of the present day, indicating the thoroughness of the authors' approach to this field of research. The importance of temperature, body size, dissolved oxygen concentration, nutritional status, activity, diurnal periodicity

and water quality as factors influencing fish metabolism are discussed in depth, and many of the relationships developed from a vast array of collated data produced by both the author and other researchers. It is clear that the measurement of fish metabolic rate is not a new undertaking in the scientific community, and the framework for studies of this type has been delineated to a certain degree. With this in mind, it is disappointing to find there are difficulties in drawing inter-species comparisons even in current studies, due to the presence of uncontrolled or variable parameters within the experimental protocol. The temperature range of the Atlantic halibut is outwith that of many of the species which have been chosen for metabolic studies, limiting even further the data which could be used for comparative purposes.

Throughout this thesis, the importance of the development of and adherence to a strict protocol has been a common theme underlying the experimental work. Nutritional, thermal and photoperiodic histories have all been documented in the text, since these are important factors in fish energetic studies. Appropriate attention to the acclimation of the test fish to the experimental conditions has also been given, and these are all areas in which there is considerable variation in the experimental procedure applied to other published work. It has become increasingly clear throughout the interpretation and comparison of the results of this study that the field of fish bioenergetics is in need of a well defined protocol, adherence to which will facilitate inter-species comparisons in energetic studies.

7.3. General Discussion of Results

The starting point for this study was a determination of the allocation of energy to the M_M component - the energy costs of homeostasis - ascertaining a minimum energy cost of maintaining the integrity of the organism. In this way, the lower limit of the "metabolic scope" as defined by Priede (1977) has been evaluated for the Atlantic halibut over a very large size range of 50 to 6000g at temperatures of 6, 10 and 14°C.

A comparison of the values of Chapter 3 with those observed for other species of fish showed that the results of this study agreed closely with many published studies for other temperate marine flatfish of similar size, at the same test temperatures. Generally, resting oxygen consumption rates in flatfish have been found to be lower than roundfish species under corresponding conditions of measurement, and the results of this thesis show that the Atlantic halibut is similar in this regard. These results quantify the lower limit of the metabolic scope in this species, and with comparatively low values obtained, it is clear that the Atlantic halibut is a relatively energy efficient organism.

Thus the lower limits of the metabolic scope have been determined under controlled environmental conditions of 6, 10 and 14°C, 12L:12D photoperiod, 34‰ salinity and for a body size within the range of 50 to 6000g for sexually immature fish. In this study the determination of active metabolic rate was not attempted, however it is possible to draw conclusions from other published work on likely values for active metabolic rate, and thus extrapolate an hypothetical value on the range of the metabolic scope in this species.

The calculation of active metabolic rate in fish has been relatively infrequently attempted, partly arising from the difficulties in the arrangement of experimental apparatus and the accuracy of active metabolic rate determinations. The works of Brett (1964, 1965) on the swimming energetics of the sockeye salmon, and Webb (1971) on the swimming energetics of the brown trout are historically important studies reporting results on the energy costs associated with sustained aerobic locomotion of these species in the aquatic environment. One of the few studies on the swimming energetics of a marine teleost is that of Tytler (1969), who measured the costs of locomotion in the haddock, *Melanogrammus aeglefinus*. An excellent review on the study of the energetics of active fish is provided by Hammer (1995), who summarises much of the research literature in this field. Due to the inherent problems in designing respirometers for flatfish, swimming studies on these species are relatively few, although the work of Priede and Holliday (1980) on the plaice, and that of Duthie (1982) on the dab, lemon sole and flounder do provide excellent

comparative information on the swimming energetics of other species of temperate marine flatfish of body weights within the range of the work in this study. Recordings of oxygen consumption in swimming flatfish had further practical difficulties when it was found that these fish could maintain a stationary body position on the floor of the respirometer at flow rates of greater than one body length s^{-1} through body posturing. These difficulties were circumvented by tilting the respirometer system and inducing the fish to swim down a slope of 60°. The conclusion of these authors was that even though flatfish possess a relatively low resting oxygen consumption rate, the critical swimming speed is low in comparison to roundfish, and thus the metabolic scope of flatfish is small. Although there is no data on swimming oxygen consumption in the Atlantic halibut, the similarities in the physiology of this species with those of other members of the Pleuronectidae suggests that such a conclusion would also hold true for this species.

Ultimately, the hypothesis that the Atlantic halibut has a relatively restricted metabolic scope in comparison to roundfish emerges. Priede and Holliday's (1980) results for swimming plaice provide a value for active metabolic rate (AMR) of 148 mg O_2 kg^{-1} h^{-1} for a fish of approximately 310g at 10°C. Assuming that this value provides a relevant estimate for a similar sized Atlantic halibut at 10°C, this figure is 3.4 times the calculated resting oxygen consumption rate of 43.4 mg kg^{-1} h^{-1}.

Such a limited metabolic scope has clear implications from an energetic perspective, since the observation of Priede (1977) that an organism must operate within the limits of its metabolic scope or death will ensue, must be realised. Clearly, a small metabolic scope requires a precise partitioning of energy within the M_A and M_F components on a temporal scale in order that the maximum limit is not exceeded. The results of this thesis (Chapter 5) show that the energy allocation towards locomotion in a tank environment is relatively low, with individuals inactive for periods of between 75 and 94% of time within a 24 hour period. Such a response may be a metabolic trade-off for an organism limited by a relatively small metabolic scope, where a reduction in the proportion of energy allocated to the M_A component may allow a correspondingly greater allocation of energy towards the M_F component. In a

study of the feeding energetics of small tank populations of Atlantic halibut in Chapter 4, it was observed that oxygen consumption rate following feeding took a relatively long time to reach peak value at approximately 18 hours, and was also relatively prolonged showing a minimum length of time of 24 hours and a maximum predicted time of 57 hours to reach pre-feeding values. This is a very similar pattern of post-prandial oxygen consumption to that observed by Soofiani and Hawkins (1982) in the Atlantic cod. These authors suggested that such patterns of oxygen consumption are a reflection of long digestion times, themselves a reflection of large stomach size and large meal size. The similarity with the Atlantic halibut is clear, and perhaps such a physiological pattern of feeding metabolism is the evolutionary response of an organism with a relatively limited metabolic scope, the long duration of post-prandial oxygen consumption ensuring that the energetic requirements of the organism are maintained within the limits of the metabolic scope as defined by Priede (1977, 1985). In terms of the culture of the organism, such results show that peak oxygen demand in feeding populations of Atlantic halibut will not exert as great a requirement for oxygen in tank systems as perhaps more active fish species consuming small meals which are metabolised relatively quickly.

The quantification of oxygen consumption rate over the temperature range 6 to 14°C and for a range of body weights of 50 to 5000g allows the development of models to describe the influence of these parameters. Equations derived in Chapters 3 and 4 of this thesis allow the prediction of resting and routine oxygen consumption rates in the Atlantic halibut. Utilising values for percentage increase of peak post-prandial over routine oxygen consumption rate in fed fish, fed oxygen consumption rates may also be modelled over this range of body weights and temperatures. At temperatures of 8, 12 and 14°C fed oxygen consumption rates showed mean peaks of 69.0% increase over routine values, and at 10°C the increase was 86.8%. These results are of direct relevance to the farming of the Atlantic halibut in tank systems, and further allow the quantification of water flow rates for such systems (see section 7.6.). Resting, routine and fed oxygen consumption rate in the Atlantic halibut over the temperature range 6 to 14°C and for a range of body weights of 50 to 5000g is shown in Fig. 7.1. All curves show increasing values with increasing temperature

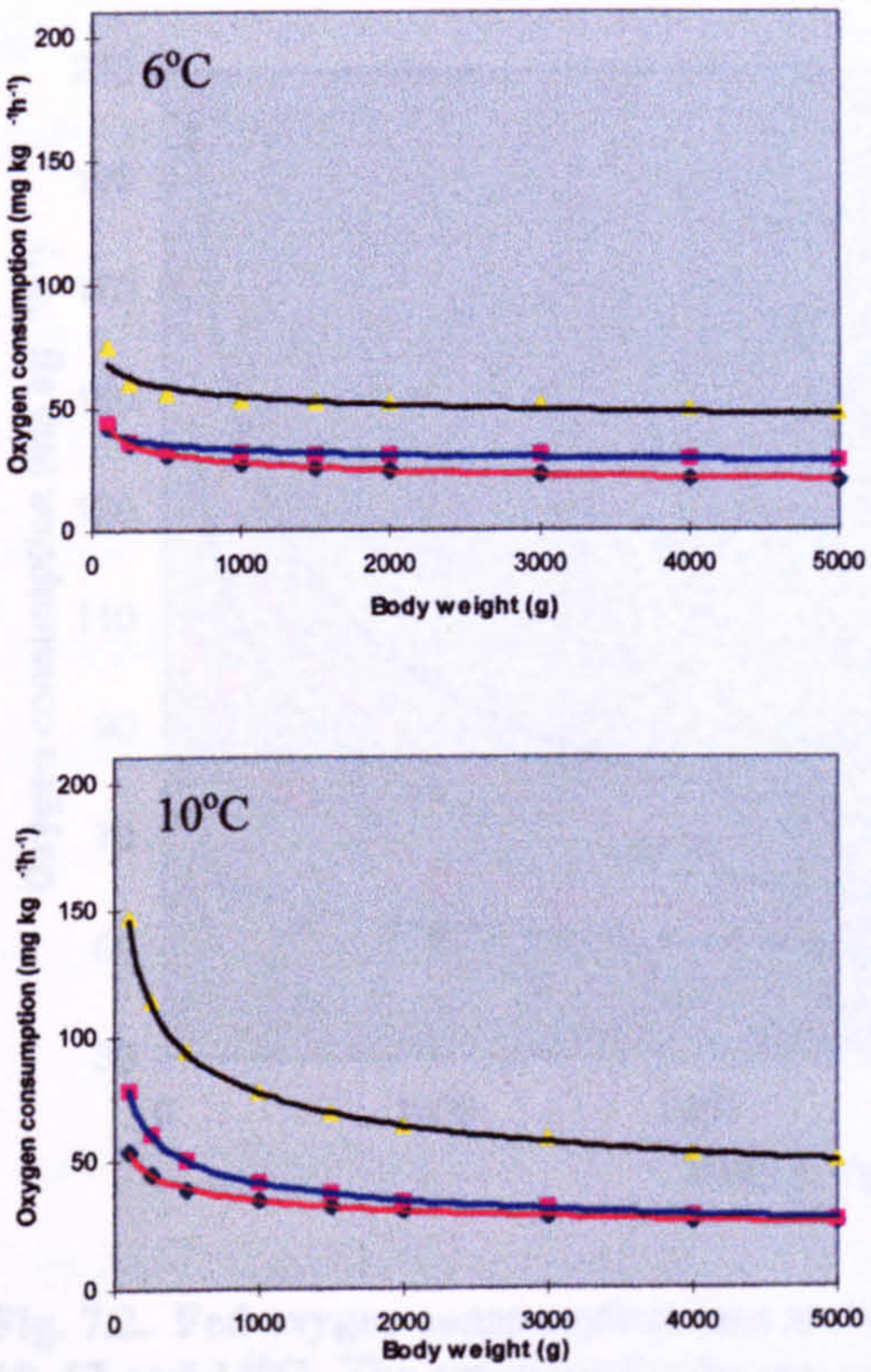

Fig. 7.1. Resting, routine and fed oxygen consumption in Atlantic halibut of 100 to 5000g at temperatures of 6, 8, 10, 12 and 14°C. Red curves represent resting oxygen consumption rate, blue curves represent routine oxygen consumption rate, and black curves represent fed oxygen consumption rate.

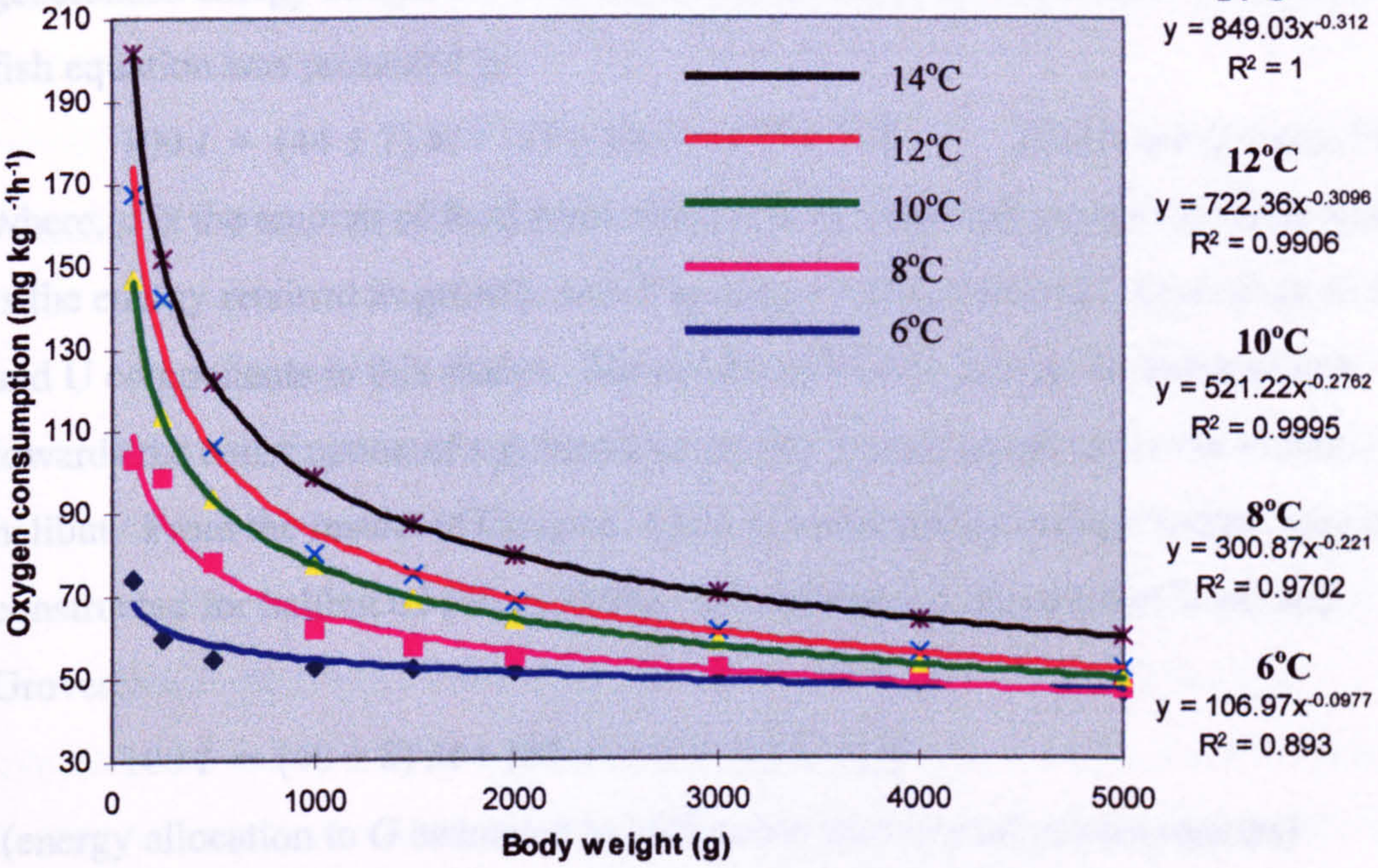

Fig. 7.2. Fed oxygen consumption rate in Atlantic halibut of 100 to 5000g at temperatures of 6, 8, 10, 12 and 14°C. The equations for the curves are presented in the form $Y = aX^b$, with the associated r^2 values, where Y is mass-specific oxygen consumption (mg kg^{-1} h^{-1}) and X is body weight (g).

7.4. The development of a Bioenergetic Model

Brett (1983) collated a series of published data from his own work, and the work of others in the field of fish energetics, in the formulation of a treatise on the energetics of the sockeye salmon in its natural environment throughout all stages of life history. In elucidating the energetics of the sockeye salmon, this well detailed study produces information which is fundamental to an understanding of this species and vital in the formulation of an accurate fishery model where estimates of allowable catch may be made with some confidence. In much the same way, the construction of an accurate energetic model for a cultured fish species facilitates the estimation of rates of production in such systems, and are vital to economic success and survival in aquaculture. For this reason, the aim of this study was to provide information on the partitioning of energy in the Atlantic halibut, which may be used to determine production models for this fish in culture systems.

In reviewing the literature at that time, Brett and Groves (1979) constructed a generalised energy budget for both carnivorous and herbivorous fish. The carnivorous fish equation was presented as:

$$100\,I = (44 \pm 7)\,M + (29 \pm 6)\,G + (27 \pm 3)\,E \qquad \text{(Brett and Groves, 1979)}$$

where, I is the amount of food consumed, M is the total energy cost of metabolism, G is the energy retained as growth, and E is energy lost as excretion (equivalent to the F and U components in this study). The results of this thesis may be extrapolated towards the construction of a generalised energy budget equation for the Atlantic halibut. From the results of Chapters 4 and 6, a generalised energy budget may be constructed for halibut of 100 to 1500g, and presented in the form of Brett and Groves:

$$100\,I = (40 \pm 8)\,M + (37 \pm 9)\,G + (23 \pm 2)\,E$$

(energy allocation to G estimated by difference from the other components)

For halibut of varying size range, this equation may be separated for fish of 100 to 150g and 500 to 1500g weight:

$100\,I = (38 \pm 4)\,M + (34 \pm 8)\,G + (22 \pm 2)\,E$ 100-150g Atlantic halibut

$100\,I = (41 \pm 12)\,M + (36 \pm 12)\,G + (24 \pm 1)\,E$ 500-1500g Atlantic halibut

These equations compare favourably with the general equation of Brett and Groves (1979), although as previously discussed the allocation of energy to metabolism is comparatively lower reflecting the dominance of roundfish species in the formulation of the energy budget of these authors. A slight reduction in the energy allocation to metabolism allows this difference to be allocated towards growth, suggesting that the Atlantic halibut is a relatively efficient fish species in terms of converting ingested feed to new fish tissue. This point enhances the position of this fish as a potential species for culture. The energy budget equations produced in this study are all constructed from data obtained from small populations of fish maintained under conditions as close as possible to those likely to be experienced by the fish under conditions of intensive culture. As explored in Chapter 4, the ration size ingested by the fish utilised in the experiments of this thesis does seem to be comparatively low, and there may be potential to design feeding regimes and culture systems in which this parameter is further optimised. With an increase in the quantity

of ingested feed, there is clear potential to also increase the production of this species in intensive culture.

7.5. Implications of the Experimental Results for Culture of the Atlantic halibut

The information gathered from the experimental work of this thesis provides the basis for the first bioenergetic model relevant to the ongrowing of the Atlantic halibut. Some of the most important information to be gleaned from this experimental work concerns the often nocturnally-located oxygen consumption rate peaks, implying a nocturnal rhythmicity in this species. As previously discussed, it may be hypothesised that a nocturnal rhythmicity would provide a general adaptive response for the Atlantic halibut to its natural environment. However, such a rhythmicity has implications for the energetics of this species under conditions of intensive culture, particularly in the areas of timing and presentation of feed, and efficiency of utilisation. Currently, the tank culture of Atlantic halibut is carried out in conjunction with other species such as Atlantic salmon, since as a result of the small numbers of juveniles produced by halibut hatcheries, there are insufficient numbers to maintain a fully dedicated halibut culture unit. Husbandry practices on such sites tend to be a reflection of the culture of tank held salmonids with associated feeding regimes. The results of this study question the suitability of a twice daily (a.m. and p.m.) feeding regime within the daylight hours as the most efficient means of ongrowing the Atlantic halibut.

As well as providing important information concerning the U component in the energy budget equation, the measurement of rate of ammonia excretion is also important in terms of the potential impact on fish health. The daily pattern of ammonia excretion rate is directly relevant to the metabolic loading of culture systems, important in throughflow systems, and especially important in recirculation technology. The results of this study show a clear 24 hour pattern in ammonia excretion rate, rising steadily to a peak at a point approximately 8 hours after feeding, and then declining at approximately the same rate to reach the pre-feeding level at a

point one to two hours prior to feeding. An elevated concentration of ammonia in the fish environment has serious implications for fish health, with the level of toxicity associated with the proportion of unionised ammonia according to the dissociation equation:

$$NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^-$$

Ammonia is considered second only to oxygen as the parameter which may affect fish health (Wickins, 1981), and it is the unionised fraction which has a far greater toxicity to fish (Wuhrman and Woker, 1948). Both temperature and pH influence the equilibrium of the dissociation equation, although pH is the most important factor with the proportion of unionised ammonia increasing with an increasing pH value (Smart, 1981). The results presented in Chapter 4 allow the prediction of the rate of ammonia excretion within an Atlantic halibut intensive culture system, and if other environmental variables are measured the likely impact on the health of the fish may be predicted. Such information is of great importance in the maintenance and operation of closed recirculation systems, and these results enable the calculation of the ammonia loading within such a system. With the primary impact of ammonia toxicity on the nervous system it has been hypothesised that cerebral energy metabolism is impaired during exposure (Smart, 1981), although the pathology associated with exposure to levels effecting chronic toxicity in fish is manifest as aneurysms and hyperplasia of the gill epithelial tissue (Larmoyeux and Piper, 1973). There is no published data on toxic levels of unionised ammonia in the Atlantic halibut, although the work of Alderson (1979) on Dover sole and turbot showed that the growth of these species was unaffected at unionised ammonia concentrations of 0.066 and 0.10 mg NH_3-N l^{-1}, slightly below the levels for growth impairment observed in work on salmonids published in the review of Wickins (1981). The prediction of metabolically produced ammonia within the culture system is an important step in furthering the knowledge required to culture the Atlantic halibut successfully.

7.6. The Water Requirements of Atlantic halibut in commercial scale single-pass tank systems, and simple economics culture model.

The tank culture of the Atlantic halibut is at the highest level of intensification of fish culture as defined by Meade (1989), in terms of increasing the metabolic load, or biomass, on a unit of water. Concomitant to an increasing degree of intensification is an additional increase of risk of loss of stock associated with potential for disease epidemics and a dependence on high technology systems as back up. Culture options for the ongrowing of the Atlantic halibut are limited to cage, single pass tank, and recirculation tank. Although tank and cage systems were both utilised successfully in the early years of the culture of the Atlantic salmon, economically cage systems are seen as the more viable option for this species at current market prices for feed and fish. However, in comparison to a potential Atlantic halibut farming industry there are important differences in the cost of juvenile stock, and in the market price for the finished product. The high technological costs of operating marine hatchery equipment are currently passed on to the halibut ongrower, with juvenile Atlantic halibut of 10-20g in weight fetching a price of between £5.00 and £6.00 each on the open market in 1998 (D. F. Mitchell, pers. comm.). As more efficient production of halibut juveniles is achieved in the future, and economies of scale begin to operate, the high price of juvenile stock will decrease.

With the relative high cost of individual fish, there may be a particular advantage on the culture of Atlantic halibut in tanks over the cage culture of this species, in that a greater element of security over stock from predation, theft and escape is ensured. With tank systems providing an environment where abiotic and biotic parameters may be more carefully controlled, the increased cost of operating a tank system with Atlantic halibut relative to a cage system may also be offset to some extent by higher survivability and more efficient growth through environmental manipulation.

The areas where this species is likely to be cultured are prone to violent weather conditions, at least during part of the year. Under such conditions, fish held

in cages may lose growth at times when husbandry staff may not be able to reach the site and feed fish; such a situation is unlikely to arise on a land-based site, and it is unlikely that feeding would be hindered in such a system. Additionally, in cage systems the Atlantic halibut has been found to be sensitive to cage movement in rough weather conditions (Martinez-Cordero *et al.*, 1994). The tank system provides a much more stable environment from this perspective. Even if cage technology improves in the future to the extent where the ongrowing of the Atlantic halibut may be carried out successfully at reduced cost in these systems, it is likely that tanks will be required for the ongrowing of halibut from the post-hatchery phase at body weights of about 10-20g to weights of 300-600g prior to transfer to cage systems. Improvements and developments in hatchery techniques and husbandry occur annually and the increase in the supply of juveniles is likely to result in a rapid expansion in the ongrowing of this species. Economically and scientifically therefore, the climate is right for the development of research studies into the ongrowing of this species, maximising the efficiency of production and the return on stock which require a high initial capital investment.

The foregoing chapters provide important information generated through a study into the energetics of this species, which may be applied to facilitate the culture of the Atlantic halibut, particularly in tank systems. The production cost of operating aquaculture units (£ kg fish^{-1} produced) is determined by the cost of maintaining the fish biomass within the unit (Summerfelt *et al.*, 1993). Meade (1989) lists dissolved oxygen concentration as the most important parameter affecting fish health and growth within aquaculture systems, with other important factors including the concentration of metabolites within the system, space, suspended solids, food and temperature. Colt and Orwicz (1991) state that oxygen is the limiting factor for production in tank flow-through aquaculture systems only in a single-pass unit. In recirculation units involving aeration or the addition of oxygen through injection systems these authors assert that the maximum carrying capacity for the unit is determined by pH (in response to the build-up of carbon dioxide in the system), and by unionised ammonia. The study of Colt and Orwicz (1991) was wholly concerned with modelling the production capacity of freshwater systems, and with seawater

possessing a high absorption and buffering potential for carbon dioxide (Libes, 1992), ammonia concentration - and the unionised fraction in particular - will conceivably be the limiting factor in Atlantic halibut tanks systems employing supplementary addition of oxygen.

The limiting factors for the production of Atlantic halibut in tank systems are therefore defined: in single pass systems carrying capacity and production are determined by the rate entry of oxygen into the system through tank flow rates; in water reuse systems or systems where flow rates have been reduced through application of additional oxygenation strategies, carrying capacity is determined by both the rate of removal of ammonia from the system and the pKa value for the dissociation equation determining the concentration of unionised ammonia (as influenced by pH value, temperature and salinity - see previous discussion). From the work of this thesis, it is possible to estimate water flow requirements for Atlantic halibut held in commercial size single pass tank systems, and provide information relevant to the carrying capacity of these units. Further expansion of this information allows for the construction of production models for these systems, and the economic viability may therefore be ascertained.

In constructing a model to maximise the production of a land-based freshwater rainbow trout farm, Bromage *et al.* (1988) assumed a minimum dissolved oxygen concentration of 5.5 mg l^{-1} in the tank effluent for fish health and growth. Given the lack of available information on the dissolved oxygen requirements for Atlantic halibut for health and growth, the assumption that this value is relevant for this species aids in the construction of a model predicting the water flow requirements. The assumed minimum figure of 5.5 mg l^{-1} in the tank effluent enables the calculation of oxygen available to the fish. Available oxygen over the temperature range 6 to 14°C is calculated according to the figures of Colt (1984) and presented in Table 7.1.

The figures presented in Table 7.1. may then be further applied to the oxygen consumption data curves derived for fed Atlantic halibut shown in Fig. 7.2., to calculate water flow requirements in tank systems. The results for the water flow rate

requirements for fed Atlantic halibut of 100 to 5000g body weight at temperatures of 6 to 14°C are shown in Fig. 7.3.

Table 7.1. Quantity of oxygen available to fish, assuming a tank effluent minimum dissolved oxygen concentration of 5.5 mg l^{-1}.

	Dissolved Oxygen (mg l^{-1})		
Temperature (°C)	**Inflowing**	**Minimum**	**Available to fish**
6	9.956	5.5	4.456
7	9.725	5.5	4.225
8	9.504	5.5	4.004
9	9.291	5.5	3.791
10	9.087	5.5	3.587
11	8.891	5.5	3.391
12	8.702	5.5	3.202
13	8.521	5.5	3.021
14	8.346	5.5	2.846

Inflowing dissolved oxygen concentrations are reproduced from the tables of Colt (1984), at a salinity of 34‰ and at a barometric pressure of 760 mm Hg.

The prediction of water flows in a pump ashore single pass seawater ongrowing site are an important aspect in the generation of an economics model for the culture of fish. When water flow rates have been quantified, the constant and variable costs of a seawater pump system may be introduced into the model, increasing the accuracy of economic predictions.

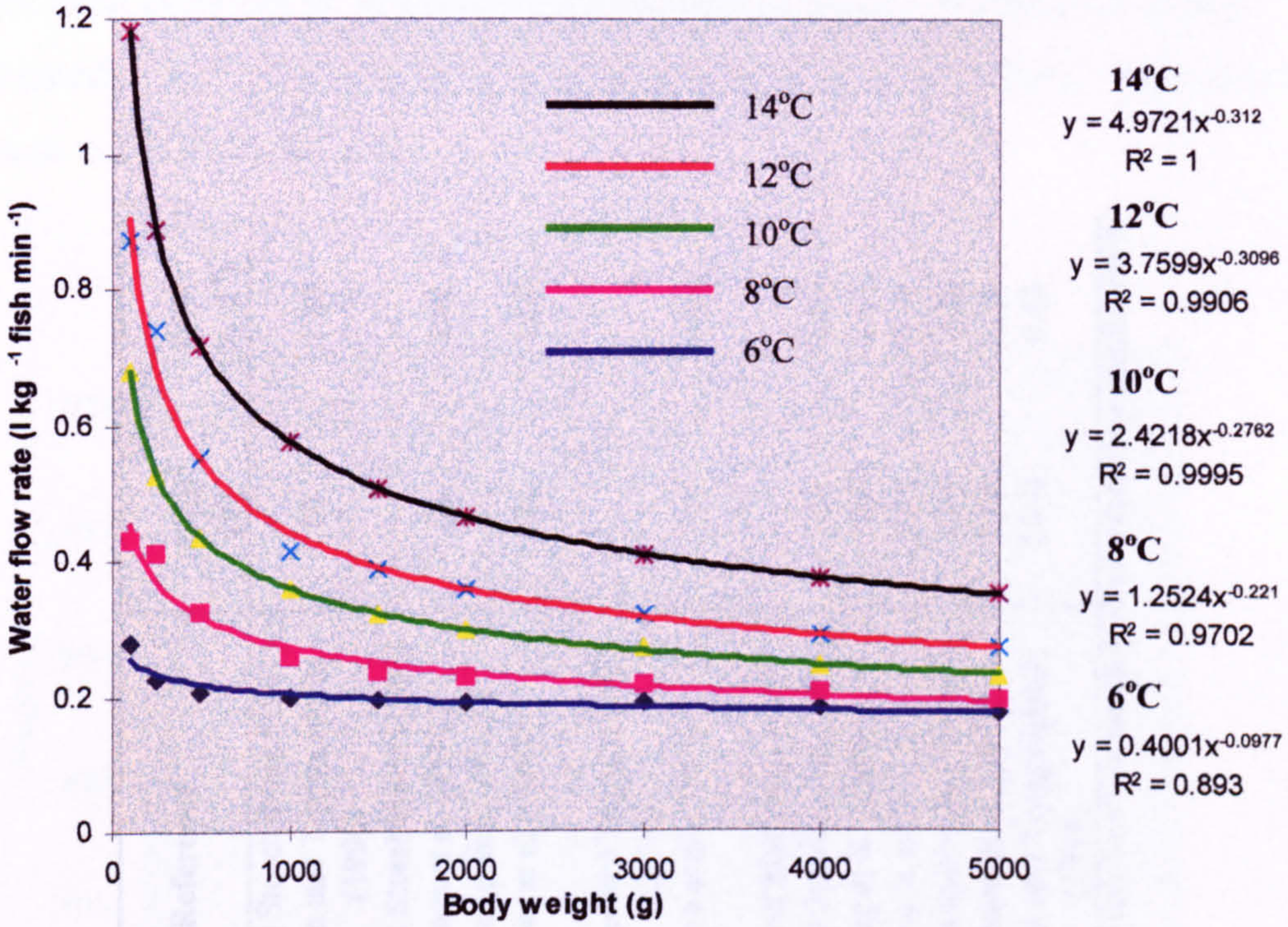

Fig. 7.3. Water flow rate requirements for Atlantic halibut held in a single pass tank system, for body weights of between 100 and 5000g, at temperatures of 6, 8, 10, 12 and 14°C. The equations for the curves are presented in the form $Y = aX^b$, with the associated r^2 values, where Y is water flow rate ($l\ kg^{-1}\ min^{-1}$) and X is body weight (g). Water flow rates are calculated to provide a dissolved oxygen concentration of 5.5 mg l^{-1} in the tank effluent.

Incorporation of the data presented above into a model for the operation of a hypothetical Atlantic halibut ongrowing unit provides a preliminary investigation into the economic viability of such a unit. The construction of this model will centre around a ongrowing unit for Atlantic halibut of approximately 250 tonne per annum production. Further information is vital to the construction of such a model, and in particular accurate information on the growth rate of halibut in culture is required. Due to the early stage of the halibut farming industry, such knowledge is limited, however the accumulation of growth data obtained from a variety of research trials makes the estimation of growth rate throughout an ongrowing cycle possible. Literature Atlantic halibut growth rate values, and also estimated figures for culture (assuming that the specific growth rates recorded are in agreement with those likely to be achieved in culture) are presented in Table 7.2. From these figures a likely

Table 7.2. Literature values for specific growth rate in the Atlantic halibut according to fish size and experimental temperature, and projected specific growth rate figures for an Atlantic halibut ongrowing unit assuming an optimum temperature regime.

Literature Values					Model	
Weight (g)	Temperature (°C)	Diet	SGR (% d^{-1})	Reference	weight (g)	SGR (% d^{-1})
6.1	7 - 9	commercial, dry	1.2-2.2	Berge and Storebakken (1991)		
8	7.3 -12.8	dry	1.3-2.2	Björnsson and Tryggvádottir (1996)	10	1.80
11.6	7 - 9	commercial, dry	1.2-2.2	Berge and Storebakken (1991)		
34	7 - 12	dry	0.68-0.71	Hjertnes et al. (1991)	50	1.40
20-90	13	dry, commercial	2.0-2.2	Hallaraker et al. (1995)		
77	9 - 13	experimental pelleted	1.31	Fraser et al. (1997)	100	1.00
140	5.0 - 14.9	dry	0.3-0.61	Björnsson and Tryggvádottir (1996)		
140	11 - 14	dry, commercial	1.4-1.8 to 0.4-0.8	Aune et al. (1997)	200	0.60
422	8 - 9	dry	0.217-0.365	Tuene and Nortvedt (1995)		
600-1500	7 - 9	moist	0.27	Berge and Storebakken (1991)	500	0.40
1100	0.2 - 13	moist	0.33	Haug et al. (1989)	1000	0.35
1320	0.2 - 13	moist	0.27	Haug et al. (1989)	2000	0.30
800-2000	5.8 - 11.8	salmon, semi-moist	0.22	Rabben and Huse (1986)	3000	0.20
2500	7	moist/dry/capelin	0.114-0.141	Björnsson et al. (1991)	4000	0.20
2900	2.4 - 15.1	moist	0.1-0.23	Björnsson and Tryggvádottir (1996)	5000	0.15

The tabulated values represented a wide range of experimental conditions under which Atlantic halibut growth rates have been recorded. Model values show specific growth rates predicted on an ongrowing site assuming a comparable temperature regime.

production cycle can be identified, with the time to reach a harvest size of 5kg estimated at approximately 147.3 weeks from a starting size of 100g. This predicted growth curve is shown in Fig. 7.4.

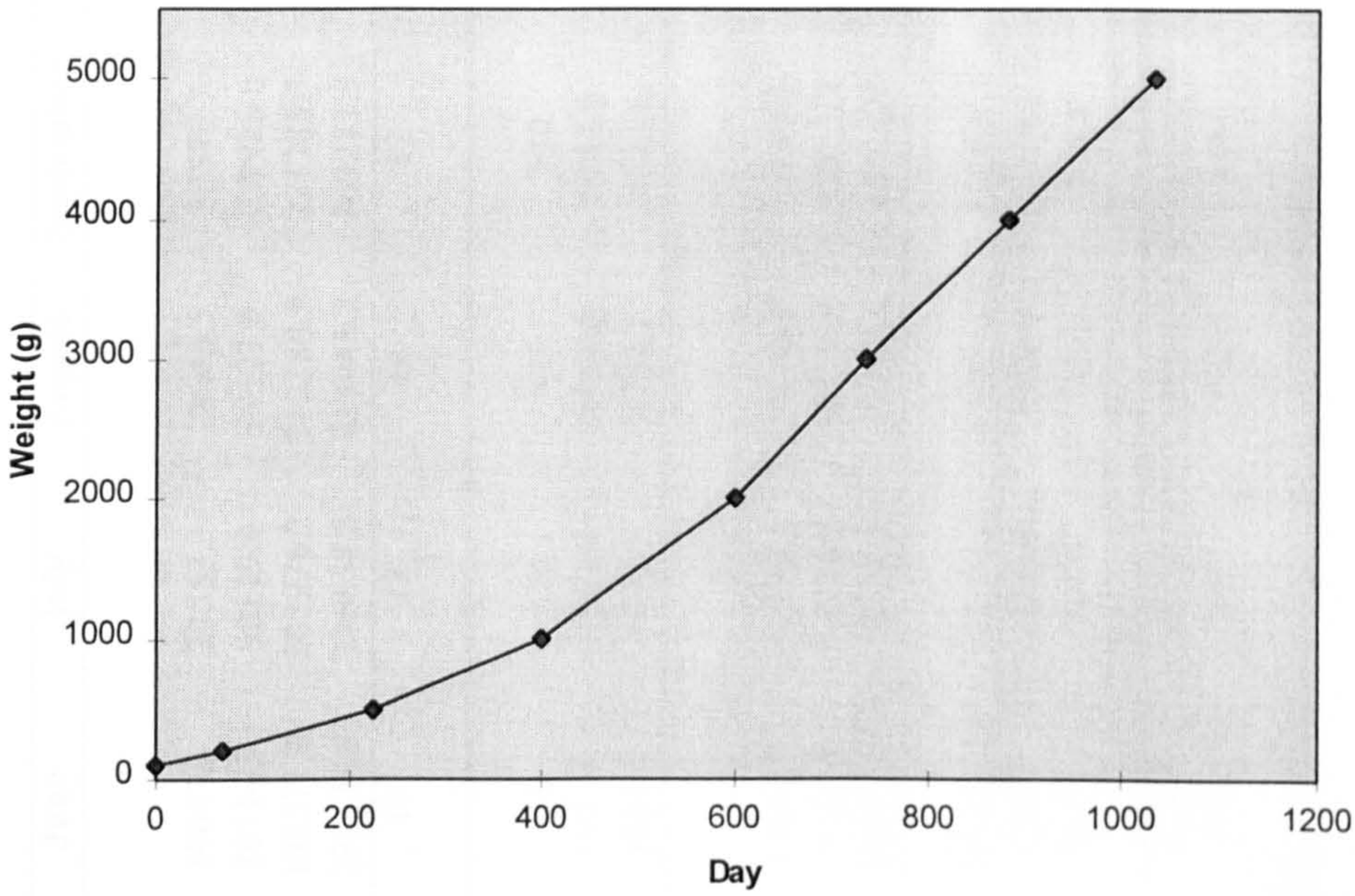

Fig. 7.4. Predicted growth curve for Atlantic halibut ongrowing from 100 to 5000g body weight, based on literature values from studies on a range of fish size presented in Table 7.2.

Taking the hypothetical scenario of a 250-tonne per annum production single-pass tank ongrowing unit, it is possible to calculate the standing biomass on site for every month of the year. Given the growth curve presented in Fig. 7.4. it is also possible to estimate the average weight of each year class on site through the production cycle, and together with information on water temperature, this may be used to calculate site flow rates. This information is shown in Table 7.3.

Importantly, the calculated flow rates presented in Table 7.3. allow further calculation of pumping costs according to the equation:

$$kW = \frac{\text{system head (m)} \times \text{Flow } (m^3\ s^{-1}) \times 9.81 \times \text{water density}}{\text{Pump Efficiency}}$$

Table 7.3. Flow Rate requirement for a hypothetical 250 tonne per annum Atlantic halibut ongrowing unit.

	January	February	March	April	May	June	July	August	September	October	November	December
Year Class	**Biomass (kg)**											
1+			5512.5	10022.7	14532.9	19043.1	23553.3	28063.5	32573.7	37083.9	41594.1	46104.3
2+	50614.5	55125	52500	61904.8	71309.5	80714.3	90119.0	99523.8	108928.6	118333.3	127738.1	137142.8
3+	146547.6	155952.4	165357.1	174761.9	184166.6	193571.4	202976.2	212380.9	221785.7	231190.4	240595.2	250000.0
Total	197162.1	211077.4	223369.6	246689.3	270009.1	293328.8	316648.5	339968.2	363287.9	386607.7	409927.4	433247.1
Temperature (°C)	7	6	7	8	10	11	12	14	12	11	10	9
Average weight by year class (g)												
1+			100	175	250	325	400	475	550	625	700	775
2+	850	925	1000	1190.5	1381.0	1571.4	1761.9	1952.4	2142.9	2333.4	2523.8	2714.3
3+	2904.8	3095.3	3285.8	3476.2	3666.7	3857.2	4047.7	4238.2	4428.6	4619.1	4809.6	5000.1
Flow requirment for each year class ($l\ kg^{-1}\ min^{-1}$)												
1+			2369.9	4308.9	7660.4	14045.7	17372.4	24916.8	18008.4	20501.9	18086.9	20048.1
2+	16428.0	11316.5	13504.6	15923.8	25561.8	31342.8	34994.9	50530.6	38831.7	42184.4	37773.5	40554.5
3+	33263.5	29712.7	35737.3	37769.8	49920.3	61837.3	58864.0	79387.8	64318.8	67046.3	58658.8	57306.3
Site total flow l/min	49691.5	41029.2	51611.8	58002.6	83142.5	107225.9	111231.2	154835.2	121158.9	129732.5	114519.1	117909.0
l/sec	828.2	683.8	860.2	966.7	1385.7	1787.1	1853.9	2580.6	2019.3	2162.2	1908.7	1965.1
l/sec per 100tonne	420.1	324.0	385.1	391.9	513.2	609.2	585.5	759.1	555.8	559.3	465.6	453.6
kW	59.5	49.2	61.8	69.5	99.6	128.5	133.3	185.5	145.2	155.4	137.2	141.3
Power cost (£)	1772	1322	1841	2002	2965	3700	3967	5521	4181	4626	3952	4205

Model is based on a 250mt production cycle with a single point harvest. 1+ year class fish are introduced to the site in March at an average weight of 100g A typical temperature regime for a marine site on the west coast of Scotland is assumed. Power costs are worked out assuming £0.04 kWh^{-1}.

Seawater of 34‰ salinity at 10°C possesses a density of 1.026g ml^{-1} (Weyl, 1970). An average head of 5m may be assumed for a pump ashore site on the west coast of Scotland (J. Bostock, pers. comm.). These values may then be incorporated into a model which takes account of all capital costs associated with the operation of such a site, and provide a greater accuracy in the prediction and economic forecasting of halibut ongrowing. Estimated costs for the operation of a 250 tonne per annum unit, together with costs for the operation of a 200 tonne per annum Atlantic salmon ongrowing unit as defined by Shaw and Muir (1987) are presented in Table 7.4.

Table 7.4. Capital costs and economic analysis for the ongrowing of Atlantic halibut in a 250 tonne per annum single-pass tank system, as fitted into the model of Muir and Shaw (1987) for the operation of a 200 tonne per annum Atlantic salmon ongrowing unit.

Variable costs (£1000)	Halibut	Salmon
Stock	303.19	130
Food	265.6	180
Insurance	20	20
Transport/Packing	40	40
Fixed/Semi-variable costs		
Management	25	25
Labour	28	28
Power/fuel	40	42
Consumables	10	10
Administration	10	10
Maintenance	10	10
Contingencies (5%)	24.7	24.7
Financial Charges		
Working capital	26	26
Fixed capital	72.9	72.9
Total	875.39	618.6
Unit cost (£ per tonne)	3.502	3.094

The Atlantic salmon production figures of Shaw and Muir (1987) allow for a production weight of 2.5kg; those for the Atlantic halibut in this study are for a production weight of 5kg. The Atlantic halibut figures incorporate current juvenile prices at approximately £5.50 per fish, current feed prices at approximately £800 $tonne^{-1}$, and power figures developed from the results of this study. Muir and Shaw assumed a figure of 160kW @ £0.03 kWh^{-1}. This study showed a monthly variation in power requirement with standing biomass, varying between 49 and 186kW, and was calculated at the rate of £0.04 kWh^{-1}.

The results of Table 7.4. clearly show a higher unit cost of production for one tonne of halibut against one tonne of Atlantic salmon, although in practice this margin may be slightly less since the salmon figures include feed and stock prices from 1987.

With a current market price of £7.00 to £7.50 kg^{-1} (D. F. Mitchell, pers. comm.) a 250 tonne per annum unit would have a return of approximately £1.8m from fish sold to market. Clearly there will be a relatively long period of culture for the first year class stocked onto the site, and thus a long period to recoup the initial capital investment. As further halibut ongrowing units develop and the market is better supplied with fish there will also be an associated decrease in the market price, and a declining profit margin.

This is very much a simplified model for the ongrowing of halibut, and under normal circumstances the actual farming of halibut could be more complex. In particular, the large variation in growth rate seen amongst individuals of the same year class is more likely to result in a "concurrent-mixed stocking and graded harvesting" technique as defined by Summerfelt *et al.* (1993). Even so, it is clear that at current market prices, and taking into account artificially inflated juvenile costs there is a great potential for the ongrowing of Atlantic halibut in single-pass tank systems provided the supply of juveniles and feed is geared up accordingly.

7.7. Conclusion

In the British Isles, as well as other temperate regions such as Norway, Iceland and Canada, marine aquaculture is dominated by the culture of one species, the Atlantic salmon. While the farming of Atlantic salmon is very much a success story, developing from small beginnings only as long ago as the 1960s, an industry which is effectively a monoculture is extremely vulnerable to economic and marketing pressures. In addition to this, the location of several farm sites containing the same organism, in close proximity within the same body of water provides an ideal environment for a panzootic of disease organisms. The widespread economic effect of the *Aeromonas salmonicida* bacterium, and the parasitic copepod *Lepeophtheirus salmonis*, on the Scottish and Norwegian salmon farming industries prior to advances in vaccination and chemotherapy, are testimony to this. A diversification into the farming of other marine species in temperate aquaculture is long overdue, and culture of the Atlantic halibut possesses great potential in this regard, owing to its high

market value and perceived position as a luxury food item. This fact has been known for some time and although Tilseth (1990) identified this potential, only very recently (Anonymous, 1997) have the first cultured Atlantic halibut in the British Isles been sold to the market place. A large amount of research money has been spent on the critical stages of yolk sac rearing and first feeding in this species, which remains something of a setback to commercial farming. This study was conducted on the ongrowing cycle of the Atlantic halibut, and provides some of the first information relevant to the oxygen consumption and energetics of production sized fish. The results of this thesis are of interest not only in providing a comparison with the energetics of other teleosts, but also importantly with those species currently associated with marine aquaculture in temperate regions. Of primary importance is the fact that the basis for an energy budget equation for the ongrowing of the Atlantic halibut has been developed.

Bibliography

Alanara, A. (1997) Feeding behaviour and growth performance of fish within a dominance hierarchy. pp10 In: *Voluntary food intake in fish.* Proceedings of the First COST 827 workshop. (Houlihan, D., Kiessling, A., and Boujard, T. eds.). Aberdeen 3-6 April 1997.

Alexander, R. McN. (1967) *Functional Design in Fishes.* London, Hutchison.

Altmann, J. (1974) Observational study of behaviour: sampling methods. *Behaviour* **49**, 227-267.

Andersen, N. G. and Alsted, N. S. (1991) Growth and body composition of turbot (*Scophthalmus maximus* (L.)) in relation to different lipid/protein ratios in the diet. pp. 479-491. In: *Fish Nutrition in Practice.* (eds. Kaushik, S. J. and Luquet, P.) Biarritz, France, June 24-27, 1991. Ed. INRA, Paris 1993 (Les Colloques, no.61.)

Anonymous (1997) Flat market is something to smile about. *Glasgow Herald* 16th October 1997.

Ansell, A. D. and Gibson, R. N. (1990) Patterns of feeding and movement of juvenile flatfishes on an open sandy beach. pp. 191-207 In: *Trophic Relationships in the Marine Environment.* (Barnes, M. and Gibson, R. N. eds.).

Aune, A., Imsland, A. K. and Pittman, K. (1997) Growth of juvenile halibut, *Hippoglossus hippoglossus* (L.), under a constant and switched temperature regime. *Aquacult. Res.* **28**, 931-939.

Aursand, A., Bleivik, B., Rainuzzo, J. R., Jørgensen, L. and Mohr, V. (1994) Lipid distribution and composition of commercially farmed Atlantic salmon (*Salmo salar*). *J. Sci. Food Agric.* **64**, 239-248.

Austreng, E., Storebakken, T and Åsgård, T. (1987) Growth estimates for cultured Atlantic salmon and Rainbow trout. *Aquaculture*, **60**, 157-160.

Beamish, F.W.H. (1964a) Respiration of fishes with special emphasis on standard oxygen consumption. II. Influence of weight and temperature on respiration of several species. *Can. J. Zool.* **42**, 177-188.

Beamish, F.W.H. (1964b) Influence of starvation on standard and routine oxygen consumption. *Trans. Am. Fish. Soc.* **93**, 103-107.

Beamish, F.W.H. (1964c) Seasonal changes in the standard rate of oxygen consumption of fishes. *Can. J. Zool.* **42**, 189-194.

Beamish, F.W.H. (1970) Oxygen consumption of largemouth bass *Micropterus salmoides* in relation to swimming speed and temperature. *Can. J. Zool.* **48**, 1221-1228.

Beamish, F.W.H. (1974) Apparent specific dynamic action of largemouth bass *Micropterus salmoides*. *J. Fish. Res. Bd. Can.* **31**, 1763-1769.

Beamish, F.W.H. and Mookherjii, P.S. (1964) Respiration of fishes with special emphasis on standard oxygen consumption. I. Influence of Weight and Temperature on Respiration of Goldfish *Carassius auratus* L. *Can. J. Zool.* **42**, 161-175.

Beamish, F.W.H. and Trippel, E.A. (1990) Heat Increment: A Static or Dynamic Dimension in Bioenergetic Models? *Trans. Am. Fish. Soc.* **119**, 649-661.

Bell, F.H. and Pruter, A.T. (1958) Climatic temperature changes and commercial yields of some marine fisheries. *J. Fish. Res. Bd. Can.* **15**, 625-683.

Berg, L. and Oiestad, V. (1986) Growth and survival studies of halibut (*Hippoglossus hippoglossus*) from hatching to beyond metamorphosis carried out in mesocosms. Coucil Meeting of I.C.E.S. F:16 19pp.

Berge, G. and Storebakken, T. (1991) Effect of dietary fat level on weight gain, digestibility, and fillet compostion of Atlantic halibut. *Aquaculture* **99**, 331-338.

Berge, G. M., Krogdahl, A., Strømsnes, Ø., Grønseth, F. A., Myhre, P. and Austreng, E. (1991) Digestibility determination in Atlantic halibut (*Hippoglossus hippoglossus*). *Fisk. Dir. Skr. Ser. Ernæring* **IV**, (2), 117-125.

Von Bertalanffy, L. (1957) Quantitative laws in metabolism and growth. *Quart. Rev. Biol.* **32**, (3), 217 - 231.

Björnsson, B. (1994) Effects of stocking density on growth rate of halibut (*Hippoglossus hippoglossus*) reared in large circular tanks for three years. *Aquaculture* **123**, 259-270.

Björnsson, B. and Tryggvadáttir (1996) Effects of size on optimal temperature for growth and growth efficiency of immature Atlantic halibut (*Hippoglossus hippoglossus* L.) *Aquaculture* **142**, 33-42.

Björnsson, B., Sigurthorsson, G., Lie, O. and Hemre, G.-I. (1991) Growth rate and food conversion of young halibut (*Hippoglossus hippoglossus* L.) fed six different diets. *I.C.E.S. C.M.* 1991/F:13.

Bligh, E. G. and Dyer, W. S. (1959) A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37**, 911-917.

Boisclair, D. and Tang, M. (1993) Empirical analysis of the influence of swimming pattern on the net energetic cost of swimming in fishes. *J. Fish Biol.* **42**, 169-183.

Bowering, W.R. (1986) The distribution, age and growth, and sexual maturity of Atlantic halibut (*Hippoglossus hippoglossus*) in the Newfoundland area of northwest Atlantic. *Can. Tech. Rep. of Fish. and Aquat. Sci.* **1432**, 34pp.

Bowman, R. E. and Bowman, E. W. (1980) Diurnal variation in the feeding intensity and catchability of silver hake (*Merluccius bilinearis*). *Can. J. Fish. Aq. Sci.* **37**, 1565-1572.

Braaten, B.R. (1979) Bioenergetics - a review on methodology. In: *Finfish Nutrition and Fishfeed Technology*, Vol. II. Eds. Halver, J.E. and Tiews, K. pp461-504.

Brafield, A.E. (1985) Laboratory studies on energy budgets. In: Fish Energetics New Perspectives. Eds. Tytler, P. and Calow, P. Croom Helm, London. pp257-282.

Brafield, A.E. and Llewellyn, M. J. (1982) *Animal Energetics*, Blackie, Glsagow, U.K.

Brett, J.R. (1962) Some considerations in the study of respiratory metabolism in fish, particularly in salmon. *J. Fish. Res. Bd. Can.* **19**, (5), 1025-1038.

Brett, J.R. (1964) The respiratory metabolism and swimming performance of young sockeye salmon. *J. Fish. Res. Bd. Can.* **21**, (5), 1183-1226.

Brett, J.R. (1965) The relation of size to rate of oxygen consumption and sustained swimming speed of sockeye salmon (*Oncorhynchus nerka*). *J. Fish. Res. Bd. Can.* **22**, 1491-1501.

Brett, J.R. (1972) The metabolic demand for oxygen in fish, particularly salmonids, and a comparison with other vertebrates. *Respir. Physiol.* **14**, 151-170.

Brett, J. R. (1976) Feeding metabolic rates of young sockeye salmon, *Oncorhynchus nerka*, in relation to ration level and temperature. *Fish. Mar. Ser. Res. Dev. Tech. Rep.* No. **675**. 43pp.

Brett, J.R. (1979) Environmental Factors and Growth. pp599-675. In: *Fish Physiology* Vol. VIII, Chap 10. Eds. Hoar, W.S. and Randall, D.J. Academic Press, New York.

Brett, J.R. (1983) Life Energetics of Sockeye Salmon. pp. 29-63 In: *Behavioural Energetics: The cost of survival in vertebrates.* Eds. Apsey, W. P. and Lustick, S. I. Ohio State University Press.

Brett, J.R. and Glass, N,R, (1973) Metabolic rates and critical swimming speeds of sockeye salmon (*Oncorhynchus nerka*) in relation to size and temperature. *J. Fish. Res. Bd. Can.* **30**, 379-387.

Brett, J.R. and Groves, T.D.D. (1979) Physiological Energetics. In: *Fish Physiology* Vol. VIII, Chap. 6. Eds. Hoar, W.S. and Randall, D.J. Academic Press, New York. pp279-352.

Brett, J.R. and Zala, C. A. (1975) Daily pattern of nitrogen excretion and oxygen consumption of sockeye salmon (*Oncorhynchus nerka*) under controlled conditions. *J. Fish. Res. Bd. Can.* **32**, 2479-2486.

B. H. A. (1997) Proceedings of British Halibut Association workshop, held 20 - 22 October 1997, Seafish Aquaculture, Marine Farming Unit, Ardtoe, Scotland.

Brody, S. (1945) Bioenergetics and Growth, with special reference to the efficiency complex in domestic animals. New York, Reinholds Publishing Corporation., 1023pp.

Bromage, N., Shepherd, J. and Roberts, J. (1988) Farming systems and husbandry practice. pp.50-102 In: *Intensive Fish Farming* (eds. Shepherd, J. and Bromage, N.), BSP Professional Books, Oxford.

Brown, J. A. and Keough, T. (1994) Atlantic halibut. *Bull. Aquacult. Assoc. Canada* **94**, 1, 9-12.

Bulow, F. J., Zeman, M. E., Winningham, J. R. and Hudson, W. F. (1981) Seasonal variation in RNA-DNA ratios and in indicators of feeding, reproduction, energy storage, and condition in a population of bluegill, *Lepomis macrochirus* Rafinesque. *J. Fish Biol.* **18**, 237-244.

Burel, C., Person-Le Ruyet, J., Gaumet, F., Le Roux, A., Sévère, A. and Bœuf, G. (1996) Effects of temperature on growth and metabolism in juvenile turbot. *J. Fish Biol.* **49**, 678-692.

Bushnell, P. G., Steffensen, J.F., Schurmann, H. and Jones, D. R. (1994) Exercise metabolism in two species of cod in arctic waters. *Polar Biol.* **14**, 43-48.

Calow, P. (1985) Adaptive aspects of energy allocation. In: *Fish Energetics : New Perspectives* Eds. Tytler, P. and Calow, P. Croom Helm, London. pp13-32.

Carter, C. G. and Brafiled, A. E. (1991) The bioenergetics of grass carp, *Ctenopharyngodon idella* (Val.): energy allocation at different planes of nutrition. *J. Fish Biol.* **39**, 873-887.

Cech, J. J. (1990) Respirometry In: *Methods in biology of fishes.* (Schreck, C. B. and Moyle, P. B. eds.) American Fisheries Society, Bethesda, Md. pp335-362.

Cech, J. J., Castleberry, D. T., Hopkins, T. E., and Petersen, J. H. (1994) Northern squawfish, *Ptychocheilus oregonensis*, O_2 consumption rate and respiration model: Effects of Temperature and Body size. *Can. J. Fish. Aquat. Sci.* **51**, 8-12.

Chakraborty, S. C. (1992) Energy budget and aspects of energy metabolism in common carp, *Cyprinus carpio*. Ph.D. thesis, Institute of Aquaculture, University of Stirling, Stirling, U.K. 235pp.

Chakraborty, S. C., Ross, L. G. and Ross, B. (1992a) The effect of photoperiod on the resting metabolism of the carp (*Cyprinus carpio*). *Comp. Biochem. Physiol.* **101A**, (1), 77-82.

Chakraborty, S. C., Ross, L. G. and Ross, B. (1992b) The effect of dietary protein level and ration level on excretion of ammonia in common carp, *Cyprinus carpio* L. *Comp. Biochem. Physiol.* **103A**, (4), 801-808.

Chakraborty, S. C., Ross, L. G. and Ross, B. (1992c) Specific dynamic action and feeding metabolism in common carp, *Cyprinus carpio* L. *Comp. Biochem. Physiol.* **103A**, (4), 809-815.

Chakraborty, S. C., Ross, L. G. and Ross, B. (1995) Energy budget and metabolism in common carp, *Cyprinus carpio* L., fed on different dietary protein levels and at different ration levels. *Aquaculture Nutrition* **1**, 179-187.

Cho, C. Y. (1982) Effects of dietary protein and lipid level on energy metabolism of rainbow trout, *Salmo gairdneri. Proc. 9th Symp. on Energy Metabolism of Farm Animals.* European Association of Animal Production, Pub. no. 29, 250-254.

Cho, C. Y. and Kaushik, S. (1985) Effects of protein intake on metabolizable and net energy values of fish diets. pp. 95-117. In: *Nutrition and Feeding in Fish.* Academic Press, London.

Cho, C. Y. and Slinger, S. J. (1980) Effect of water temperature on energy utilisation in rainbow trout (*Salmo gairdneri*). *Proc. 8th Symp. on Energy Metabolism in Farm Animals.* European Association Animal Producers, Pub. no. **26**, 287-291.

Cho, C.Y., Slinger, S. J. and Bayley, H. S. (1976) Influence of level and type of dietary protein, and of level of feeding on feed utilisation by rainbow trout. *J. Nutr.* **106**, 1547-1557.

Cho, C. Y., Slinger, S. J. and Bayley, H. S. (1982) Bioenergetics of salmonid fishes: Energy intake, expenditure and productivity. *Comp. Biochem. Physiol.* **73B**, (1), 25-41.

Clarke, A. (1991) What is Cold Adaptation and how should we measure it? *Amer. Zool.* **31**, 81-92.

Colt, J. (1984) Computation of Dissolved Gas Concentrations in water as functions of Temperature, Salinity and Pressure. *Am. Fisher. Soc. Special Pub.* **14**. Bethesda, Maryland.

Colt, J. and Orwicz, K. (1991) Modeling production capacity of aquaculture systems under freshwater conditions. *Aquacult. Eng.* **10**, 1-29.

Cui, Y. and Liu, J. (1990) Comparison of energy budget among six teleosts - IV. Individual differences in growth and energy budget. *Comp. Biochem. Physiol.* **97A**, (4), 551-554.

Cui, Y. and Wootton, R. J. (1988a) Pattern of energy allocation in the minnow *Phoxinus phoxinus* (L.) (Pisces: Cyprinidae). *Funct. Ecol.* **2**, 57-62.

Cui, Y. and Wootton, R. J. (1988b) Effect of ration temperature and body size on the body composition, energy content and condition of the minnow *Phoxinus phoxinus* (L.). *J. Fish Biol.* **32**, 749-764.

Danielsen, D. S. and Hjërtnes, T. (1991) Effect of dietary protein levels in diets for turbot (*Scophthalmus maximus* L.) to market size. pp. 89-96. In: *Fish Nutrition in Practice*. (eds. Kaushik, S. J. and Luquet, P.) Biarritz, France, June 24-27, 1991. Ed. INRA, Paris 1993 (Les Colloques, no.61.)

Davenport, J., Kjørsvik, E. and Haug, T. (1990) Appetite, gut transit, oxygen uptake and nitrogen excretion in captive Atlantic halibut *Hippoglossus hippoglossus* L., and lemon sole *Microstomus kitt* (Walbaum). *Aquaculture* **90**, 267-277.

Degani, G., Gallagher, M. L. and Meltzer, A. (1989) The influence of body size and temperature on oxygen consumption of the European eel, *Anguilla anguilla*. *J. Fish Biol.* **34**, 19-24.

Dejours, P. (1988) Principles of Comparative Respiratory Physiology (2nd revised edition). Elsevier/North Holland Biomedical Press; Amsterdam, New York, Oxford. 265pp.

De Silva, C. D., Premawansa, S. and Keembiyahetty, C. N. (1986) Oxygen consumption in *Oreochromis niloticus* (L.) in relation to development of salinity, temperature and time of day. *J. Fish Biol.* **29**, 267-277.

Diana, J. S. (1980) Diel activity pattern and swimming speed of northern pike (*Esox lucius*) in Lac Ste. Anne, Alberta. *Can. J. Fish. Aq. Sci.* **37**, 1454-1458.

Dosdat, A., Metailler, R., Tetu, N., Servais, F., Chartois, H., Huelvan, C. and Desbruyeres, E. (1995) Nitrogenous excretion in juvenile turbot, *Scophthalmus maximus* (L.), under controlled conditions. *Aquaculture Research* **26**, 639-650.

Dosdat, A., Servais, F., Metailler, R., Huelvan, C. and Debruyeres, E. (1996) Comparison of nitrogenous losses in five teleost fish species. *Aquaculture* **141**, 107-127.

Duthie, G.G. (1980) Physiological responses of flatfish to temperature; a comparative study. Ph.D. thesis, University of Aberdeen.

Duthie, G.G. (1982) The respiratory metabolism of temperature-adapted flatfish at rest and during swimming activity and the use of anaerobic metabolism at moderate swimming speeds. *J. Exp. Biol.* **97**, 359-373.

DuPreez, H. H., Strydom, W. and Winter, P. E. D. (1986) Oxygen consumption of two marine teleosts, *Lithognathus mormyrus* and *L. lithognathus* (Cuvier, 1830) (Teleostei: Sparidae). *Comp. Biochem. Physiol.* **85A**, (2), 313-331.

Eastmann, J. T. (1993) *Antarctic Fish Biology; Evolution in a unique Environment.* Academic Press. California. 322pp.

Edwards, R. R. C. (1971) An assessment of the energy cost of gill ventilation in the plaice (*Pleuronectes platessa* L.). *Comp. Biochem. Physiol.* **40A**, 391-398.

Edwards, R.C., Finlayson, D.M. and Steel, J.H. (1969) The ecology of O-group plaice and common dabs in Loch Ewe. III. Experimental studies on metabolism. *J. Exp. Mar. Biol. Ecol.* **3**, 1-17.

Edwards, R. R. C., Blaxter, J. H. S., Gopalan, U. K., Mathew, C. V. and Finlayson, D. M. (1971) Feeding, Metabolism, and Growth of Tropical Flatfish. *J. Exp. Mar. Biol. Ecol.* **6**, 279-300.

Edwards R. R. C., Finlayson, D. M., and Steele, J. H. (1972) An experimental study of the oxygen consumption, growth, and metabolism of the cod (*Gadus morhua* L.). *J. Exp. Mar. Biol. Ecol.* **8**, 299-309.

Elliott, J. M. (1976) The energetics of feeding, metabolism and growth of brown trout (*Salmo trutta* L.) in relation to body weight, water temperature and ration size. *J. Anim. Ecol.* **45**, 923-948.

Elliott, J. M. (1979) Energetics of freshwater teleosts. *Symp. zool. Soc. Lond.* **44,** 29-61.

Elliott, J. M. and Davison, W. (1975) Energy equivalents of oxygen consumption in animal energetics. *Oecologia*, **19**, 195-201.

Eriksson, L-O. (1978) Nocturnalism versus Diurnalism; Dualism within fish individuals. pp. 69-90 In: *Rhythmic Activity of Fishes* (Thorpe, J. E. ed.). Academic Press, London.

F.A.O. (1991) Fishery statistics, Catches and Landings. Rome: Food and Agricultural Organisation of the United Nations Vol. 72.

Farmer, G. J. and Beamish, F. W. H. (1969) Oxygen consumption of *Tilapia nilotica* in relation to swimming speed and salinity. *J. Fish. Res. Bd. Can.* **26**, 2807-2821.

Farmer, G. J., Ritter, J. A. and Ashfield, D. (1977) Seawater adaptation and parr-smolt transformation of juvenile Atlantic salmon, *Salmo salar*. *J. Fish. Res. Bd. Can.* **35**, 93-100.

Feldmeth, C. R. (1983) Costs of aggression in trout and pupfish. In: *Behavioural Energetics, The cost of survival in vertebrates*. (Apsey, W. P. and Lustick, S. I. eds.) pp.117-138. Columbus, Ohio State University Press.

Fessler, J. L. and Wagner, H. H. (1969) Some morphological and biochemical changes in Steelhead trout during the parr-smolt transformation. *J. Fish. Res. Bd. Can.* **26**, 2823-2841.

Fevolden, S.E. and Haug, T. (1988) Genetic population structure of Atlantic halibut, *Hippoglossus hippoglossus* (L.). *Can. J. Fish. Aq. Sci.* **45**, 2-7.

Flowerdew, M. W. and Groves, D. J. (1980) An energy budget for juvenile thicklipped mullet, *Crenimugil labrosus* (Risso). *J. Fish Biol.* **17**, 395-410.

Fonds, M., Cronie, R., Vethaak, A. D. and van der Puyl, P. (1992) Metabolism, food consumption and growth of plaice (*Pleuronectes platessa*) and flounder (*Platichthys flesus*) in relation to fish size and temperature. *Neth. J. Sea Res.* **29**, (1-3), 127-143.

Forsberg, O. I. (1994) Modelling of oxygen consumption of post-smolt Atlantic salmon in commercial-scale, land-based farms. *Aquacult. Int.* **2**, 180-196.

Forster, M. E., Franklin, C. E., Taylor, H. H. and Davison, W. (1987) The aerobic scope of an Antarctic fish, *Pagothenia borchgrevinki* and its significance for Metabolic Cold Adaptation. *Polar Biology* **8**, 155-159.

Forster, R. P. and Goldstein, L. (1969) Formation of excretory products. In: *Fish Physiology* Vol.1. (eds. Hoar, W.S. and Randall, D.J.) pp. 313-350. Academic Press, New York and London.

Fraser, K. P. P., Lyndon, A. R. and Houlihan, D. F. (1998) Protein synthesis and growth in juvenile Atlantic halibut, *Hippoglossus hippoglossus* L.: application of ^{15}N stable isotope tracer. *Aquaculture Research* **29**, 289-298.

Fry, F.E.J. (1947) Effects of environment on animal activity. *Pub. Ont. Fish. Res. Lab.* **55**, 1.

Fry, F.E.J. (1957) The aquatic respiration of fish. In: *The Physiology of Fishes* (Brown, M. E. ed.) Vol. I., 1-63; Academic Press, New York.

Fry, F.E.J. (1971) The effect of environmental factors on the physiology of fish. In: *Fish Physiology* (Hoar, W.S. and Randall, D.J. eds.) Vol. VI. pp 1-98. Academic Press, New York.

Furnell, D. J. (1987) Partitioning of locomotor and feeding metabolism in sablefish (*Anoplopoma fimbria*). *Can. J. Zool.* **65**, 486-489.

Gaumet, F., Dosdat, A., Desbruyères, E. and Chartois, H. (1994) Monitoring of excretion in marine fish: Methodological approach and application to ammonia production in turbot (*Scophthalmus maximus*). pp.251-260 In: *Turbot Culture: Problems and Prospects* (Lavens, P. and Remmerswaal, R. A. M. eds.), European Aquaculture Society, Special Pub. no. **22**, Gent, Belgium. 1994.

Gasca-Leyva, J. F. E., Martinez-Palcios, C. A. and Ross, L. G. (1991) The respiratory requirements of *Macrobrachium acanthurus* (Weigman) at different temperatures and salinities. *Aquaculture* **93**, 191 - 197.

Gerking, S. D. (1955) Endogenous nitrogen excretion of bluegill sunfish. *Physiol. Zool.* **28**, 283-289.

Gibson, R. N. (1973) Tidal and circadian activity rhythms in juvenile plaice, *Pleuronectes platessa*. *Mar. Biol.* **22**, 379-386.

Giguère, L., Côté, B. and St.-Pierre, J.-F. (1988) Metabolic rate scales isometrically in larval fishes. *Mar. Ecol. Prog. Ser.* 50 : 13-19.

Glass, N. R. (1968) The effect of time of food deprivation on the routine oxygen consumption of largemouth black bass (*Micropterus salmoides*). *Ecology* **49**, (2), 340-343.

Glass, N. R. (1969) Discussion of calculation of power function with special reference to respiratory metabolism in fish. *J. Fish. Res. Bd. Can.* **26**, 2643-2650.

Gnaiger, E. (1983a) Microcalorimetric monitoring of biological activities. Ecological and toxicological studies in aquatic animals. *Science Tools* **30**, (2), 21-26.

Gnaiger, E. (1983b) The twin-flow microrespirometer and simultaneous calorimetry. pp. 134-166. In: *Polarographic Oxygen Sensors - Aquatic and physiological Applications.* (Gnaiger, E. and Forstner, H., eds.). Springer, Berlin.

Graham-Brown, J.A., Jones, A. and Matty, A.J. (1984) Oxygen metabolism of farmed turbot (*Scophthalmus maximus*). 1. The influence of fish size and water temperature on metabolic rate. *Aquaculture* **36**, 273-281.

Greer Walker, M., Harden Jones, F. R., and Arnold, G. P. (1978) The movements of plaice (*Pleuronectes platessa* L.) tracked in the open sea. *J. Cons. Int. Explor. Mer.* **38**, (1), 58-86.

Gries, G., Whalen, K. G., Juanes, F. and Parrish, D. L. (1997) Nocturnal activity of juvenile Atlantic salmon (*Salmo salar*) in late summer: evidence of diel activity partitioning. *Can. J. Fish. Aq. Sci.* **54**, 1408-1413.

de Groot, S. J. (1971) On the Interrelationships between Morphology of the Alimentary tract, Food and Feeding behaviour in Flatfishes (Pisces: *Pleuronectiformes*). *Neth. J. Sea Res.* **5**, (2), 121-196.

Guinea, J. and Fernandez, F. (1997) Effect of feeding frequency, feeding level and temperature on energy metabolism in *Sparus aurata. Aquacult.* **148**, (2-3), 125-142.

Hallaråker, H., Folkvord, A. and Stefansson, S. O. (1995) Growth of juvenile halibut (*Hippoglossus hippoglossus*) related to temperature, day length and feeding regime. *Neth. J. Sea Res.* **34**, (1-3), 139-147.

Hamada, A. and Ida, T. (1973) Studies on Specific Dynamic Action of fishes - I. Various Factors affecting the values of Measurement. *Bull. Jap. Soc. Sci. Fish.* **39**, (12), 1231-1235.

Hammer, C. (1995) Fatigue and exercise tests with fish. *Comp. Biochem. Physiol.* **112A**, (1), 1-20.

Harden Jones, F. R. (1980) The migration of plaice (*Pleuronectes platessa*) in relation to the environment. pp. 383-399 In: *Fish behaviour and its use in the culture and capture of fishes.* (Bardach, J. E., Magnuson, J. J., May, R. C. and Reinhart, J. M. eds.) I.C.L.A.R.M. Conference Proceedings 5, 512p. International Center for Living Aquatic Resource Management, Manila, Phillipines.

Harden Jones, F. R., Arnold, G. P., Greer Walker, M. and Scholes, P. (1979) Selective tidal stream transport and the migration of plaice (*Pleuronectes platessa* L.) in the southern North Sea. *J. Cons. Int. Explor. Mer.* **38**, (3), 331-337.

Haug, T. (1990a) Biology of the Atlantic halibut *Hippoglossus hippoglossus* (L. 1758). *Adv. Mar. Biol.* **26**, 70pp.

Haug, T. (1990b) Om ulike livshistoriestrategier hos fisk og spesielt om kveitas plass I denne sammenhengen. *Fauna*, **43**, 105-117.

Haug, T. and Fevolden, S.E. (1986) Morphology and biochemical genetics of Atlantic halibut *Hippoglossus hippoglossus* (L.) from various spawning grounds. *J. Fish Biol.* **28**, 367-378.

Haug, T. and Gulliksen, B. (1988) Fecundity and egg sizes in ovaries of female Atlantic halibut *Hippoglossus hippoglossus* (L.). *Sarsia* **73**, 259-261.

Haug, T., Kjørsvik, E. and Solemdal, P. (1984) Vertical distribution of Atlantic halibut (*Hippoglossus hippoglossus*) eggs. *Can. J. Fish. Aquat. Sci.* **41**, 798-804.

Haug, T., Ringø, E. and Pettersen, G. W. (1988) Total lipid and fatty acid composition of polar and neutral lipids in different tissues of Atlantic halibut, *Hippoglossus hippoglossus* (L.). *Sarsia* **73**, 163-168.

Haug, T., Huse, I., Kjørsvik, E. and Rabben, H. (1989) Observations on the growth of juvenile Atlantic halibut (*Hippoglossus hippoglossus* L.) in captivity. *Aquaculture* **80**, 79-86.

Heggenes, J., Krog, O. M. W., Lindås, O. R., Dokk, J. G. and Bremnes, T. (1993) Homeostatic behavioural responses in a changing environment: brown trout (*Salmo trutta*) become nocturnal during winter. *J. Anim. Ecol.* **62**, 295-308.

Heinsbroek, L.T.N., Tussen, P.A.T., Flach, R.B. and DeJong, G.D.C. (1993) Energy and nitrogen balance studies in fish. pp. 375-389 In: *Fish Nutrition in Practice*, Biarritz, France, June 24-27, 1991. Ed. INRA, Paris 1993 (Les Colloques, no.61.)

Hepher, B. (1988) *Nutrition of Pond Fishes.* Cambridge University Press, Cambridge, England. 388pp.

Hettler, W.F. (1976) Influence of temperature and salinity on routine metabolic rate and growth of young Atlantic menhaden. *J. Fish Biol.* **8**, 55-65.

Hirata, H. (1973) Diurnal rhythm of metabolic and activity rates in juvenile Atlantic salmon, *Salmo salar* Linnaeus. *Mem. Fac. Fish., Kagashima Univ.* **22**, (1), 73-93.

Hjërtnes, T. and Opstvedt, J. (1990) Effects of dietary protein levels on growth in juvenile halibut (*Hippoglossus hippoglossus* L.). In: *Proc. 3rd Int. Symp. on Feeding and Nutrition in Fish.* pp189-193, August 28-September 1, 1989, Tokyo, Japan.

Hjërtnes, T., Gulbrandsen, K. E., Johnsen, F. and OPstvedt, J. (1991) Effect of dietary protein, carbohydrate and fat levels in feed for juvenile Atlantic halibut

(*Hippoglossus hippoglossus*). pp. 493-496. In: *Fish Nutrition in Practice.* Biarritz, France, June 24-27, 1991. Ed. INRA, Paris 1993 (Les Colloques, no.61.)

Holliday, F.G.T., Tytler, P. and Young, A. H. (1974) Activity levels of trout (*Salmo trutta*)in Airthrey Loch and Loch Leven, Kinross. *Proc. R. Soc. Edinb.* **74B,** 315.

Houlihan, D. F. (1991) Protein turnover in Ectotherms and its relationship to Energetics. pp.1-43 In: *Advances in Comparative and Environmental Physiology* Vol.7. (Gilles, R. ed.). Springer-Verlag, Berlin, Heidelberg.

Houlihan, D. F. and Allan, D. (1982) Oxygen consumption of some Antarctic and British gastropods: An evaluation of cold adaptation. *Comp. Biochem. Physiol.* **73A,** 383-387.

Houlihan, D. F., Hall, S. J., Gray, C. and Noble, B. S. (1987) Growth rates and protein turnover in Atlantic cod, *Gadus morhua. Can. J. Fish. Aq. Sci.* **45,** 951-964.

Houlihan, D. F., Hall, S. J. and Gray, C. (1989) Effects of ration on protein turnover in cod. *Aquaculture* **79,** 103-110.

Hughes, G. M. (1981) Effects of Low Oxygen and Pollution on the Respiratory Systems of Fish. In: *Stress and Fish* (Pickering, A. D. ed.). pp. 121-146. Academic Press, London.

Hughes, G. M., Albers, C., Muster, D. and Götz, K. H. (1983) Respiration of the carp, *Cyprinus carpio* L., at 10 and 20°C and the effects of hypoxia. *J. Fish Biol.* **22,** 613-628.

Ingram, M. (1987) The flatfish are coming. *Aquacult. Mag.* May/June : 44-47.

Ivlev, V. S. (1939) Energy balance in carps. *Zool. Zh.* **18,** 303-318.

Jákupsstovu, S.H.I. (1986) Kalvakanninger vid Magnusi Heinasyni November 1983-Januar 1984, *Fiskirannsokir* 1986 (3), 31-46.

Jakupsstovu, S.H. and Haug, T. (1988) Growth, sexual maturation, and spawning season of Atlantic halibut *Hippoglossus hippoglossus*, in Faroese waters. *Fish. Res.* **6,** 201-215.

Jirásek, J. and Adámek, Z. (1977) Effect of a type of food on oxygen consumption by carp fry. *Živočišná Výroba* **22,** (11), 833-838.

Jobling, M. (1981a) The influence of feeding on the metabolic rate of fishes: a short review. *J. Fish Biol.* **18,** 385-400.

Jobling, M. (1981b) Some effects of temperature, feeding and body weight on nitrogenous excretion in young plaice *Pleuronectes platessa* L. *J. Fish Biol.* **18,** 87-96.

Jobling, M. (1982) A study of some factors affecting rates of oxygen consumption of plaice, *Pleuronectes platessa* L. *J. Fish Biol.* **20**, 501-516.

Jobling, M. (1983) Towards an explanation of specific dynamic action (S.D.A.). *J. Fish Biol.* **23**, 549-555.

Jobling, M. (1985) Growth. In: "Fish Energetics New Perspectives" Eds. Tytler, P. and Calow, P. Croom Helm, London. pp213-230.

Jobling, M. (1993) Bioenergetics: feed intake and energy partitioning. In: "*Fish Ecophysiology*" (Rankin, J. C. and Jensen, F. B. eds.) Chapman and Hall Fish and Fisheries Series.

Jobling, M. (1994) Fish Bioenergetics. Chapman and Hall Fish and Fisheries Series No.13. 309pp.

Jobling, M. and Davies, P. S. (1980) Effects of feeding on metabolic rate, and the Specific Dynamic Action in plaice, *Pleuronectes platessa* L. J. Fish Biol. **16, 629-638.**

Jobling, M., Gwyther, D. and Grove, D. J. (1977) Some effects of temperature, meal size and body weight on gastric evacuation time in the dab *Limanda limanda* (L.). *J. Fish Biol.* **10**, 291-298.

Johnston, I. A. and Dunn, J. (1978) Temperature acclimation and metabolism in ectotherms with particular reference to teleost fish. pp. 67-93. In: *Temperature and Animal Cells* (Bowler, K. and Fuller, B. J. eds.). *Society for Experimental Biology Symposium XXXXI.*

Kadri, S., Metcalfe, N. B., Huntingford, F. A. and Thorpe, J. E. (1991) Daily feeding rhythms in Atlantic salmon in sea cages. *Aquaculture* **92**, 219-224.

Karamushko, -L. I. (1993) Effect of feeding on metabolic rate and specific dynamic action in *Gadus morhua, Anarhicas lupus* and *Pleuronectes platessa. J. Ichthyol.* **33**, (9), 148-158.

Kaufmann, R. (1983) VAMP: A video activity monitoring processor for the registration of animal locomotor activity. *J. Exp. Biol.* **104**, 295-298.

Kaushik, S. J. (1980) Influence of nutritional status on the daily patterns of nitrogen excretion in the carp (*Cyprinus carpio* L.) and the rainbow trout (*Salmo gairdneri* R.). *Reprod. Nutr. Dévelop.* **20**, (6), 1751-1765.

Kaushik, S. J. (1986) Environmental effects on feed utilisation. *Fish Phys. Biochem.* **2**, (1-4), 131-140.

Kjørsvik, E., Haug, T. and Tjemsland, J. (1987) Spawning season of the Atlantic halibut (*Hippoglossus hippoglossus*) in northern Norway. *Council Meeting of I.C.E.S.* **43**, 285-293.

Kleiber, M. (1961) "The Fire of Life" - An introduction to Animal Energetics. pub. John Wiley, New York. pp. 453.

Knights, B. (1985) Energetics and Fish Farming. In:"Fish Energetics New Perspectives" Eds. Tytler, P. and Calow, P. Croom Helm, London. pp309-340.

Knights, B. (1987) Agonistic behaviour and growth in the European eel, *Anguilla anguilla* L., in relation to warm-water aquaculture. *J. Fish Biol.* **31**, (2), 265-276.

Koch, F. and Wieser, W. (1983) Partitioning of energy in fish: Can reduction of swimming activity compensate for the cost of production? *J. Exp. Biol.* **107**, 141-146.

Kruuk, H. (1963) Diurnal periodicity in the activity of the common sole, *Solea vulgaris* Quensel. *Neth. J. Sea Res.* **2**, (1), 1-28.

Kutty, M. N. (1968) Respiratory quotients in goldfish and rainbow trout. *J. Fish. Res. Bd. Can.* **25**, (8), 1689-1728.

Larmouyeux, J. D. and Piper, R. G. (1973) Effects of water re-use on rainbow trout in hatcheries. *Prog. Fish Cult.* **35**, 2-8.

Leim, A.H. and Scott W. B. (1966) Fishes of the Atlantic Coasts of Canada. *Fish. Res. Bd. Can.* Bull. No. **155**. Ottawa.

Libes, S. M. (1992) An Introduction to Marine Biogeochemistry. John Wiley and Sons, Inc. U. S. A. 734pp.

Lied, E. and Braaten, B. (1987) The effect of feeding and starving, and different ratios of protein energy to total energy in the feed on the excretion of ammonia in the Atlantic cod (*Gadus morhua*). *Comp. Biochem. Physiol.* **78A**, (1), 49-52.

Liewes, E.W. (1984) Culture, Feeding and Diseases of Commercial Flatfish species. A.A.Balkema 103pp.

Lucas, A. (1996) Bioenergetics of Aquatic Animals. Taylor and Francis, Ltd. London, U. K. 169pp.

McCracken, F.D. (1958) On the biology and fishery of the Canadian Atlantic halibut (*Hippoglossus hippoglossus*). *J. Fish. Res. Bd. Can.* **15**, (6), 1269-1311.

Macdonald, J. A., Montgomery, J. C. and Wells, R. M. G. (1987) Comparative Physiology of Antarctic fishes. In: *"Advances in Marine Biology"* (J. H. S. Blaxter and A. J. Southward, eds.), Vol. 24., pp. 321-388. Academic Press, London.

McGregor, I. S. and Lee, A. M. (1995) Metabolic changes associated with ingestion of different macronutrients and different meal sizes in rats. *Physiol. and Behav.* **57**, (2), 277-286.

McIntyre, A. D. (1952) Statistics of the Scottish halibut fishery 1930-1949. *Marine Research, Scotland* 1952 (1), 1-24.

MacIsaac, P. F., Goff, G. P. and Speare, D. J. (1996) Comparison of routine oxygen consumption rates of three species of pleuronectids conducted at three temperatures. *Bull. Aquacult. Assoc. Can.* **96**, (3), 18-20.

McKenzie, R.A. (1946) The Canadian Atlantic halibut fishery. *Bull. Fish. Res. Bd. Can.* **71**, 29pp.

Manteifel, B. P., Girsa, I. I. and Pavlov, D. S. (1978) On rhythms of fish behaviour. pp. 215-224. In: *Rhythmic Activity of Fishes* (Thorpe, J. E. ed.). Academic Press, London.

Marais, J.F.K. (1987) Routine oxygen consumption of *Mugil cephalus*, *Liza dumerili* and *L. richardsoni* at different temperatures and salinities. *Mar. Biol.* **50**, 9-16.

Martinez-Cordero, F. J., Beveridge, M. C. M., Muir, J. F., Mitchell, D. F. and Gillespie, M. (1994) A note on the behaviour of adult Atlantic halibut, *Hippoglossus hippoglossus* (L.), in cages. *Aquacult. and Fish. Man.* **25**, 475 -481.

Martinez-Palacios, C. A. and Ross, L. G. (1986) The effects of temperature, body weight and hypoxia on the oxygen consumption of the Mexican mojarra, *Cichlasoma urophthalmus* (Günther). *Aquacult. Fish. Man.* **17**, 243-248.

Meade, J. W. (1989) *Aquaculture Management.* Van Nostrand Reinhold. 175pp.

Medland, T. E. and Beamish, F. W. H. (1985) The influence of diet and fish density on apparent heat increment in rainbow trout, *Salmo gairdneri*. *Aquaculture*, **47**, 1-10.

Mork, J. and Haug, T. (1983) Genetic variation in halibut *Hippoglossus hippoglossus* (L.) from Norwegian waters. *Hereditas* **98**, 167-174.

Moser, M. L. and Miller, J. M.(1994) Effects of salinity fluctuation on routine metabolism in juvenile spot, *Leiostomus xanthurus*. *J. Fish Biol.* **45**, 335-340.

Muir, B. S. and Niimi, A. J. (1972) Oxygen consumption of the euryhaline fish aholehole, *Kuhlia sandvicensis*, with reference to salinity, swimming and food consumption. *J. Fish. Res. Bd. Can.* **29**, 67-77.

Müller, K. (1978) The flexibility of the circadian system of fish at different latitudes. pp. 91-104 In: *Rhythmic Activity of Fishes* (Thorpe, J. E. ed.). Academic Press, London.

Muller-Fuega, A., Petit, J. and Sabaut, J.J. (1978) The influence of temperature and wet weight on the oxygen demand of rainbow trout (*Salmo gairdnei* Richardson) in fresh water. *Aquaculture* **14**, 355-364.

Musisi, M. L. (1984) The nutrition, growth and energetics of tilapia, *Sarotherodon mossambicus*. Ph.D. thesis, University of London.

Naas, K.E., Berg, L., Klungsoyr, J. and Pittman, K. (1987) Natural and cultivated zooplankton as food for Atlantic halibut larvae. I.C.E.S. CM.1987/F:17.

Nagarajan, K. andGopal, V. (1983) Effect of photoperiod on oxygen consumption and food utilisation in *Tilapia mossambica* Peters. *Proc. Nat. Acd. Sci. India* **53**, (B), III, 217-225.

Neilson, J.D. and Bowering, W.R. (1989) Minimum size regulations and the implications for yield and value in the Canadian Atlantic halibut (*Hippoglossus hippoglossus*) fishery. *Can. J. Fish. Aquat. Sci.* **46**, 1899-1903.

Neilson, J.D., Bowering, W.R. and Frechet, A. (1987) Management concerns for Atlantic halibut (*Hippoglossus hippoglossus*) in the Canadian North Atlantic. *C.A.F.S.A.C. Res. Doc.* 87/73; 22pp.

Neilson, J.D., Kearney, J.F., Perley, P. and Sampson, H. (1993) Reproductive biology of Atlantic halibut (*Hippoglossus hippoglossus*) in Canadian waters. *Can. J. Fish. Aq. Sci.* **50**, (3), 551-563.

Nelson, J.S. (1984) Fishes of the World. 2nd edition. John Wiley and Sons, Ltd., Chichester, U.S.A. 523pp.

Nickerson, J.T.R. (1978) The Atlantic halibut and its utilisation. *Mar. Fish. Rev.* **40**, (7), 21-25.

Niimi, A. J. and Beamish, F. W. H. (1974) Bioenergetics and growth of largemouth bass (*Micropterus salmoides*) in relation to body weight and temperature. *Can. J. Zool.* **52**, 447-456.

Nijhof, M. (1991) Effects of body weight on diet requirements of turbot (*Scophthalmus maximus*) with special reference to feed intake and body composition. pp. 501-513. In: *Fish Nutrition in Practice*. (eds. Kaushik, S. J. and Luquet, P.) Biarritz, France, June 24-27, 1991. Ed. INRA, Paris 1993 (Les Colloques, no.61.)

Nijhof, M. (1994) Research on nutrition and growth in the culture of post larval turbot (*Scophthalmus maximus* L.). pp. 137-163. In: *Turbot Culture: Problems and Prospects,* (eds. Lavens, P. and Remmerswaal, R. A. M.). European Aquaculture Society, Special Publication No.22, Gent, Belgium.

Ott, M. E., Heisler, N. and Ultsch, G. R.(1980) A re-evaluation of the relationship between temperature and the critical oxygen tension in freshwater fishes. *Comp. Biochem. Physiol.* **67A**, 337-340.

Paul, A. J., Paul, J. M. and Smith, R. L. (1994) Energy and ration requirements of juvenile Pacific halibut (*Hippoglossus stenolepis*) based on energy consumption and growth rates. *J. Fish Biol.* **44**, 1023-1031.

Paulson, L. J. (1980) Models of ammonia excretion for brook trout (*Salvelinus fontinalis*) and rainbow trout (*Salmo gairdneri*). *Can. J. Fish. and Aquat. Sci.* **37**, 1421-1425.

Pandian, T. J. (1987) Fish. In: *Animal Energetics Vol.2* (Pandian, T. J. and Vernberg, F. J. eds.). pp 357-465. Academic Press, San Diego, California.

Parsons T. R., Y. Maita, and C. M. Lalli (1984) A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press, Oxford.

Payne, A. I. (1979) Physiological and Ecological factors in the development of fish culture. *Symp. zool. Soc. Lond.* **44**, 383-415.

Peters, R. H. (1983) *The Ecological Implications of Body Size.* Cambridge Studies in Ecology, Cambridge University Press. 329pp.

Phillips, A. M. (1972) Calorie and Energy Requirement. pp. 1-28 In: *Fish Nutrition* (Halver, J. E. ed.) Academic Press, New York and London.

Pitcher, T. J. and Hart, P. J. B. (1982) *Fisheries Ecology.* Croom Helm Ltd., Beckenham, Kent, U.K. 414pp.

Priede, I. G. (1977) Natural selection for energetic efficiency and the relationship between activity level and mortality. *Nature* **267**, 610-611.

Priede, I.G. (1985) Metabolic scope in fishes. In: *Fish Energetics New Perspectives* (Tytler, P. and Calow, P. eds.) Croom Helm, London. pp33-66.

Priede, I.G. and Holliday, F.G.T. (1980) The use of a new tilting tunnel respirometer to investigate some aspects of metabolism and swimming activity of the plaice (*Pleuronectes platessa* L.). *J. Expl. Biol.* **85**, 295-309.

Priede, I. G. and Tytler, P. (1977) Heart rate as a measure of metabolic rate in telesot fishes: *Salmo gairdneri*, *Salmo trutta* and *Gadus morhua. J. Fish Biol.* **10**, 299-318.

Priede, I. G. and Young, A. H. (1977) The ultrasonic telemetry of cardiac rhythms of wild brown trout (*Salmo trutta* L.) as an indicator of bio-energetics and behaviour. *J. Fish Biol.* **10**, 231-242.

Rabben, H. (1987) A stripping method for Atlantic halibut (*Hippoglossus hippoglossus* L.). *Council Meeting of I.C.E.S.* F:40 6pp.

Rabben, H., Nilsen, T.O., Huse, I. and Jelmert, A. (1986) Production experiment of halibut fry in large enclosed water columns. I.C.E.S. CM.1986/F:19 27pp.

Rae, B. B. (1959) Halibut - Observations on its size at first maturity, sex ratio and length/weight relationship. *Marine Research, Scotland* **4**, 19pp.

Ramnarine, I. W., Pirie, J. M., Johnstone, A. D. F. and Smith, G. W. (1987) The influence of ration size and feeding frequency on ammonia excretion by juvenile Atlantic cod, *Gadus morhua* L. *J. Fish Biol.* **31**, 545-559.

Randell Brown, C. and Cameron, J. N. (1991a) The induction of specific dynamic action in channel catfish by infusion of essential amino acids. *Physiol. Zool.* **64**, 276-297.

Randell Brown, C. and Cameron, J. N. (1991b) The relationship between specific dynamic action (SDA) and protein synthesis in the channel catfish. *Physiol. Zool.* **64**, 298-309.

Rao, G. M. M. (1968) Oxygen consumption of rainbow trout (*Salmo gairdneri*) in relation to activity and salinity. *Can. J. Zool.* **46**, 781-786.

Ronnestad, I. and Naas, K.E. (1993) Oxygen consumption and ammonia excretion in larval Atlantic halibut (*Hippoglossus hippoglossus* L.) at first feeding: A first step towards an energetic model. In: *Physiological and Biochemical Aspects of Fish Development.* (Walther, B.T. and Fyhn, H.J. eds.) p279-284.

Ross, L. G. (1985) Oxygen Electrodes pp. 33-44 In: *Chemical and Biological Methods of Water Analysis for Aquaculturists.* (ed. Stirling, H. P.) Institute of Aquaculture, University of Stirling, Stirling.

Ross, L. G. and McKinney, R. W. (1988a) Respiratory cycles in *Oreochromis niloticus* (L.), measured using a six-channel microcomputer-operated respirometer. *Comp. Biochem. Physiol.* **89A**, (4), 637-643.

Ross, L. G. and McKinney, R. W. (1988b) Photoperiod mediated variation in respiratory rate of *Oreochromis niloticus* and its implication for tilapia culture. p. 421 - 428 In: *The Second International Symposium on Tilapia in Aquaculture* (Pullin, R. S. V., Bhukaswan, T., Tonguthai, K. and MacLean, J. L. eds.) I.C.L.A.R.M. Conference Proceedings 15, pp623.

Ross, L. G., McKinney, R. W. and Ross, B. (1988c) Energy Budgets for Cultured Tilapias. p.83-89. In: *The Second International Symposium on Tilapia in*

Aquaculture (Pullin, R. S. V., Bhukaswan, T., Tonguthai, K. and MacLean, J. L. eds.) I.C.L.A.R.M. Conference Proceedings 15, pp623.

Ross, L. G., McKinney, R. W., Cardwell, S. K., Fullarton, J. G., Roberts, S. E. J. and Ross, B. (1992) The effects of dietary protein content, lipid content and ration level on oxygen consumption and specific dynamic action in *Oreochromis niloticus* L. *Comp. Biochem. Physiol.* **103A**, (3), 573-578.

Rowan, D. J. and Rasmussen, J. B. (1996) Measuring the bioenergetic cost of fish activity in situ using a globally dispersed radiotracer (^{137}Cs). *Can. J. Fish. Aq. Sci.* **53**, 734-745.

Rubner, M. (1902) "Die Gesetze des Energieverbrauchs bei der Ernahrung," Liepzig and Vienna, Franz Dauticke.

Sánchez-Vázquez, F. J. and Tabata, M. (1998) Circadian rhythms of demand-feeding and locomotor activity in rainbow trout. *J. Fish Biol.* **52**, 255-267.

Sánchez-Vázquez, F. J., Madrid, J. A. and Zamora, S. (1995a) Circadian Rhythms of Feeding Activity in Sea Bass, *Dicentrarchus labrax* L.: Dual phasing Capacity of Diel Demand-Feeding Pattern. *J. Biol. Rhythms* **10**, (3), 256-266.

Sánchez-Vázquez, F. J., Zamora, S. and Madrid, J. A. (1995b) Light-dark and food restriction cycles in sea bass: Effect of conflicting zeitgebers on demand-feeding rhythms. *Physiol. & Behav.* **58**, (4), 705-714.

Sánchez-Vázquez, F. J., Madrid, J. A. Zamora, S., Iigo, M. and Tabata, M. (1996) Demand-feeding and locomotor circadian rhythms in the goldfish, *Carassius auratus*: dual and independent phasing. *Physiol. Behav.* **60**, 665-674.

Sánchez-Vázquez, F. J., Madrid, J. A., Zamora, S. and Tabata, M. (1997) Feeding entrainment of locomotor activity rhythms in the goldfish is mediated by a feeding-entrainable circadian oscillator. *J. Comp. Physiol.* A., **181**, 121-132.

Saunders, R. L. (1963) Respiration of the Atlantic cod. *J. Fish. Res. Bd. Can.* **20**, (2), 373-386.

Sayer, M. D. J. and Davenport, J. (1987) The relative importance of fish gills to ammonia and urea excretion in five seawater and one freshwater teleost species. *J. Fish Biol.* **31**, 561-570.

Schram, T. A. and Haug, T. (1988) Ectoparasites on the Atlantic halibut, *Hippoglossus hippoglossus* (L.), from northern Norway - potential pests in halibut aquaculture. *Sarsia* **73**, 213-227.

Schurmann, H. and Steffensen, J. F. (1997) Effects of temperature, hypoxia and activity on the metabolism of juvenile cod. *J. Fish Biol.* **50**, 1166-1180.

Schuur, A. M. (1991) A bioenergetic model for application to intensive fish culture system management. *Am. Fish. Soc. Symp.* **10, 393-401.**

Scott, W.B. and Scott, M.G. (1988) *Atlantic Fishes of Canada.* Univ. of Toronto Press. 731pp.

Shaw, S. A. and Muir, J. F. (1987) *Salmon: Economics and Marketing.* Croom Helm Ltd., Beckenham, Kent. 270p.

Shields, R.J., Davenport, J., Young, C. and Smith, P.L. (1993) Oocyte maturation and ovulation in the Atlantic halibut, *Hippoglossus hippoglossus*, examined using ultrasonography. *Aquacult. Fish. Man.* **24**, 181-186.

Sims, D. W. and Davies, S. J. (1994) Does specific dynamic action (SDA) regulate return of appetite in the lesser spotted dogfish, *Scyliorhinus canicula*? *J. Fish Biol.* **45**, 341-348.

Sims, D. W., Davies, S. J. and Bone, Q. (1994) On the diel rhythms in metabolism and activity of post-hatching lesser spotted dogfish, *Scyliorhinus canicula*. *J. Fish Biol.* **43**, 749-754.

Smart, G. R. (1981) Aspects of water quality producing stress in intensive fish culture. pp. 277-293 In: *Stress and Fish* (ed. Pickering, A. D.). Academic Press, London.

Summerfelt, S. T., Hankins, J. A., Summerfelt, S. R. and Heinen, J. M. (1993) Modeling continuous culture with periodic stocking and selective harvesting to measure the effect on productivity and biomass capacity of fish culture systems. pp. 581-595 In: *Techniques for Modern Aquaculture* Proc. Aquac. Eng. Conference 21-23 June 1993, Spokane, Washington.

Smith, R. R. (1989) Nutritional Energetics pp. 1-29. In: *Fish Nutrition*, 2nd edition, (ed. Halver, J. E.). Academic Press, London, New York.

Smith, R. R., Rumsey, G. L. and Scott, M. L. (1978a) Net energy maintenance requirements of salmonids as measured by direct calorimetry: effect of body size and environmental temperature. *J. Nutr.* **108**, 1017 - 1023.

Smith, R. R., Rumsey, G. L. and Scott, M. L. (1978b) Heat increment associated with dietary protein, fat, carbohydrate and complete diets in salmonids: Comparative energetic efficiency. *J. Nutr.* **108**, 1025-1032.

Solomon, D. J. and Brafield, A. E. (1972) The energetics of feeding, metabolism and growth of perch (*Perca fluviatilis*). *J. Anim. Ecol.* **41**, 699-718.

Soofiani, N. M. and Hawkins, A. D. (1982) Energetic costs at different levels of feeding in juvenile cod, *Gadus morhua*, L. *J. Fish Biol.* **21**, 577-592.

Spoor, W. A. (1946) A quantitative study on the relationship between the activity and oxygen consumption of the goldfish, and its application to the measurement of respiratory metabolism in fishes. *Biol. Bull.* **91**, 312-325.

Staples, D.J. and Nomura, M. (1976) Influence of body size and food ration on the energy budget of rainbow trout *Salmo gairdneri* Richardson. *J. Fish Biol.* **9**, 29-43.

Steffens, W. (1989) Principles of Fish Nutrition. Ellis Horwood Ltd., Chichester, England. pp. 384.

Steffensen, J. F., Bushnell, P. G. and Schurmann, H. (1994) Oxygen consumption in four species of teleosts from Greenland: no evidence of metabolic cold adaptation. *Polar Biology* **14**, 49-54.

Stewart, D. J., Weininger, D., Rottiers, D. V. and Edsall, T. A. (1983) An energetics model for lake trout, *Salvelinus namaycush*: application to Lake Michigan pollution. *Can. J. Fish. Aq. Sci.* **40**, 681-698.

Stickney, R. and Liu, H. (1993) Culture of Atlantic halibut and Pacific halibut. *Rev. Fish. Sci.* **1**, (3), 285-309.

Stirling, H. (1985) *Chemical and Biological Methods of Water Analysis for Aquaculturists*. Institute of Aquaculture, University of Stirling, Stirling. 119pp.

Stobo, W. T., Neilson, J. D. and Simpson, P. G. (1987) Movements of Atlantic halibut (*Hippoglossus hippoglossus*) in the Canadian North Atlantic. *Can. J. Fish. Aquat. Sci.* **45**, 484-491.

Tandler, A. and Beamish, F. W. H. (1979) Mechanical and biochemical component of apparent S.D.A. in largemouth bass, *Micropterus salmoides* Lacépède. *J. Fish Biol.* **14**, 343-350.

Tandler, A. and Beamish, F. W. H. (1980) Specific Dynamic Action and diet in largemouth bass, *Micropterus salmoides* (Lacépède). *J. Nutrition* **110**, 750-764.

Tandler, A. and Beamish, F. W. H. (1981) Apparent S.D.A., fish weight, and level of caloric intake in largemouth bass. *Aquaculture*, **23**, 231-242.

Tátrai, I. (1980) On the standard metabolism of bream (*Abramis brama* L.). *Aquacultura Hungarica* (*Szarvas*), II, 31-36.

Tilseth, S. (1990) New Marine fish species for cold-water farming. *Aquaculture* **85**, 235-245.

Trudel, M. and Boisclair, D. (1996) Estimation of fish activity costs using underwater video cameras. *J. Fish Biol.* **48**, 40-53.

Tuene, S. and Nortvedt, R. (1995) Feed intake, growth and feed conversion efficiency of Atlantic halibut, *Hippoglossus hippoglossus* (L.). *Aquacult. Nutr.* **1**, 27-35.

Tytler, P. (1969) Relationships between oxygen consumption and swimming speed in the haddock, *Melanogrammus aeglefinus*. *Nature, Lond.* **221**, 274.

Umezawa, S-I, Adachi, S. and Taneda, K. (1983) Group effect on oxygen consumption of the ayu (*Plecoglossus altivelis*) in relation to growth stage. *Jap. J. Ichthyol.* **30**, (3), 261-267.

Ursin, E. (1979) Principles of growth in fishes. *Symp. zool. Soc. Lond.* **44**, 63-87.

Vahl, O. and Davenport, J. (1979) Apparent Specific Dynamic Action of food in the fish *Blennius pholis*. *Mar. Ecol. Prog. Ser.* **1**, 109-113.

Verheijen, F.J. and de Groot, S. J. (1967) Diurnal activity pattern of plaice and flounder (*Pleuronectidae*) in aquaria. *Neth. J. Sea Res.* **3**, (3), 383-390.

Voyer, R. A. and Morrison, G. E. (1971) Factors affecting respiration rates of winter flounder (*Pseudopleuronectes americana*). *J. Fish. Res. Bd. Can.* **28**, 1907-1911.

Waller, U. (1992) Factors influencing routine oxygen consumption in turbot, *Scophthalmus maximus*. *J. Appl. Ichthyol.* **8**, 62-71.

Ware, D. M. (1975) Growth, metabolism and optimal swimming speed of a pelagic fish. *J. Fish. Res. Bd. Can.* **32**, 33-41.

Weatherley, A.H. (1976) Factors affecting maximisation of fish growth. *J. Fish. Res. Bd. Can.* **33**, 1046-1058.

Webb, P. W. (1971) The swimming energetics of trout. II. Oxygen consumption and swimming efficiency. *J. Exp. Biol.* **55**, 521-540.

Webb, P. W. (1984) Form and function in fish swimming. *Scient. Am.* **251**, 58-68.

Weihs, D. (1978) Tidal stream transport as an efficient method for migration. *J. Cons. Int. Explor. Mer.* **38**, (1), 92-99.

Weyl, P. K. (1970) Oceanography: An introduction to the marine environment. John Wiley and Sons, New York. 535p.

Wheeler, A. (1969) The fishes of the British Isles and North West Europe. McMillan and Co., London. 613p.

Whitehead, P.J.P., Buuchof, M.-L., Hureau, J.-C., Nielsen, J. and Tortonese, E. (1986) Fishes of the North-eastern Atlantic and the Mediterranean Vol III. Pub. United Nations Educational Scientific and Cultural Organisation. The Chaucer Press, U.K. 458pp.

Wickins, J. F. (1981) Water quality requirements for intensive aquaculture: A review. pp 18-37. In: *Proc. World Symp. on Aquaculture in Heated Effluents and Recirculation Systems.* Stavanger, 28-30 May, 1980. Vol. 1. Berlin, 1981.

Wiggs, A. J., Henderson, E. B., Saunders, R. L. and Kutty, M. N. (1989) Activity, respiration, and excretion of ammonia by Atlantic salmon (*Salmo salar*) smolt and postsmolt. *Can. J. Fish. Aquat. Sci.* **46**, (5), 790-795.

Wilhelmj, C. M., Bollman, J. L. and Mann, F. C. (1928) Studies on the physiology of the liver. XVII. The effect of removal of the liver on the Specific Dynamic Action of Amino Acids administered intravenously. *Am. J. Physiol.* **87**, 497-509.

Williams, D. L., Wall, A. E., Branson, E., Hopcroft, T., Poole, A. and Brancker, W. M. (1995) Preliminary findings of ophthalmological abnormalities in farmed halibut. *Vet. Rec.* **136**, 610-612.

Winberg, G.G. (1956) Rate of metabolism and food requirements of fishes. Beloruss. State Univ. Minsk. [*Fish. Res. Bd. Can., Transl. Ser.* No. 194 (1960)].

Wohlschlag, D. E. (1960) Metabolism of an Antarctic fish and the phenomenon of Cold Adaptation. *Ecology* **41**, (2), 287-292.

Wood, C. M., McMahon, B. R. and McDonald, D. G. (1979) Respiratory gas exchange in the resting starry flounder, *Platichthys stellatus*; a comparison with other teleosts. *J. Exp. Biol.* **78**, 167-180.

Wuhrman, K. and Woker, H. (1948) Experimentelle Untersuchungen über die Ammoniak- und Blausäurevergiftung. *Schweiz. Z. Hydrol.* **11**, 210-244.

Xie, X. J. and Sun, R. (1993) Pattern of energy allocation in the southern catfish (*Silurus meridionalis*). *J. Fish Biol.* **42**, 197-207.

Zar, J. H. (1984) Biostatistical Analysis. Second Edition. Prentice-Hall International Editions, New Jersey, U. S. A. pp. 718.