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Specific Dynamic Action, Growth and Development in Larval Atlantic Cod, Gadus Morhua

Jessica A. Geubtner

SPECIFIC DYNAMIC ACTION, GROWTH AND DEVELOPMENT IN LARVAL ATLANTIC COD, *GADUS MORHUA*

By

Jessica A. Geubtner

B.A. University of Delaware, 1998

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Advisory Committee:

Dr. Ione Hunt von Herbing, Assistant Professor of Marine Sciences, Advisor

Dr. Linda Kling, Associate Professor of Aquaculture and Fish Nutrition

Dr. Bruce Sidell, Professor of Marine Sciences

Dr. James McCleave, Professor of Marine Sciences

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An Abstract of the Thesis Presented
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The larval stage of marine fish is a period of rapid growth and development. Atlantic cod (*Gadus morhua*) are approximately 4-5 mm in length upon hatch and feed endogenously from their yolk sac for the first week. After this time, larval cod must successfully capture live prey to survive and to fuel high growth rates of greater than $10\%d^{-1}$. Previous studies have found that during exogenous feeding and at growth rates greater than $8\%d^{-1}$ larvae experience what appears to be cost free growth, where mass specific metabolic rate does not decrease with increasing mass. Due to size and condition constraints involved in working with larval marine fish, few data exist on the metabolism of early larval growth. This study was done using microcalorimetry to identify specific dynamic action (SDA) which represents the cost of feeding, digestion, and protein assimilation (growth) in larval cod. Cod were used in the experiments only after the yolk sac was completely utilized, and the larvae were feeding exogenously. This study was the first to record changes in metabolic rates as a function of feeding in larval fish using a

microcalorimeter. Growth rates for two populations (Rhode Island and Newfoundland) of Atlantic cod were measured and ranged from 6.9%d^{-1} to 13.1%d^{-1}, showing that larvae grew well under culture conditions. After comparing mean growth rates between the two populations and finding them to be significantly different, only Newfoundland cod were used in the experiments to measure SDA.

Using a Thermometric© LKB 2277 microcalorimeter (TAM) the total heat output of larval cod from 10 – 40 days post hatch was determined under two different feeding conditions. Two cod of the same age (days post hatch) were placed concurrently into the microcalorimeter in one of two channels. One cod larva was left unfed for at least 12 hours prior to the experiment (unfed) while the other was fed to satiation immediately before the experiment was run (fed).

TAM measurements showed that for the first 30 days post hatch (dph), both fed and unfed larval cod showed increases in mean heat output on a daily basis. During this time fed larvae had a significantly higher average heat output than unfed larvae. From day 30 through 40 post hatch, fed larvae were observed to have a lower mean daily heat output when compared to unfed larvae. This change in mean daily heat output may be attributed to factors involved in the transformation stage of the larvae to the juvenile stage such as more efficient digestion and increased swimming and searching activity.

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Chapter 1

LITERATURE REVIEW

The larval stage of marine fish is a period of rapid growth and development. Larval fish have extremely high growth rates that may exceed 30%d^{-1} in temperate fishes (Wieser, 1995). During the larval stage, fish also experience extremely high mortality rates, reaching up to 30-50%d^{-1} (Bailey and Houde, 1989). However, once larval fish transform to the juvenile stage, mortality rates decline. High rates of growth during the first few weeks of a fish's life may help a larva to reach the adult stage by minimizing the time spent in the vulnerable larval period. While metabolic rate is often measured in fish by recording their oxygen uptake at increasing swimming speeds, larval cod cannot swim against a current. Because of this constraint, few data exist on the metabolism of early larval cod growth.

Many of the physiological processes that underlie high growth rates are not understood in larval fish. For example, during exogenous feeding and at growth rates greater than 8%d^{-1}, larvae experience what appears to be cost free growth (Rombough, 1994; Wieser, 1995). Apparent cost free growth means that as mass increases, there is no decrease in mass specific metabolic rate or metabolic rates where maintenance does not increase with increasing growth rates. To understand the physiology governing such high rates of growth, more metabolic data must be collected. To do this, a precise method of data collection must be used. Historically, respirometry, an indirect measure of metabolism using oxygen uptake readings taken by electrodes, has been used to determine metabolic rates of larval fishes. However, respirometry may not show a clear

metabolic picture due to the sensitive nature of larval work. Over the past 20 years, microcalorimetry research has been successful in obtaining metabolic data. Microcalorimetry detects heat output at very high resolution (± 0.15 μW). Due to its precise nature, microcalorimetry is an excellent tool for examining the metabolism of small larval organisms, without requiring them to swim against a current.

Growth rates per unit mass decrease as most animals increase in size until leveling off when growth stops. However, larval fish may not fit into this basic metabolic curve. For example, the relationship between metabolic rate (R) and body mass (M) is; $\mathbf{R=aM^b}$, where a is the metabolic intensity and b is the mass exponent. For adult fish, b scales close to 0.8. However, for larval fish the mass exponent is highly variable and reaches a value of 1.0 in some species (Wieser, 1995; Pedersen, 1997). For example, oxygen uptake studies done by Torres et al. (1996) showed that metabolic rate scales isometrically with mass in larval red drum (*Sciaenops ocellatus*).

It has been hypothesized that to support high growth rates larval fish may not allocate energy in the same manner as their adult conspecifics. For example, juvenile and adult fish allocate energy in an additive fashion. This means that available energy is partitioned into three main areas: energy used to support life, energy used for new tissue production and energy lost from the system (Rombough, 1994). However, the same study found that in chinook salmon (*Oncorhynchus tshawytscha*) larvae, energy was not allocated in an additive way. Instead, energy may be used in a compensatory manner, where energy is suppressed from going to supply certain functions and is reallocated to other functions, such as growth (Wieser, 1989; 1991). Rombough (1994) also suggests that the mass specific cost of growth (COG) in larvae may vary. COG is defined as the

processes, which in larval fish may be considered negligible due to their small amounts of anaerobic enzymes (El-Fiky et al., 1987). Standard metabolic rate is estimated to be the resting and fasting metabolism for a fish. Generally this level is determined by back extrapolation using higher metabolic rates found as a fish swims at controlled swimming speeds. Routine rates are defined as the metabolism of a fish during daily activities, such

energy expended on new tissue production. Therefore, changes in the COG of larval fish may mean that they are able to direct more energy toward growth by taking energy away from other functions such as maintenance. Higher growth efficiencies were seen with low COG in larval herring (*Clupea harengus*) (Kiorbøe et al., 1987), and a study by Conceicao et al. (1998) found that high growth rates in larval fish seem to be associated with a low COG and high food conversion efficiencies. However, COG in larval fish remains largely unexplained.

The basic principle of bioenergetics states that all energy gained through ingestion of food is ultimately lost as waste, used in metabolic processes or deposited as new body tissue. This principle can be stated as the equation:

$$\mathbf{R = F + U + M + P}$$

Where R is the total energy ingested, F is the energy lost as feces, U is the energy lost in excretory products, M is the energy lost in metabolism and P is the energy used for production (growth). Metabolism (M) can be broken down further into aerobic and anaerobic components. Aerobic metabolism can be described by the equation:

$$\mathbf{R = R_s + R_a + R_f}$$

where R is the total aerobic metabolic energy, R_s is the energy cost required for body maintenance, R_a is the energy cost associated with activity and R_f is the energy cost related to the digestion, absorption and processing of food.

Different metabolic levels may dictate how much energy goes toward these energetic functions. The total energy available to a larval fish is defined as the difference between active and standard metabolic rates. This difference is termed the aerobic scope for activity. Above the maximum level of the scope, activity is fueled by anaerobic

Studies have shown proportionality between rates of growth and metabolic rates in adult fish (Hogendoorn, 1983; Jobling, 1985). However, this situation may not hold true for larval fish with smaller aerobic scopes and high growth rates. For example, cyprinid larvae show less than a three-fold difference between active and standard metabolism but may have growth rates as high as $50\%d^{-1}$(Wieser and Forstner, 1986). In this case, Wieser and Medgyesy (1990) suggest that sequential rather than additive energy allocation may be occurring. In larval fish with very small aerobic scopes, it is important to learn how they are able to allocate energy to support their high growth rates.

Of particular interest in understanding the physiology of rapid larval growth is studying specific dynamic action (SDA). SDA is the post-prandial increase in metabolic rate due to the digestion, absorption and assimilation of food. SDA is also termed calorigenic or thermic effect of feeding, heat increment or dietary induced thermogenesis. While SDA has been known of and studied for more than 100 years, many of its causal mechanisms are poorly understood. SDA is generally quantified using indirect calorimetry by measuring oxygen uptake in adult fish (Tandler and Beamish, 1981). An oxygen uptake peak approximately two to three times the pre-feeding level occurs within several hours, then decreases until again reaching the pre-feeding level.

SDA has three components: duration, amplitude and magnitude. Duration is the time needed for metabolism to reach prefeeding levels. Duration is affected by meal size and composition and environmental temperature. These factors each may influence the rate of food passage through the gut. Amplitude is the difference between oxygen uptake at the peak of SDA and standard metabolism. Amplitude may be affected by ration size and quality, however prefeeding oxygen uptake levels usually return within 12 hours

after ingestion. Finally, magnitude is the total area under the curve of metabolic rate vs. time and represents the total energy used for SDA. Magnitude is also affected by meal size, generally increasing with increasing meal size (Jobling, 1981). Jobling and Davies (1980) found two trends in adult fish SDA. First, magnitude increases with increasing levels of digestible energy in the diet. Second, magnitude increases with increasing proportion of digestible protein in the diet.

SDA results from the post-feeding energy required for digestion, absorption and storage of nutrients, for amino acid deamination and synthesis of excretory products, and for increased turnover and deposition of tissue components (Jobling, 1981). Studies have shown that the mechanical contribution to SDA is very small. Most of SDA is a post-absorptive phenomenon (McMillian and Houlihan, 1988; Waterlow and Millward, 1989; Brown and Cameron, 1991; Houlihan, 1991). Brown and Cameron (1991) verified the post-absorptive component of SDA by injecting amino acids intravenously into adult fish. This resulted in a significant increase in metabolic rate. Wieser and Medgyesy (1990) found a post prandial increase in oxygen uptake in larval cyprinids which largely reflected the metabolic cost of growth. However, most studies done on SDA have been on adult fish, where high post feeding oxygen demand in adult fish may reduce the available scope for activity of the fish. Scope for activity was reduced by 30-40% in adult aholehole (*Kuhlia sandvicensis*) (Muir and Niimi, 1972) and was reduced by 50% in adult largemouth bass (*Micropterus salmoides*) after feeding (Beamish, 1970; 1974). More work must be done with larval fish to determine if SDA occurs.

Determinations of aerobic metabolic rate in larval, juvenile and adult fish are generally made using respirometry. These oxygen consumption values convert easily

into energy units and are useful in comparing caloric intake to energy expended. While there are several types of respirometers used to record oxygen uptake data, there are also problems inherent with each design. Adult fish are able to swim for sustained periods of time against a current and also feed well within the respirometer. However, most larval fish do not swim well against a current, or can only hold their position in the water column for a short period of time before they exhaust their energy stores. Larval fish are also poor predators, and may not feed consistently within the respirometer. With these constraints in mind, several different respirometers have been designed to study larval fish over the past 20 years.

Several larval cod experiments were done using flasks or vials with oxygen probes inserted and then sealed. Experiments have used anesthetized and non-anesthetized larval cod to separate the contribution of activity to the oxygen readings (Solberg and Tilseth, 1984; Davenport and Lonning, 1980). Significant differences were seen between the two treatment groups, however anesthetized larval fish may not show true routine metabolism due to possible anesthesia effects on metabolism. In research done by Kiorbøe et al. (1987) larval herring were fed then anesthetized in an attempt to determine SDA. Larvae were incubated in small chambers (initially oxygen saturated) from which water samples were drawn to determine oxygen uptake by the larvae. Anesthesia controls activity in larval fish. However, even when fish readily recover once placed in fresh seawater, there may be some level of stress-induced error added to the experiment.

Most research has been done using large, fast growing larval fish. Wieser and Medgyesy (1990) studied larval roach using an intermittent respirometer. During the

experiment, the respirometer remained closed, and oxygen measurements were taken using polarigraphic oxygen sensors. After this period, the system was flushed with fresh oxygenated water and prepared for the next experiment. Larval roach fed well in this system. However, larval roach weighing 15 to 700 mg fresh weight were used. In comparison, larval cod may only reach 8-10 mg fresh weight just before transformation to juveniles.

Finally, Hunt von Herbing and Boutilier (1996) measured larval cod respiration rates using pulsed oxygen electrodes and developed a relationship between metabolism and activity in larval cod from 0-40 dph. Two to four larvae were placed in each chamber at one time (numbers decreased with larval growth), and all activity was recorded and analyzed using a Sony HI-8 video camera with a resolution of 60 frames/second. Oxygen consumption significantly increased with activity in larvae greater than 14 dph. However, larval cod were often placed together in the respirometry chambers and group effects may have confounded the data. Also, the study focused on activity – metabolism relationships, not the difference in metabolism due to feeding or SDA while larvae used in the experiments were unfed.

Respirometers, especially flow through systems equipped with sensitive oxygen electrodes, are an effective method to record metabolic data on some fishes. However, these designs work best with larval fish that are able to swim against a current for a sustained period of time which regulates activity levels. Larvae must also be able to feed effectively in the respirometry system. Respirometry data show interesting phenomena occurring during the larval stage and encourage more research in this area. Respirometry may not be the most effective means by which to measure metabolic rates

in small fish larvae due to the ideal conditions needed throughout the experimental period and small size of cod throughout the larval stage.

In contrast to the indirect method of respirometry, microcalorimetry is a direct method of measuring metabolism, and includes both aerobic and anaerobic components. Studies have shown that larval fish have few anaerobic enzymes and may not use white muscle anaerobically during the larval stage (Wieser, 1995). Microcalorimetry is useful in detecting smaller changes in metabolic rate than respirometry. Changes in heat output as small as 0.15 microwatts (μW) are detectable using a microcalorimeter. Extensive work has been done over the past two decades on bivalves and sessile intertidal organisms using microcalorimetry in connection with respirometry. This combination of methods was used to separate aerobic and anaerobic components of metabolism (Shick et al., 1983; Widdows and Shick, 1985; Shick et al., 1986; Shick et al., 1988). This same method was used in a study by Widdows and Shick (1985), in which the heat increment (SDA) associated with feeding and digestion was determined in the mussels *Mytilus edulis* and *Cardium edule*. In addition, Bookbinder and Shick (1986) used this method to detect metabolic components of sea urchin ovaries. Other successful studies using microcalorimetry have investigated changes of energy flow in *Artemia franciscana* during both aerobic and anoxic phases (Hand and Gnaiger, 1988; Hand, 1990). Microcalorimetry was used together with respirometry and high performance liquid chromatography (HPLC) analysis of metabolic end products to examine the components of metabolism. Doll et al. (1994) used this method to examine the energy profile and metabolism of turtle (*Chrysemys picta*) cortical slices by converting heat data into ATP

utilization rate. These rate changes were then monitored during periods of normoxia and anoxia, successfully showing turtle cortical sensitivity to environmental changes.

Only two studies have used microcalorimetry to record fish heat output. Paynter et al. (1991) used an LKB 2277 Thermal Activity Monitor to detect differences in metabolic rates between different Ldh-B genotypes of developing killifish (*Fundulus heteroclitus*) embryos. Within 10 to 15 hours post fertilization, microcalorimetric measurements detected significant metabolic differences between the two genotypes. Finally, Finn et al. (1995) used microcalorimetry (LKB 2277 Bioactivity Monitor) in connection with respirometry to determine aerobic and anaerobic metabolic components in embryonic and larval turbot (*Scophthalmus maximus*). Finn et al. (1995) suggested that at least from days 0 to 12 post fertilization, metabolism is completely aerobic. However, no larval feeding studies have employed microcalorimetry to determine metabolic rate differences between fed and unfed larval fish in order to identify and characterize SDA.

The physiology supporting high larval fish growth rates remains largely unknown due to difficulties in handling larvae that are small and fragile. New techniques must be used to understand the processes driving high larval growth rates fully. High growth rates and SDA have been found in some larval fish species using respirometry. However, larval cod are not good candidates for respirometry, since they are unable to swim against a current. This study uses microcalorimetry to investigate metabolic differences between fed and unfed larval cod. These metabolic differences were examined as SDA.

Chapter 2

DETERMINATION OF SPECIFIC DYNAMIC ACTION

2.1. Introduction and Objectives

Atlantic cod (*Gadus morhua*) is one of many over fished species in the world. Growing attention has been given to population dynamics in the wild. As an alternative to fishing wild stocks, farming of Atlantic cod would remove fishing pressures in the wild. Balanced energy budgets and bioenergetic models of cod metabolism contribute to future successes in aquaculture (Jobling, 1993). Larval metabolic research contributes to better understanding of high larval growth rates and larval mortality factors.

The first objective of these experiments was to measure growth rates of two different Atlantic cod stocks from Rhode Island and Newfoundland. The second and primary objective was to record daily heat output from the same larval cod. Each day of the experiment two larvae were placed into a microcalorimeter. One larva was fed immediately before microcalorimetry readings were taken (fed). The other larva was starved for at least 12 hours before heat readings were taken (unfed). Heat output readings were analyzed to determine if larval cod with food in their digestive system gave off a different amount of heat compared to larvae with empty digestive systems. This difference would be the first record of specific dynamic action (SDA) in larval fish using microcalorimetry.

2.2. Materials and Methods

2.2.1. Cod Egg Acquisition and Incubation

Eggs used in the experiments came from two separate stocks due to availability and season. Fertilized Atlantic cod eggs were obtained from the National Marine Fisheries Service laboratory in Narrangansett, R.I. in March 2000 (RI stock). For transport, eggs were placed into plastic bags with oxygenated seawater and kept in a cooler with ice packs to maintain a temperature of 6-7°C. Eggs were transported by car to the Aquaculture Research Center (ARC) at the University of Maine in Orono, ME. Eggs were then disinfected with 25% gluteraldehyde diluted to 200 ppm in seawater for 10 minutes before placement in 135 L incubation tanks. The eggs were incubated in artificial seawater (32 ppt) at 7°C in complete darkness until immediately prior to hatch. Just before hatch, eggs were counted volumetrically and moved into rearing tanks. Experiments ran from March through April 2000.

Fertilized cod eggs were also obtained from Newfoundland Aqua-Ventures in Newfoundland, Canada in June 2000 (NF stock). Eggs were shipped in plastic bags in oxygenated seawater at approximately 8°C. Eggs were flown to Halifax, Nova Scotia, Canada, from where they were transported by car to the ARC at the University of Maine in Orono, ME. Upon arrival, eggs were disinfected with PVP© Iodine in seawater (50 ppm) for 10 minutes. The eggs were counted volumetrically and placed directly into rearing tanks after disinfection because they were close to hatching upon arrival. Eggs were incubated in artificial seawater in 22 L blue tanks. Initial stocking density was 75 larvae L^{-1}. Water temperature was maintained at 9°C and salinity at 32 ppt. Eggs were

incubated under 24 hour light conditions at 300 lux. Light intensity was slowly raised to 1000 lux by hatch. After hatching, larvae remained in the same tanks for rearing.

During all incubations eggs were kept under constant aeration to ensure egg movement and uniform distribution throughout the tanks. Temperature and salinity (YSI 85 Probe), pH (Hach EC30 pH meter), ammonia and nitrite (Hach Permachem reagents) levels were monitored daily. Dead eggs were regularly siphoned off the bottom of the tanks to help maintain water quality. Experiments were run from June through August 2000. The two separate egg stocks were used to conduct intra- and inter-population comparisons.

2.2.2. Culture of Live Food

All live food was cultured at the ARC, University of Maine. Saltwater rotifer (*Brachionus plicatilis*) culture was maintained year-round. Rotifers were enriched daily with DHA Selco, Algamac 2000 and algae enrichment. Rotifers were strained through a 45 μm mesh screen every morning into buckets of artificial seawater.

Artemia were also raised at the ARC at a concentration of approximately 150 *Artemia* ml^{-1}. The culture was enriched daily with DHA Selco and Algamac 2000. *Artemia* were strained through a 150 μm mesh screen every morning into buckets of artificial seawater.

2.2.3. Culturing of Rhode Island (RI) Larvae

One batch of larval cod from RI were divided (Group I and II) and placed into two separate rearing systems upon arrival at ARC, University of Maine. Group I larvae

were reared in 50 L black tanks placed in an artificial recirculating seawater system under simulated daylight (16l:8d). Lights were turned on at 0600 at a level of 1 lux and were adjusted up every hour until 1400 when the lights reached a maximum level of 120 lux. Lights were then gradually lowered every hour until 2200 when lights were turned off. System temperature was kept at 8°C and salinity was maintained at 32 ppt. Constant aeration was provided. Temperature and salinity, pH, ammonia and nitrite levels were taken daily to ensure water quality. Tanks were siphoned regularly to remove dead larvae and excess food.

Starting at 1 dph, larvae were fed 4000 rotifers per feeding. Feedings were done daily at 0830, 1230, 1530, 1830 and 2230. At 20 dph larvae were weaned onto microparticulate diet. After this point, larvae were no longer used in experiments.

Group II larvae were reared in 121 L green plastic tanks in an artificial recirculating seawater system. Larvae were reared at 10°C and 32 ppt salinity under 17l:7d photoperiod at 80 lux. Constant aeration was provided. Temperature, salinity, pH, ammonia and nitrite tests were done daily. Tanks were siphoned regularly to remove dead larvae and excess food.

One-20 dph larvae were fed 200,000 rotifers per feeding. By 24 dph larvae had been weaned onto *Artemia* and were fed 100,000 *Artemia* per feeding. Larvae were fed at this level through 40 dph. Feedings were given at 0700, 1000, 1300, 1600, 1900 and 2200 daily.

2.2.4. Culture of Newfoundland (NF) Larvae

Larval cod from Newfoundland (NF) were reared from one through 40 dph in an artificial recirculating seawater system. Cod larvae were kept in 22 L blue plastic tanks (small) and 121 L green plastic tanks (large) within the system. Larvae were reared under constant illumination of 1000 lux. Temperature was kept at 10°C and salinity was maintained at 32 ppt. Temperature, salinity, pH, ammonia and nitrite levels were monitored daily. Tanks were siphoned regularly to remove any dead larvae and excess food.

Larvae (1-20 dph) in small tanks were fed 200,000 rotifers per feeding. At 21 dph, these larvae were weaned onto microparticulate diet and were not used in any further experiments. Larvae (1-20 dph) in large tanks were fed 800,000 rotifers per feeding. On days 21 through 24 larvae in large tanks were fed a mixture of rotifers and *Artemia*. Larvae (24-40 dph) in large tanks were fed 400,000 *Artemia* per feeding and no rotifers. Feedings were done daily at 0700, 1000, 1300, 1600, 1900 and 2200 for the duration of rearing.

2.2.5. Procedure for Larval Sampling

All larvae were sampled using the same method. Larvae that had not been fed for at least 8 hours were sampled prior to first feeding of the day (unfed larvae). Approximately 150 ml of artificial seawater from the rearing system containing the larvae were collected from the inflow pipe into a clean glass beaker wrapped in black plastic. Using a clean plastic pipette with the tapered end cut off, individual larvae were gently pipetted from the rearing tank into the glass beaker. Once two to four larvae had been

placed into the beaker, they were carried by hand to a walk-in cold room kept at 10 ± 0.5 °C. Transport time was always less than one minute. The beaker containing the larvae was placed into a water bath, maintained at 10 ± 0.5°C for one hour. This allowed the water in the beaker to slowly equilibrate to the temperature of the cold room, at which all experiments were run.

After the first feeding of the day, larvae up to 14 dph were given an hour to feed in the rearing tanks. Previous feeding studies done in the lab showed one hour to be long enough to ensure gut fullness. After approximately 14 dph, larvae were large enough to distinguish gut fullness simply by looking into the tank. After feeding for one hour, two to four larvae were sampled (fed larvae) in the same manner as unfed larvae. The fed larvae were placed in a separate glass beaker in the cold-water bath in the cold room to allow the water temperature to equilibrate.

Most of the experimental larvae had been reared at low light levels. Lights in the cold room were kept at approximately 50 lux to reduce stress in the fish. The lowered light levels in the cold room also served as a step toward preparing the larvae for complete darkness. All experiments were conducted in closed ampoules and therefore under total darkness.

2.2.6. Image Capturing of Larvae

All larvae were anesthetized using tricane methanesulfonate (MS-222) prior to image capturing. Anesthetized larvae were placed on a wet slide. All images were captured using an Olympus SZH10 research stereomicroscope fitted with a Hitachi HV-C20 camera. The camera was connected to a Dell Dimension XPS computer installed with Optimas 6.1 imagine software (Optimas, Inc.) Pictures were captured using

Optimas. All pictures clearly showed larval developmental stage, gut contents and total length.

2.2.7. Larval Dry Weight Preparation and Measurements

Larvae were rinsed thoroughly with distilled water to remove any salt water and anesthetic and were placed individually into 2.5 ml snap top plastic tubes in approximately 0.5 ml distilled water. Five larvae of the same age and treatment (representative sample) were also rinsed and placed together into a tube. The labeled tubes were placed into a freezer to preserve larvae until dry weights were taken.

To prepare for dry weight measurements, frozen larvae were removed from the tubes and placed onto individual pre-weighed squares of clean foil. Foils were cut and handled only by clean scissors and clean forceps. Foils holding larvae were placed into separate sections of a clean, labeled ice cube tray. The trays were left in an 80°C oven for 24 hours. Drying time was determined through previous experiments, which showed that larval dry weights did not changed after 24 hours at 80°C. Trays with larvae were removed from the oven and dry weights to the nearest ± 2 μg were taken using a Mettler Toledo AT 20 analytical balance. All dry weights were directly recorded from the balance into Quattro Pro ©.

2.2.8. Microcalorimeter Design, Operation and Calibration

The LKB Thermal Activity Monitor© (TAM) was used in all heat output experiments. The TAM was designed to monitor heat output accurately to ± 0.15

microwatts (μW). Full details of the mechanical design can be found in literature from Thermometric AB, Spjutvagen 5 A, S-175 61 Jarfalla, Sweden.

The TAM is designed to measure differences in heat output between a sample and reference chamber (Figure 2.1). Each signal results from one cylinder in which there are two chambers, each built to house one 5 ml stainless steel ampoule. A recirculating water bath surrounds the cylinders in the TAM, keeping the temperature stable at 10 ± 0.2°C. The dual chamber design allows for an experimental sample to be placed in one chamber (larva in seawater) while the second chamber houses only the reference material (seawater). The final signal shows only the precise heat output difference in the sample chamber minus the reference chamber for one channel (two chambers housed in one cylinder plus signal amplifiers). The TAM is designed to record data as heat flows from the ampoule into the system moving towards thermodynamic equilibrium. As heat energy moves across Peltier elements in series made of semiconductor material, the heat energy is converted into a voltage signal. This voltage signal is proportional to the heat flow measured in μW. By using a built in computer interface, the final signal is transmitted and recorded on an external computer. These experiments used a Dell Latitude LM running Digitam for Windows 4.0 software to record all heat measurements.

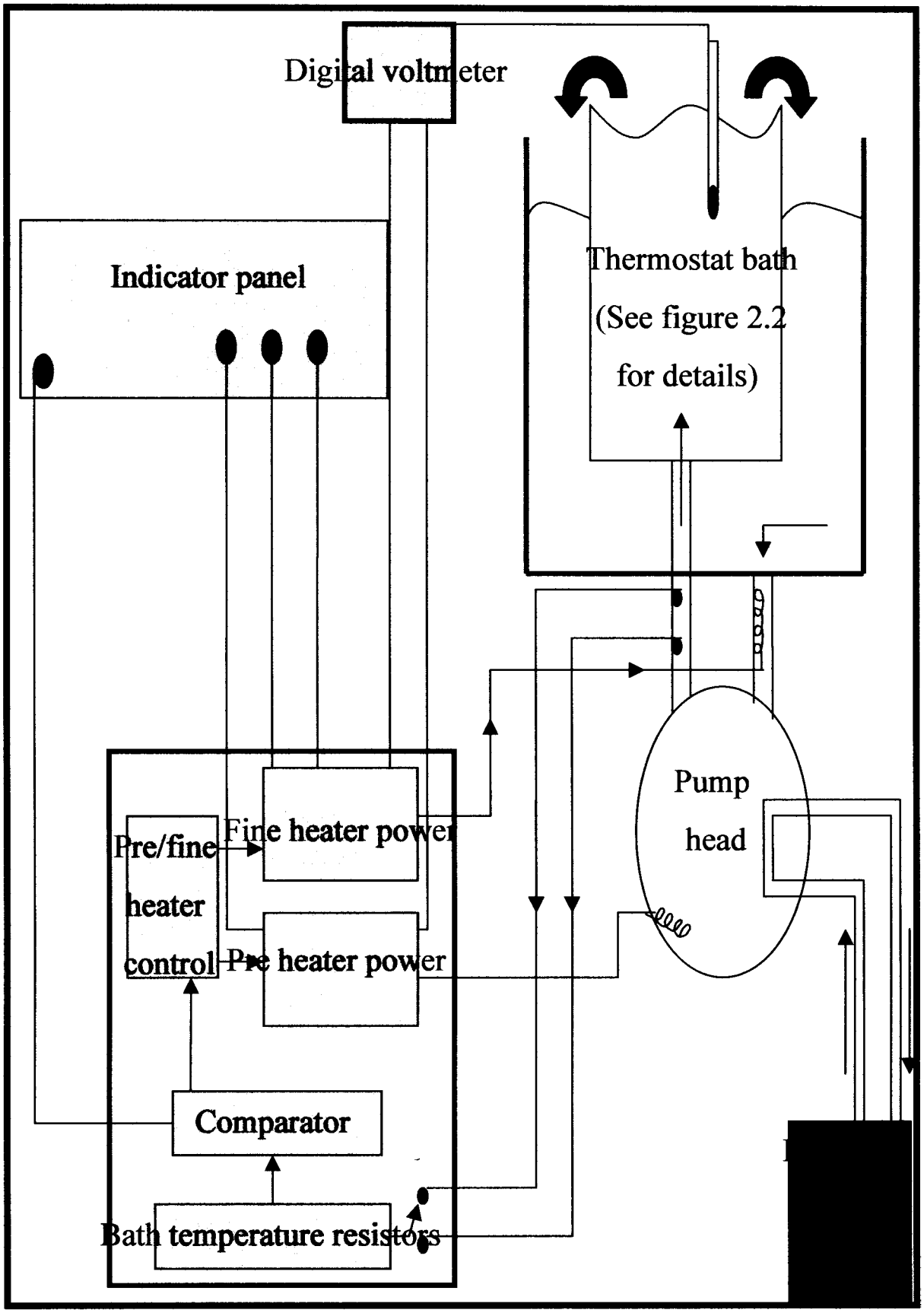
During the experiments, routine calibrations were done approximately once a week on the TAM to ensure that the baseline was not drifting from zero. Baseline data between calibrations always remained at 0 ± 0.2 μW. Calibration techniques are described in literature from Thermometric AB.

The TAM was maintained in a walk-in cold room at 10 ± 1.0°C for the

between calibrations always remained at 0 ± 0.2 μW. Calibration techniques are

Figure 2.1. Water Thermostat Control System

The figure shows how several interactive control systems maintain a constant water temperature within the water bath.


Digital voltmeter
Indicator panel
Thermostat bath
(See figure 2.2
for details)
Pump
head
Fine heater power
Pre/fine
heater
control
Pre heater power
Comparator
Bath temperature resistors

described in literature from Thermometric AB.

The TAM was maintained in a walk-in cold room at 10 ● 1.0°C for the duration of the experiments. A cold-water bath circulated water chilled to 7°C through the TAM to ensure constant temperature conditions.

2.2.9. Set-up for TAM Larval Experiments

After the one hour equilibration period in the cold room water bath, larvae were moved into 5 ml stainless steel ampoules. Ampoules and their screw-top lids were cleaned with soap, water and alcohol after each experimental run. Reusable Teflon® seals were placed into each lid, and were cleaned with soap, water and alcohol after each use. Once ampoules, lids and seals were cleaned, they were only handled by alcohol wipes to minimize external contamination. Any residue on the outside of the ampoules may have resulted in a distorted heat output reading.

Two sample larvae, one fed and one unfed, were placed into the TAM at the same time. This necessitated the use of four ampoules (one sample and one reference), of which two ampoules went into one cylinder. The two sample ampoules received 4 ml of the seawater removed earlier from the larval rearing tank which was now temperature equilibrated to 10 ● 1.0°C. The fed larva was then gently pipetted into one sample ampoule and the unfed larva was pipetted into the other sample ampoule. Lids and seals were then screwed tightly onto the ampoule. The two reference ampoules each received 4 ml of seawater taken earlier from the larval rearing tank. Lids and seals were then screwed onto reference ampoules. Each day, ampoules were randomly assigned to each chamber to reduce sampling bias.

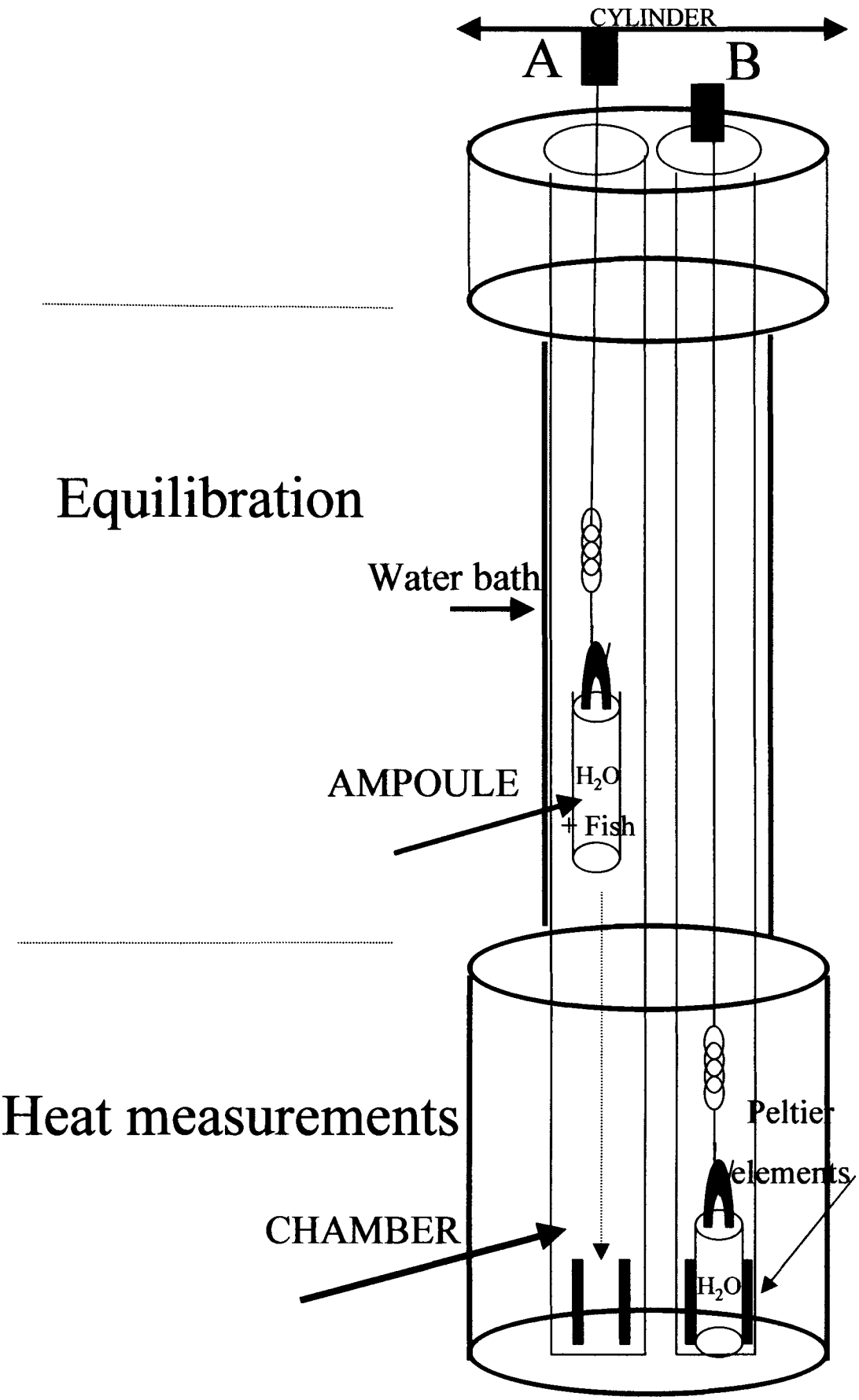
Ampoules containing samples of larvae and water were placed into the TAM (Figure 2.2). Ampoules were placed onto the hooked end of the ampoule lifters and slowly lowered over 40 seconds into the TAM until they reached the half way equilibration point in the chamber. Slow and controlled lowering of ampoules into the TAM is necessary to minimize generation of heat from friction between the ampoule and the chamber walls. Magnets attached to the lifter held the ampoule in place allowing a final temperature equilibration to occur. All ampoules were attached to separate lifters and lowered in this manner to the equilibration point. After a minimum of 15 minutes or until the TAM baseline reached zero (maximum of 30 minutes), each ampoule was slowly lowered one at a time over 40 seconds the rest of the way into the TAM for recording of experimental heat output. Larvae were left in the TAM for approximately 12 hours. Data were recorded every 10 seconds for the duration of the experiment. Experiments were run consecutively every day for 40 days.

2.2.10. Statistical Analysis

Statistics were run to make both intra- and inter-population comparisons. Analyses were used to determine whether RI and NF larvae were distinct populations. Statistics were also run to look at mass effects on heat output, as well as the effect of feeding on heat output.

Figure 2.2. Combination Measuring Cylinder

The figure demonstrates the experimental set-up for measuring changes in larval fish heat output.


CYLINDER
A
B
Equilibration
Water bath
AMPOULE
H_2O
+ Fish
Heat measurements
Peltier
elements
CHAMBER
H_2O

Dry weight data were analyzed using Analysis of Covariance (ANCOVA) (Steel et al. 1997). Independent variables were either the unfed or fed treatment. Covariables were dry weight of the larvae and larval heat output. First, the natural logs of the dry weights of individual larvae were plotted against dph and regressed. Homogeneity of slopes test was performed to determine if the slopes were significantly different ($p < 0.05$). If homogeneity of slopes was found, a t test was done to determine if the regression lines were significantly different. Specific growth rates (SGR) were calculated for each population using the equation; $G = [(\ln W_2 - \ln W_1)/(t_2 - t_1)] \times 100$. Regressions were performed using Sigma Plot™ software (version 2000). Homogeneity of slope tests were performed using Systat™ software (version 9).

2.3. Results

2.3.1. Rhode Island (RI) Intra-population Comparisons

Figure 2.3 shows the dry weights of post yolk sac RI larvae reared at 10°C under simulated daylight conditions. Both fed and unfed groups of larvae grew exponentially over the experimental period. Significant differences were found between dry weights of fed and unfed larvae (ANCOVA, $F = 8.94$, $p < 0.05$). After plotting the natural log of the dry weight data against days post hatch (dph) regressions were done (Figure 2.4). Specific growth rates (SGR) were calculated for both fed and unfed groups of larvae. Larvae fed immediately before experiments were run had an SGR of 8.5% d^{-1}, and larvae that were unfed for at least 8 hours before experiments were run had an SGR of 13.1% d^{-1}.

2.3.2. Newfoundland (NF) Intra-population Comparisons

Figure 2.5 shows the dry weights of post yolk sac larvae reared at 10°C under constant light of 1000 lux. Both fed and unfed groups of larvae grew exponentially over the experimental period. Significant differences were found between the fed and unfed groups (ANCOVA, $F = 6.38$, $p < 0.05$). After plotting the natural log of the dry weight data against dph linear regressions were done (Figure 2.6). SGR was calculated for both fed and unfed groups of larvae. Larvae fed immediately before experiments were run had an SGR of 8.6% d^{-1} and larvae that were unfed for at least 8 hours before experiments were run had an SGR of 6.9% d^{-1}. The difference in SGR between fed and unfed larvae most likely reflects dry weight added by food in the guts of fed larvae.

2.3.3. Inter-population Comparisons

Significant differences (ANCOVA, $F = 11.87$, $p < 0.05$) were found between dry weights of Rhode Island (RI) and Newfoundland (NF) larval cod throughout ontogeny. Only unfed larval data were used to eliminate the variation in dry weight resulting from a larva having food in its gut. The RI population spawns from October through April in water with temperatures between 8 – 10°C. The NF population spawns from April to June in water with temperatures between 0 – 5°C (Lear and Green, 1984). Along with the difference in water temperature, light levels differ at spawning for the two populations (Puvanendran and Brown, 1998). These two populations are also considered genetically distinct (Pogson et al., 1993). Based on the difference in weights, RI and NF data sets were not combined for any further analysis of heat output.

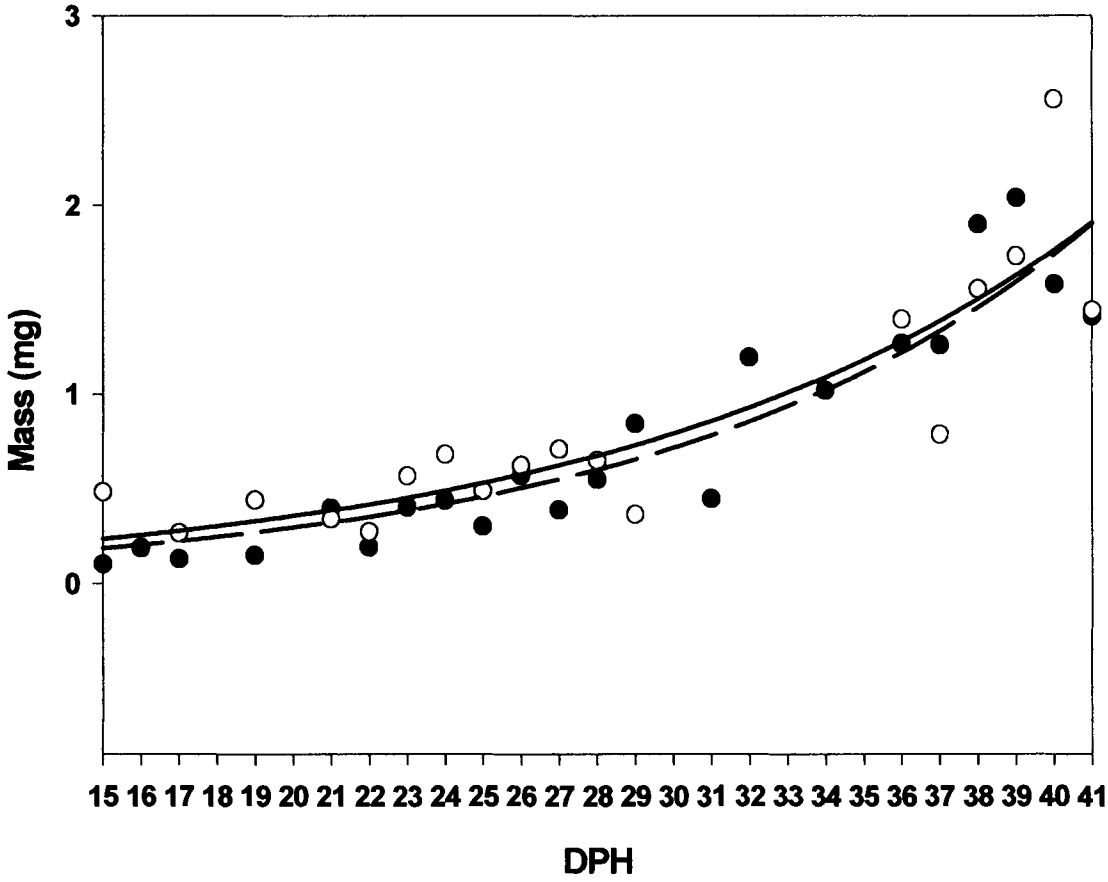


Figure 2.3. RI Unfed and Fed Larval Dry Weights vs. DPH. ● = unfed larvae, ○ = fed larvae. — unfed data regression, --- fed data regression. Unfed: $y = 1.14x^{0.139}$. Fed: $y = 1.089x^{0.28}$.

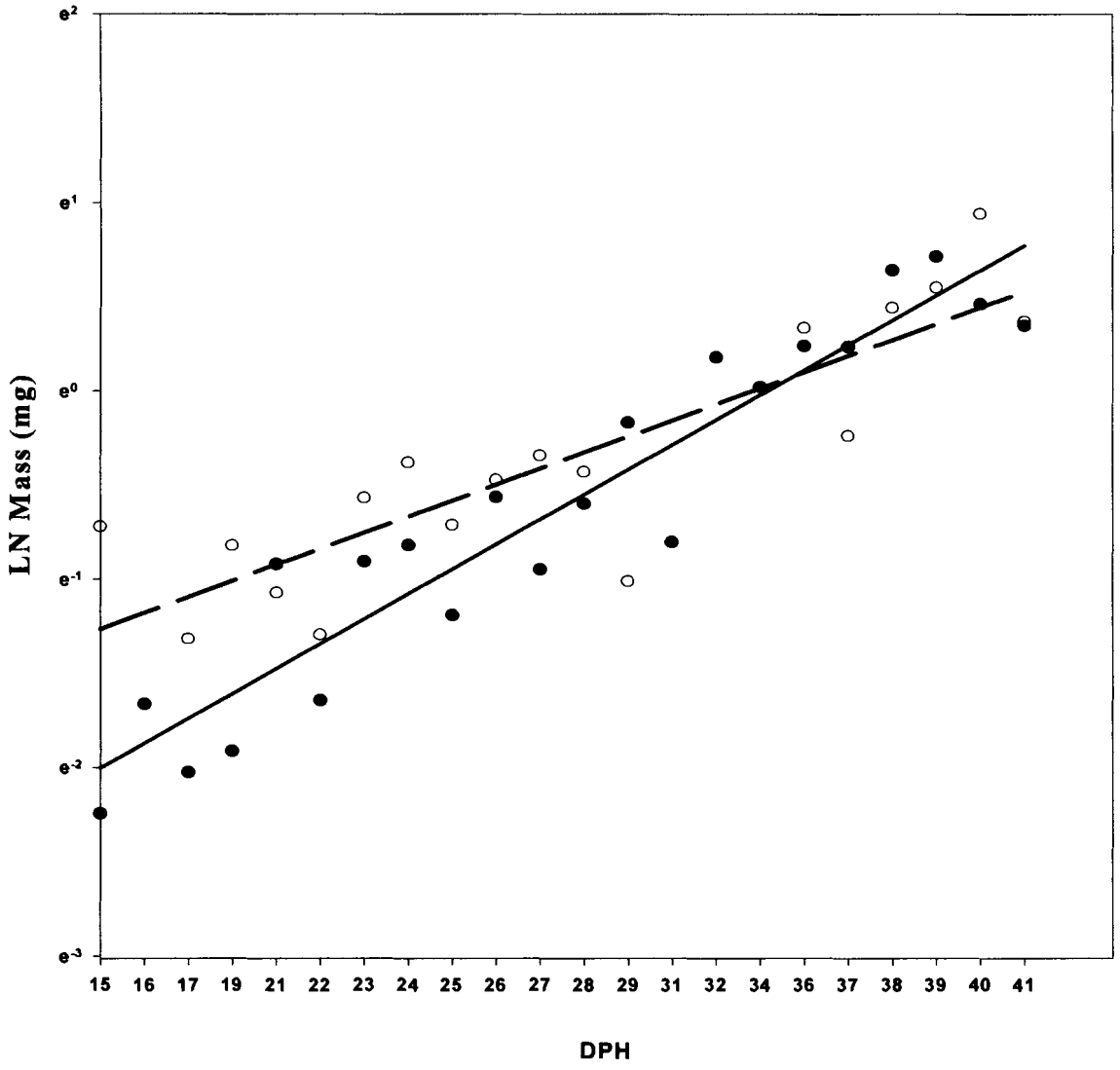


Figure 2.4. Natural Log of RI Dry Weight vs. DPH. ● = unfed, ○ = fed. — unfed data regression, --- fed data regression. Unfed : $r^2 = 0.895$, $y = 0.13\ln x - 2.004$. Fed: $r^2 = 0.74$, $y = 0.085\ln x - 1.27$.

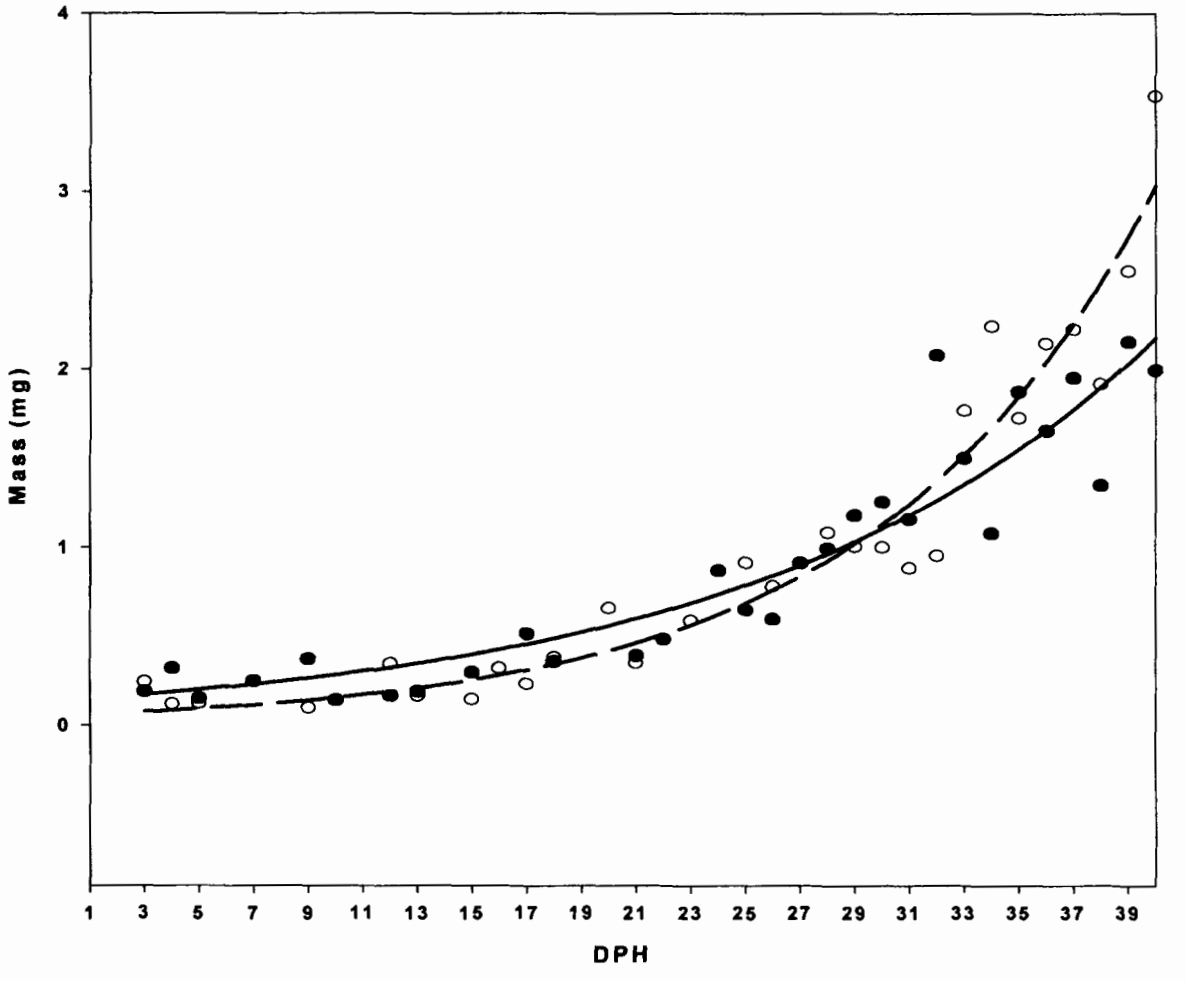


Figure 2.5. NF Unfed and Fed Larval Dry Weights vs. DPH. ● = unfed larvae, ○ = fed larvae. — unfed data regression, --- fed data regression. Unfed: $y = 1.071x^{0.126}$. Fed: $y = 1.09x^{0.08}$.

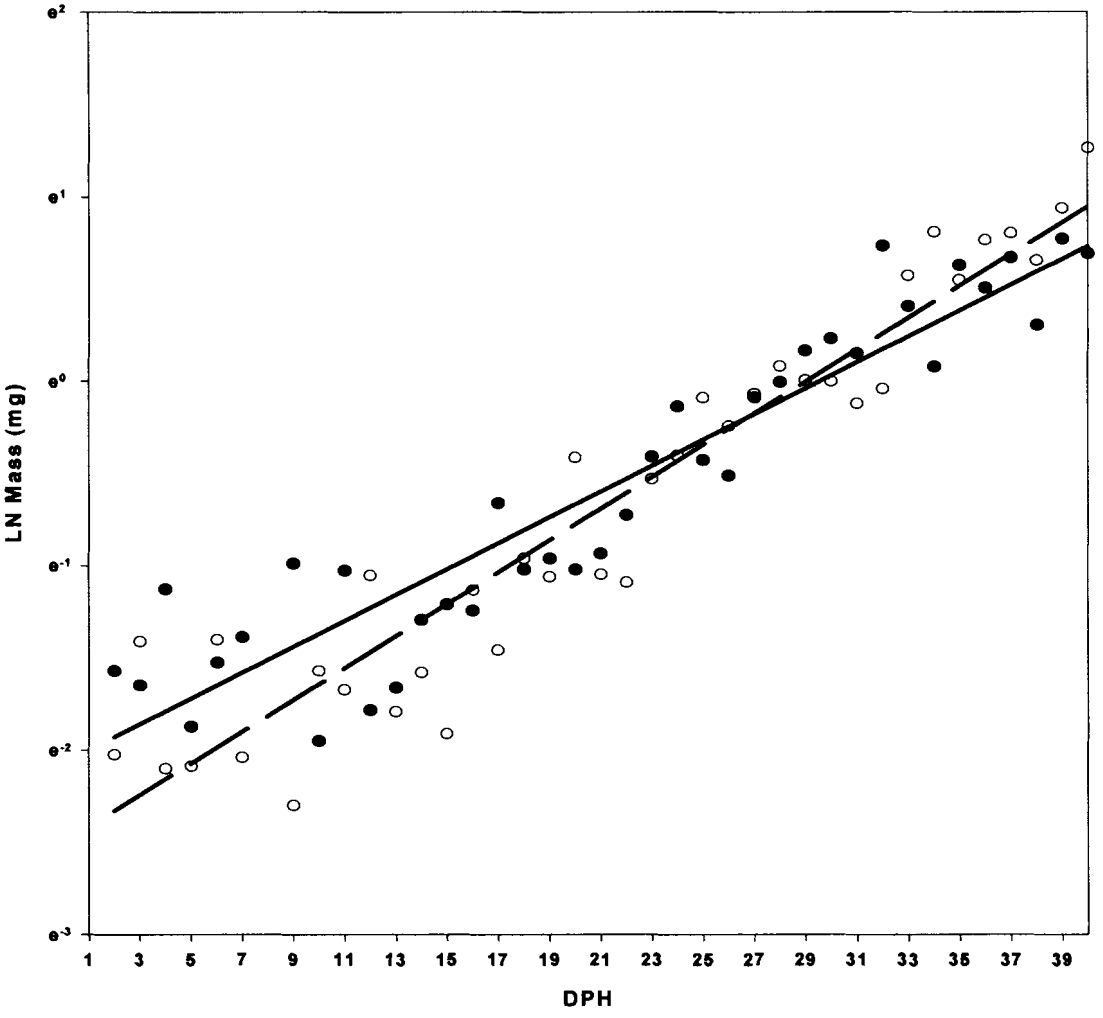


Figure 2.6. Natural Log of NF Dry Weight vs. DPH. ● = unfed larvae, ○ = fed larvae. — unfed data regression, --- fed data regression. Unfed : $r^2 = 0.88$, $y = .069\ln x - 2.07$. Fed : $r^2 = 0.90$, $y = 0.086\ln x - 2.5$.

2.3.4. RI Larval Heat Output

No significant difference was found (ANCOVA $F = 0.011$, $p > 0.05$) between fed and unfed larval heat output. Many technical problems confounded RI heat output data. On two occasions cold room temperatures reached temperatures more than 30°C. These were caused by malfunction of valves in the heater/chiller unit. Repairs prevented this from happening during future experiments. Larvae for the study were only used from 16 to 40 dph due to TAM baseline fluctuations prior to 16 dph. On many experiment days ampoules were lowered into the TAM and positive or negative heat output spikes resulted. On days when these spikes reached a plateau at extreme levels (differing from baseline by up to 3,000 μW) ampoules were removed. The TAM was very sensitive and could take over 8 hours to return to baseline levels. More than 55% of RI larval heat measurements were negative.

Near the end of the RI larval period, a TAM sales representative and technician visited Maine. The TAM was then cleaned and maintenance was done. New electronics and a new measuring cylinder for the ampoules were installed. Baselines were consistent after these repairs. Trial runs were done over a three-week period before NF heat output measurements began. During this time, the TAM performed consistently.

For these reasons, RI larval heat output data were considered unreliable. No further microcalorimetry data were analyzed for RI larvae.

2.3.5. NF Larval Heat Output

Heat data were recorded every 10 seconds and data were average by the hour for analysis. Prior to averaging, data were graphed in order to examine the details of heat

output fluctuations. Figure 2.7 shows unaveraged heat output data from both a fed and unfed 13 dph cod larva.

Data were collected for a minimum of 10 hours. Approximately 11 total hours of raw data is shown in Figure 2.7. At the beginning of each experimental measurement there was a period of TAM equilibration, seen in Figure 2.7 as the heat fluctuations before 8000 seconds. After this time, the unfed larva had a higher heat output than the fed larva for the duration of the experiment.

2.3.6. NF Heat Output Data Throughout Ontogeny

Changes in heat output by the minute were found to be very small, on the order of less than 2 μW. Therefore, heat output data for all fed and unfed larval cod were averaged by the hour. Fed cod larvae generally showed a higher heat output than unfed cod larvae of the same age. As the cod grew, both fed and unfed larvae showed higher averaged heat output. Figures 2.8-2.11 show only hours 2-10 of experimental heat output recordings. The first hour was eliminated due to microcalorimeter equilibration. The ending time for the experiments was determined by oxygen uptake studies that showed the largest larvae became oxygen limited after 12 hours.

After 30 dph, unfed cod larvae consistently showed higher heat output than fed larvae of the same dph. Figures 2.12 and 2.13 show hourly averaged heat output data from representative cod larvae of ages 32 dph and 38 dph respectively.

2.3.7. Comparison of Fed and Unfed NF Heat Output Over Development

Total heat output was averaged by day and plotted against dph from both fed and unfed larval cod (Figure 2.14). As expected, both fed and unfed groups of larval cod showed increases in heat output with increasing age.

The natural log of heat output for each fed and unfed larva was plotted against the natural log of the corresponding larval dry weight of cod age one through 40 dph. A significant difference was found between treatments (ANCOVA $F = 6.38$, $p < 0.05$). However, after larvae reached a dry weight of 1.0 mg (30 dph) fed larval heat output was consistently lower than unfed larval heat output. Data were separated into groups of one through 29 dph and 30 through 40 dph. ANCOVAs were run on the separated data set with the following results. For days one through 29 post hatch, no difference was found between fed and unfed larval cod heat output slopes ($F = 2.833$, $p > 0.05$). Also, from 30 through 40 dph, no difference was found between fed and unfed larval cod heat output ($F = 2.802$, $p > 0.05$).

Heat output averaged by week was higher in fed versus unfed larval cod until week 4 (Figure 2.15). After this time, unfed heat output trends were higher than fed. However, only averages of weeks two and six were significantly different. Weekly heat output data plotted versus weekly average dry mass resulted in the same trend (Figure 2.16).

Trends in average specific heat output were similar to absolute heat output averaged by week. However, specific heat output averages showed significant differences between treatments for weeks one and two, with fed larvae generating more heat per milligram of dry weight than unfed larvae (Figure 2.17). Significant differences

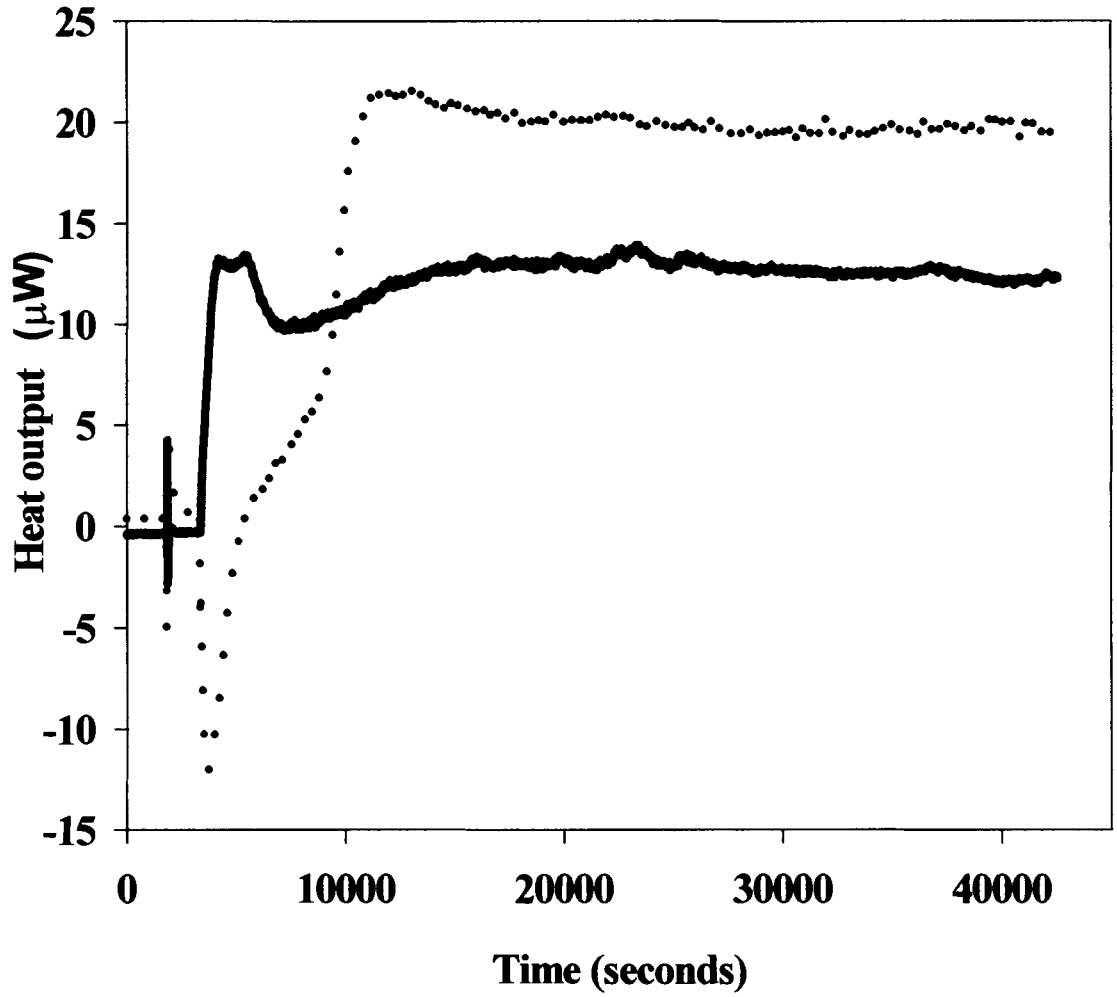


Figure 2.7. NF Fed and Unfed Raw Heat Output Data. Broken line = fed larva, solid line = unfed larva.

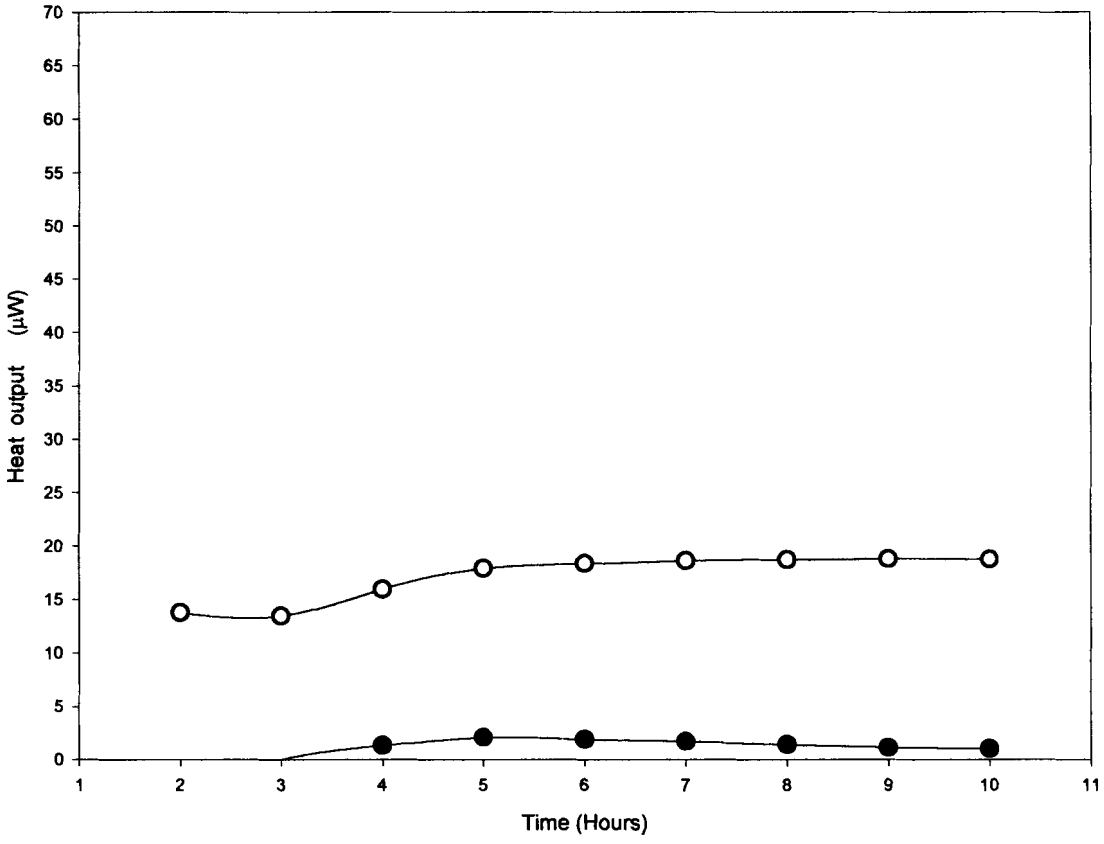
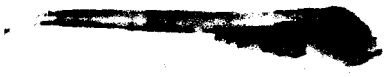


Figure 2.8.



4 DPH NF Cod Larval Heat Output. Larva is5 mm total length (TL).

Heat output data in microwatts (µW) averaged by hour plotted against time in hours for one unfed and one fed 4 dph NF cod larva. Open circles (○) represent fed cod larva. Filled circles (●) represent unfed cod larva.

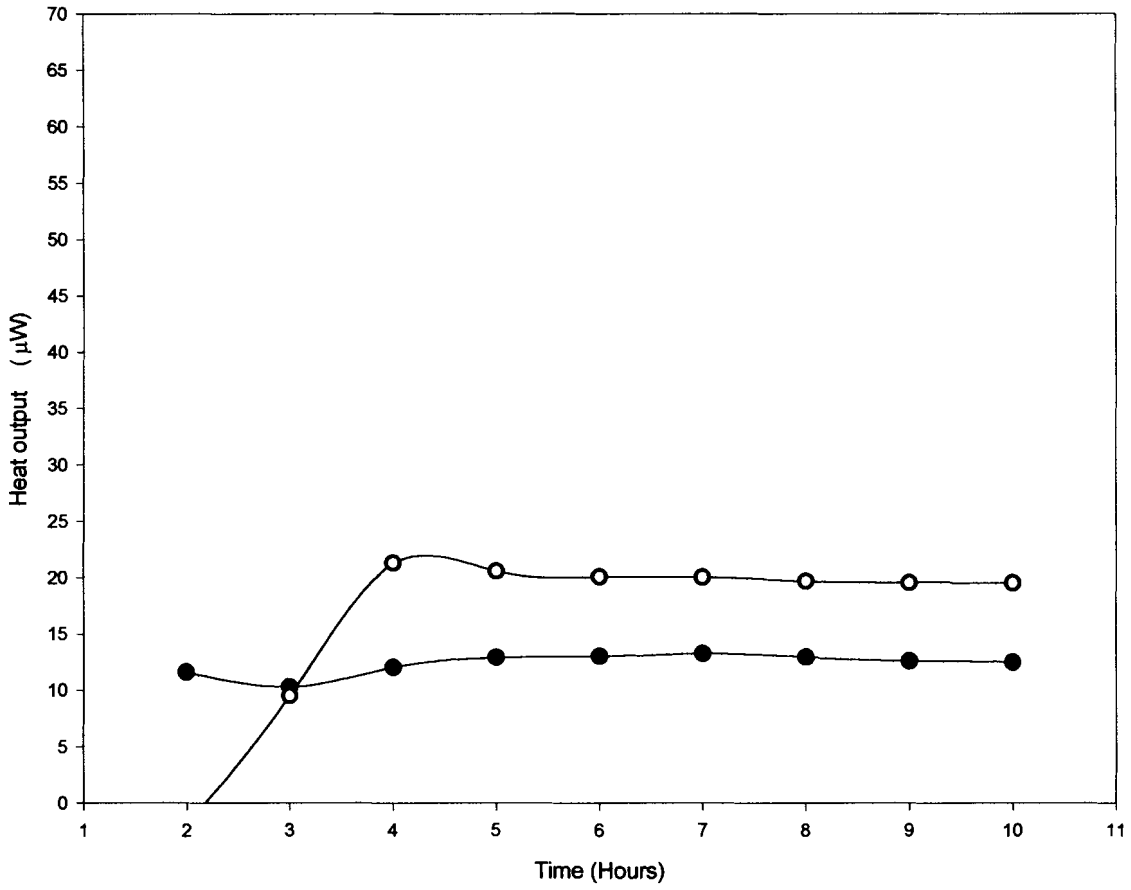
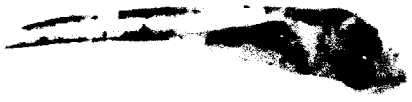


Figure 2.9.



13 DPH NF Cod Larval Heat Output. Larva is 7 mm TL.

Heat output data in microwatts (µW) averaged by hour plotted against time in hours for one unfed and one fed 13 dph NF cod larva. Open circles (○) represent fed cod larva. Filled circles (●) represent unfed cod larva.

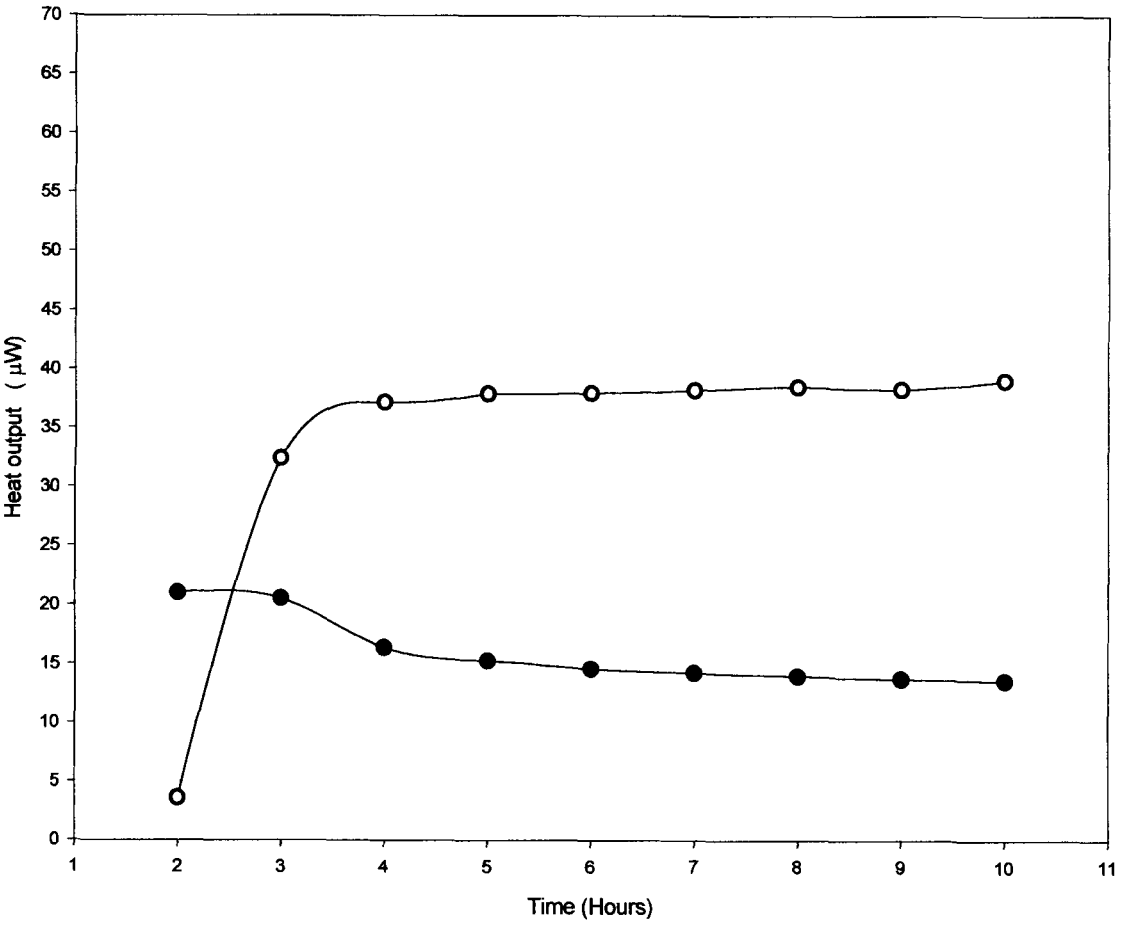


Figure 2.10.

21 DPH NF Cod Larval Heat Output. Larva is 9 mm TL.

Heat output data in microwatts (µW) averaged by hour plotted against time in hours for one unfed and one fed 21 dph NF cod larva. Open circles (○) represent fed cod larva. Filled circles (●) represent unfed cod larva.

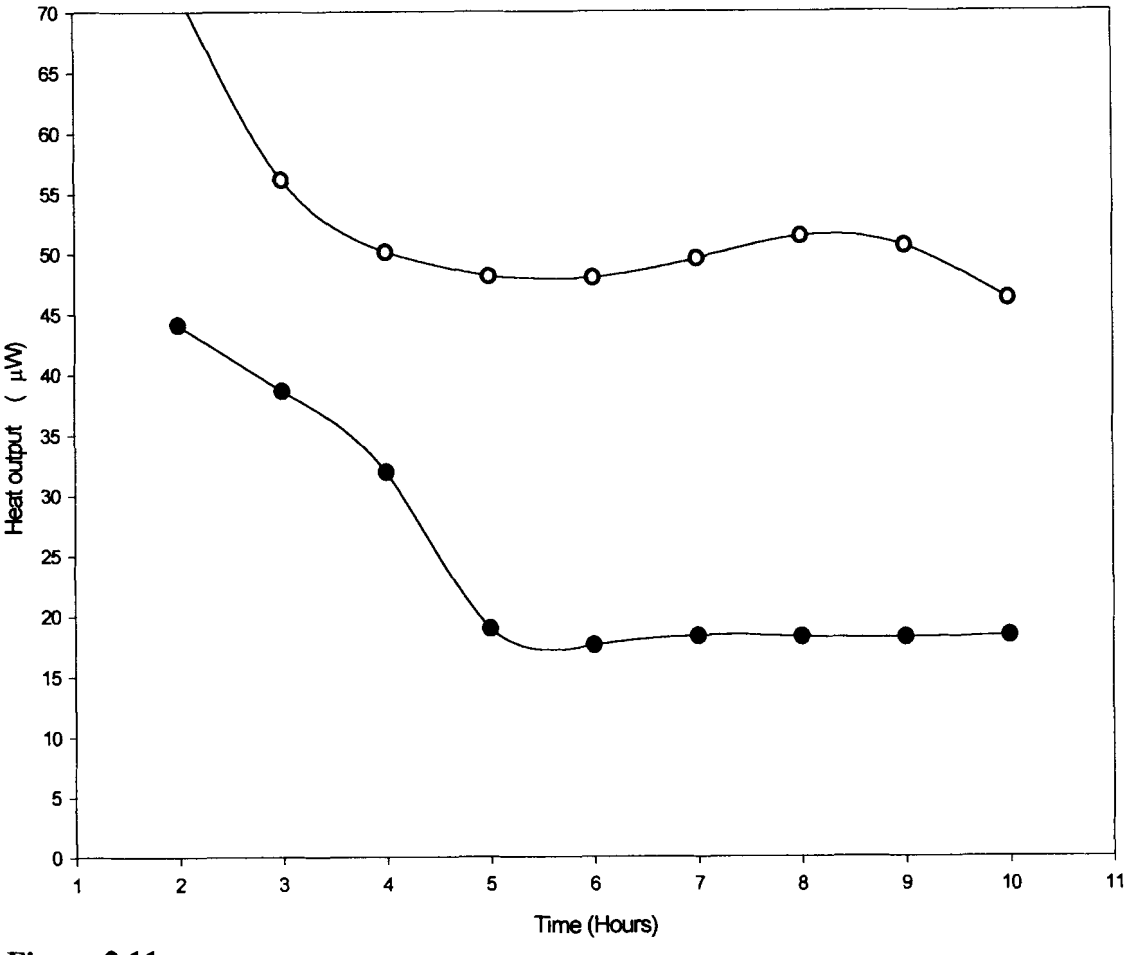


Figure 2.11.

28 DPH Cod Larval Heat Output. Larva is 13 mm TL.

Heat output data in microwatts (µW) averaged by hour plotted against time in hours for one unfed and one fed 28 dph NF cod larva. Open circles (○) represent fed cod larva. Filled circles (●) represent unfed cod larva.

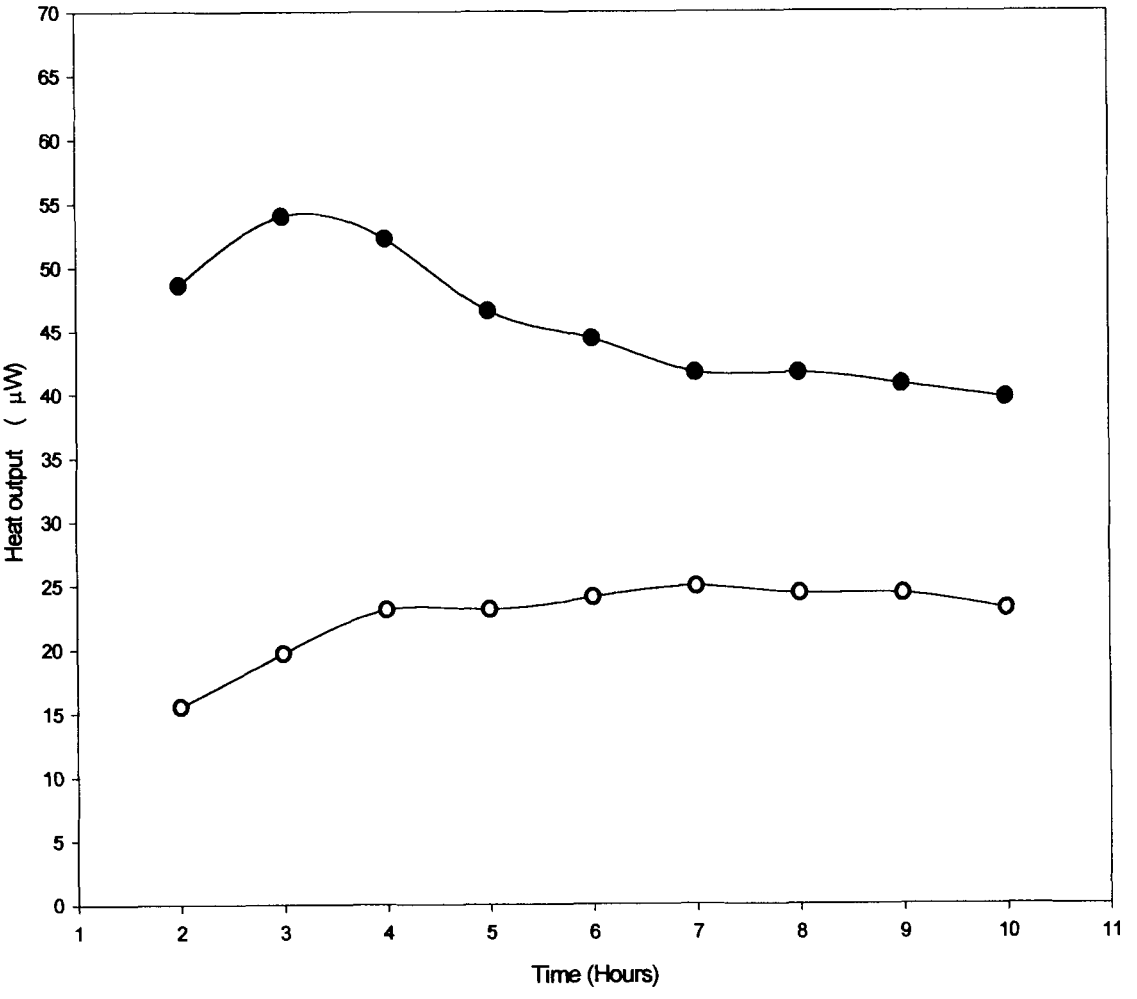


Figure 2.12.

32 DPH Cod Larval Heat Output. Larva is 15 mm TL.

Heat output data in microwatts (µW) averaged by hour plotted against time in hours for one unfed and one fed 32 dph NF cod larva. Open circles (○) represent fed cod larva. Filled circles (●) represent unfed cod larva.

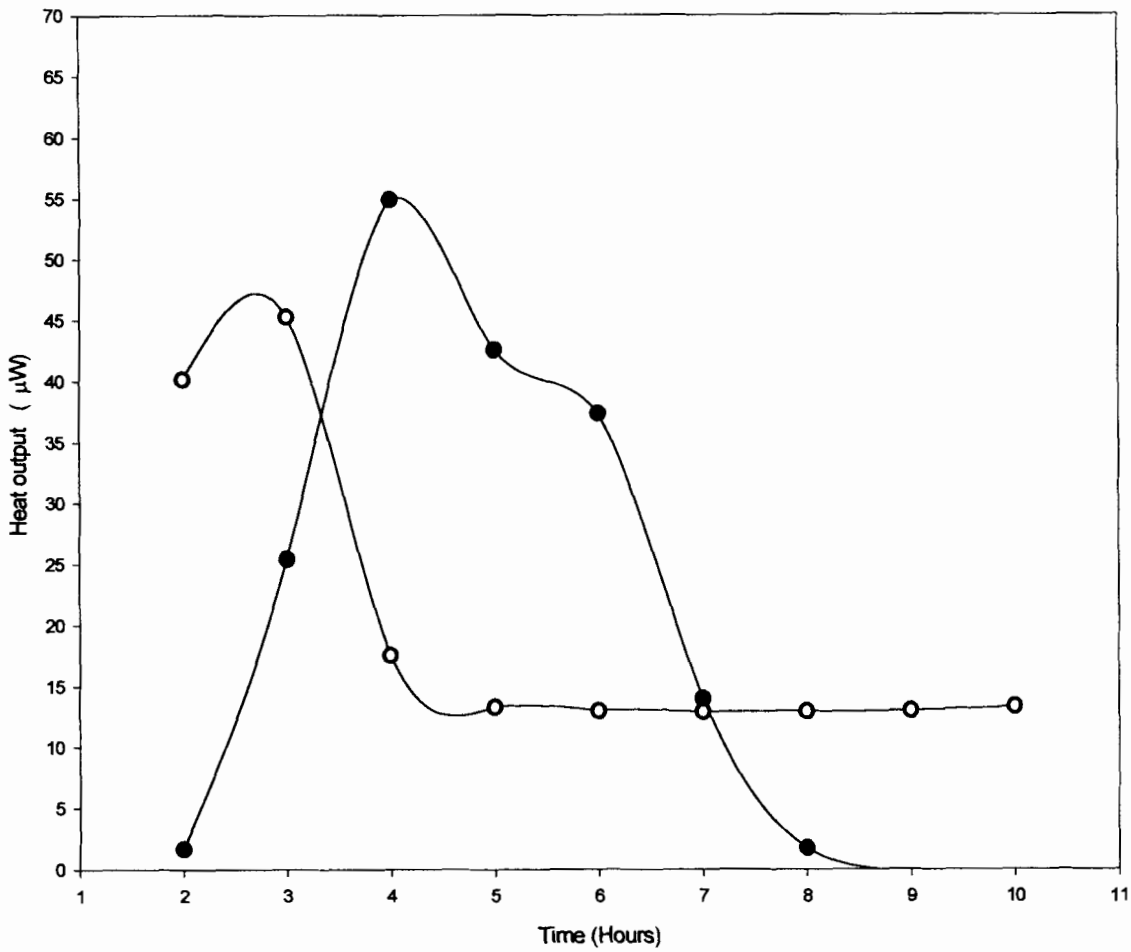


Figure 2.13.

38 DPH Cod Larval Heat Output. Larva is 16mm TL.

Heat output data in microwatts (µW) averaged by hour plotted against time in hours for one unfed and one fed 38 dph NF cod larva. Open circles (○) represent fed cod larva. Filled circles (●) represent unfed cod larva.

were also seen at week six, with unfed specific heat output being greater than fed. The same trends were seen in weekly averages of specific heat output plotted against their corresponding weekly averaged dry weight (Figure 2.18).

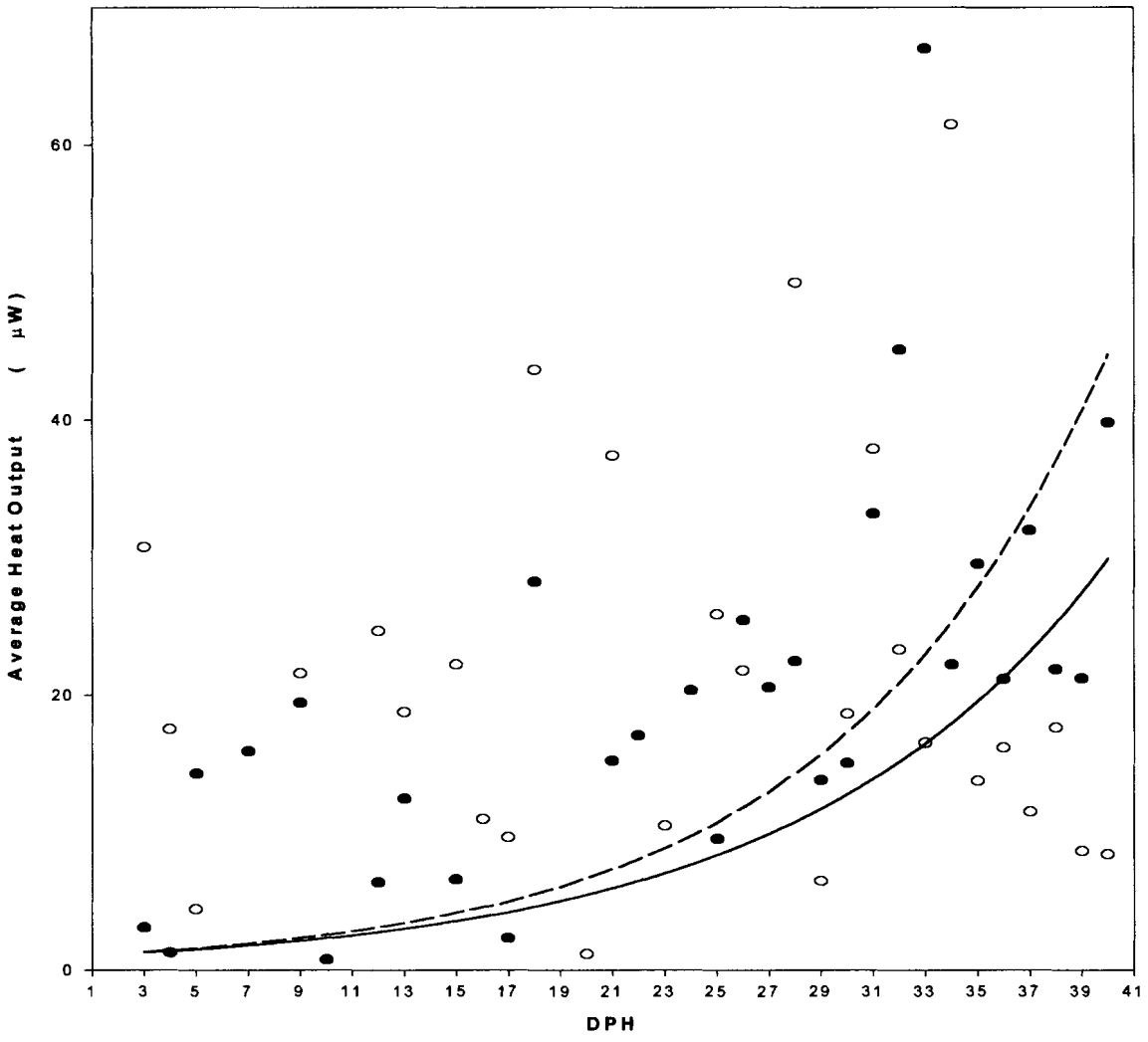


Figure 2.14. NF Fed and Unfed Daily Averaged Heat Output. ● = unfed larvae, ○ = fed larvae. — unfed data regression, --- fed data regression.

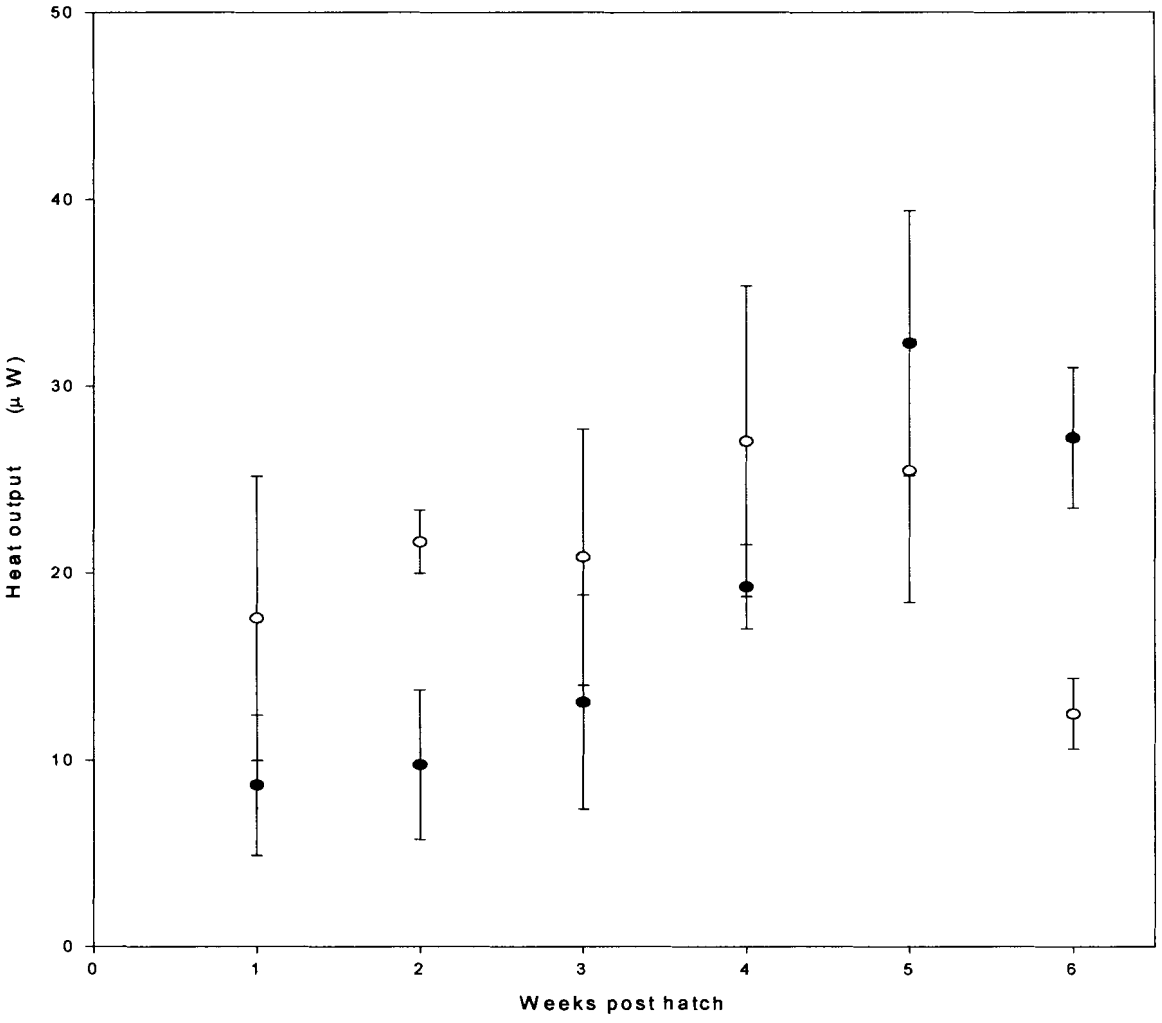


Figure 2.15. NF Fed and Unfed Weekly Averaged Heat Output. Each week is an average of data from 3 to 5 larvae. ● = unfed larvae, ○ = fed larvae.

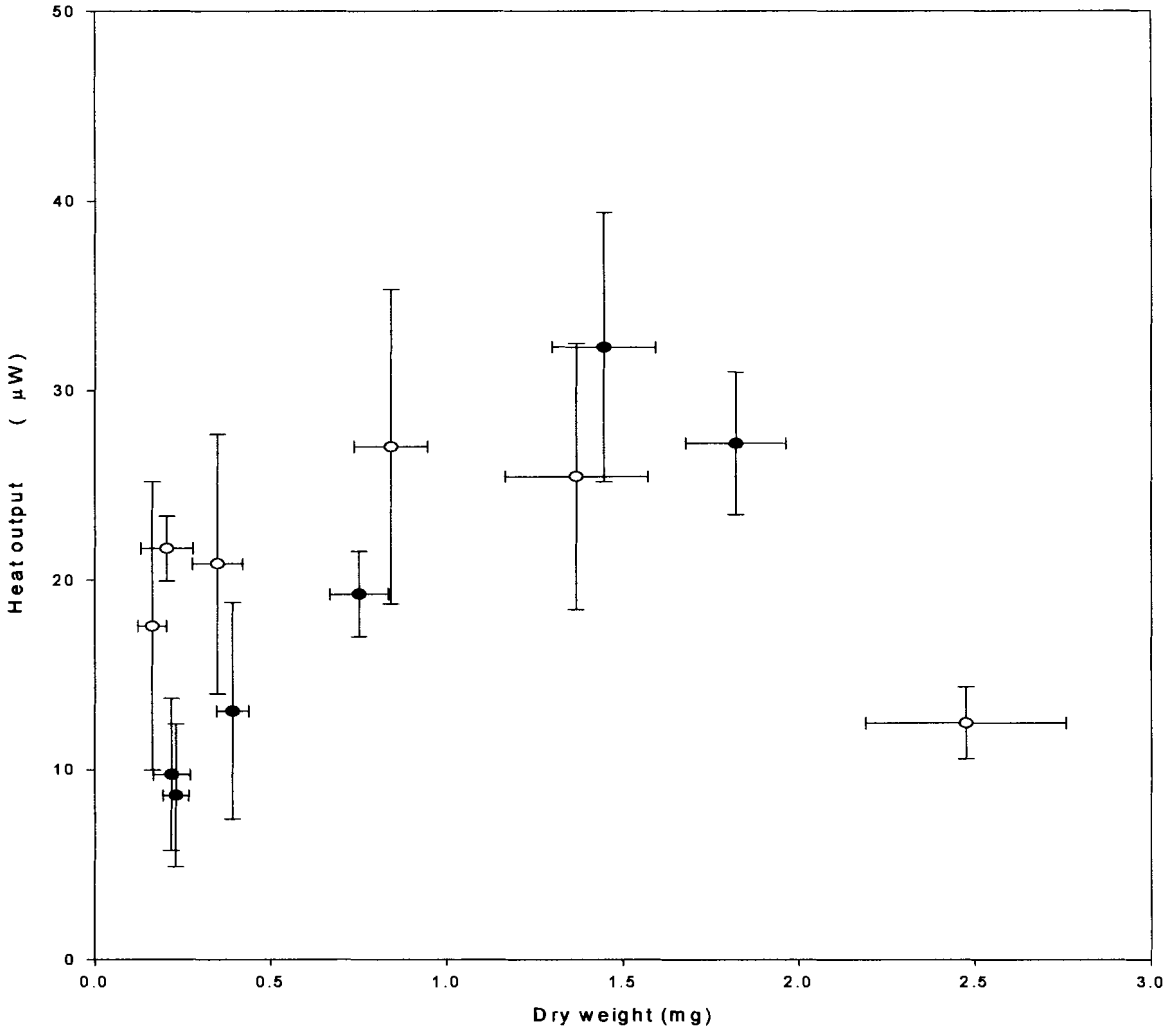


Figure 2.16. NF Fed and Unfed Weekly Averaged Heat Output vs. Dry Weight. Each week is an average of 3 to 5 larvae. Weeks are in pairs from left to right of one fed and one unfed circle. Weeks are numbered one through 6. ● = unfed larvae, ○ = fed larvae.

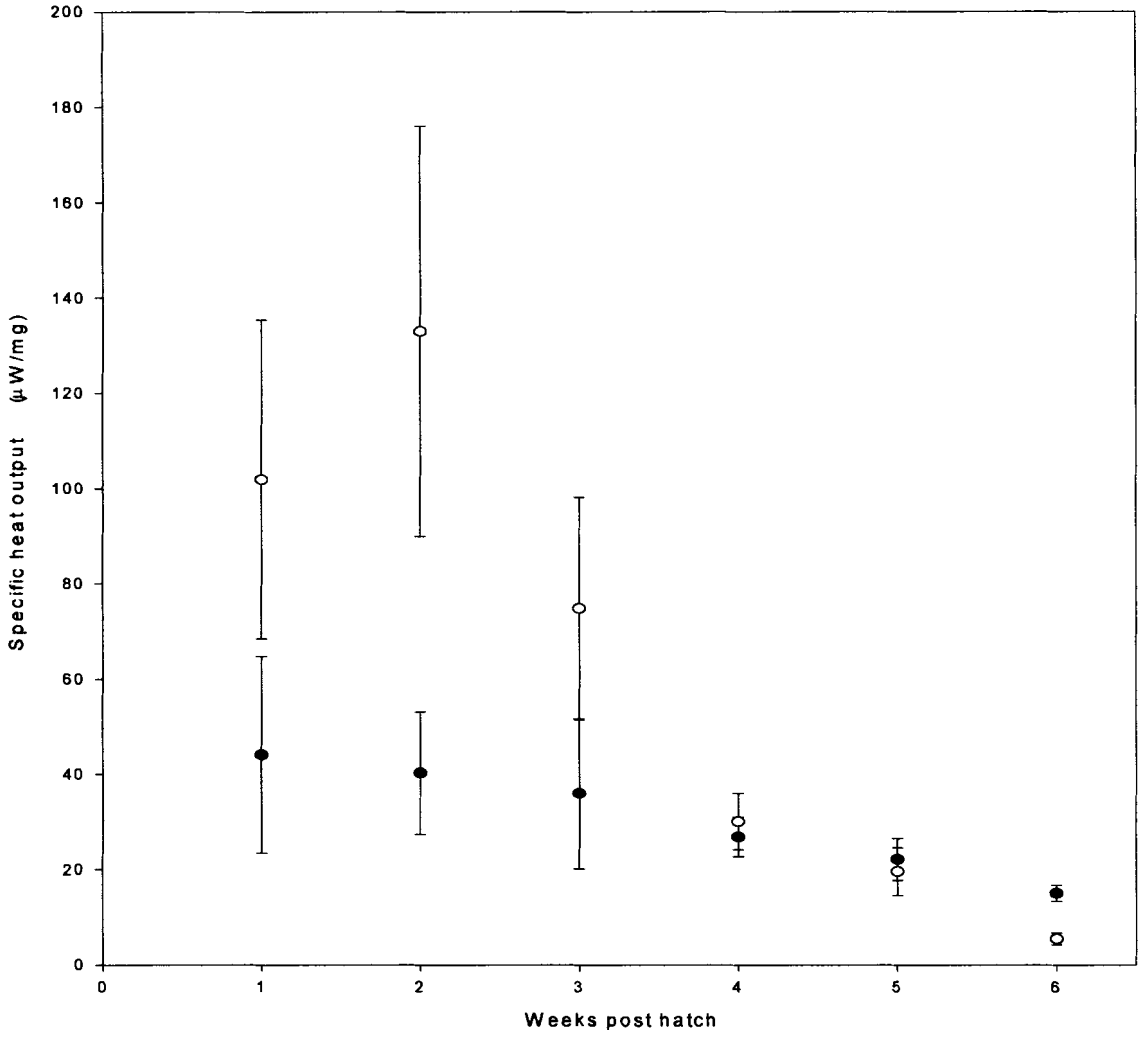


Figure 2.17. NF Fed and Unfed Weekly Averaged Specific Heat Output. Each week is an average of data from 3 to 5 larvae. ● = unfed larvae, ○ = fed larvae.

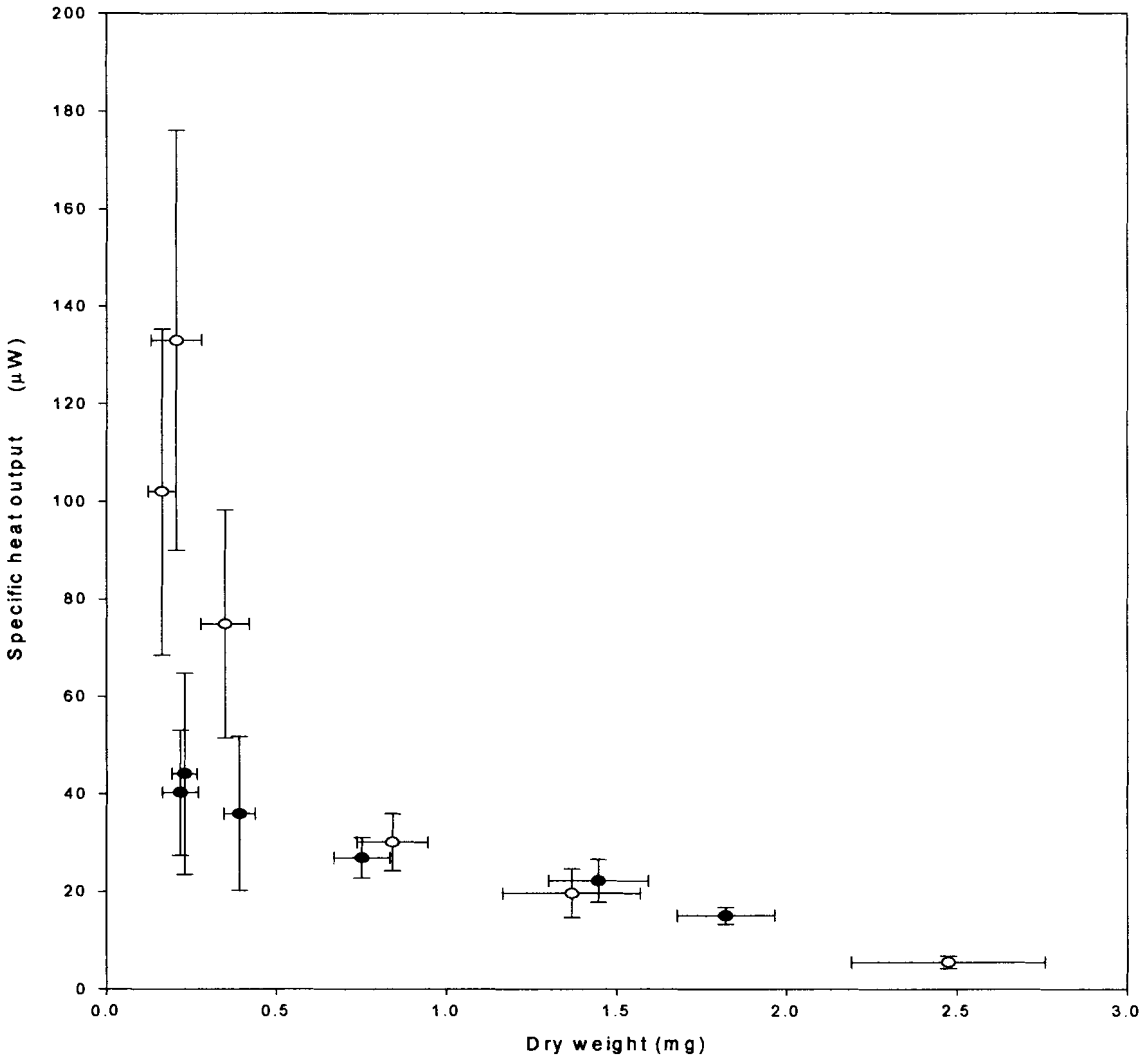


Figure 2.18. NF Fed and Unfed Weekly Averaged Specific Heat Output vs. Dry Weight. Each week is an average of data from 3 to 5 larvae. Weeks are in pairs from left to right of one fed and one unfed circle. Weeks are numbered one through 6. ● = unfed larvae, ○ = fed larvae.

2.4. Discussion

2.4.1. Introduction

This study was the first to record changes in metabolic rates as a function of feeding in larval fish using a microcalorimeter. Total (aerobic and anaerobic) metabolism was recorded as changes in enthalpic heat output (ΔH). Results from this study showed that metabolic rates increased during the first 30 days post-hatching (dph) in larval Atlantic cod (*Gadus morhua*) and were higher in the fed larvae than the unfed larvae. The difference of metabolic rate in fed versus unfed larvae in this study was interpreted as apparent Specific Dynamic Action (SDA), which occurs as a post-prandial increase in oxygen consumption. Measurements of heat output in larvae older than 30 dph were highly variable and exhibited patterns in metabolic heat output that were opposite to those of younger larvae such that metabolic rates in unfed larvae were higher than fed larvae. In general however, the results of this physiological study are unique for larval fish and provide the foundation for future investigations of metabolic physiology in rapidly growing and developing vertebrates.

2.4.2. Growth Rate

Two populations of Atlantic cod, Rhode Island (RI) and Newfoundland (NF) were used in the present study, because it was difficult to obtain fertilized cod eggs reliably on a regular basis. Growth rates ranged from 6.9% d^{-1} to 13.1% d^{-1} in both populations suggesting that larvae from both populations grew well under culture conditions and over the course of the experiment. However, mean growth rates were significantly different between RI and NF cod, and NF larvae had significantly higher growth rates than RI

larvae. Differences in growth rate may be due to inherent genetic differences in the populations as was shown by Pogson (1995) or may be due to the natural variation in larval growth rates among egg batches (Koslow et al., 1987).

Both genetic and environmental factors may contribute to the variation in larval growth and survival rates. Among many environmental factors that may potentially affect fish larvae, temperature, salinity, and food nutrient content have been shown to be very important. However, it is difficult to determine which factor is the most important in regulating growth and survival rates in developing marine fishes (Neilson et al., 1986; Koslow et al., 1987; MacKenzie et al., 1990).

The higher growth rates in NF larvae compared to the RI population can be attributable to several factors. The first of these is that eggs obtained from the RI population were eggs that were produced near the end of the natural spawning period for cod in Rhode Island. These eggs may have been of lesser quality, in that they were smaller, which produced smaller larvae that had a lower survival rate. In contrast, eggs from NF cod were collected from adults that had only been spawning for about one month. Eggs were likely to have been larger and had lower mortality rates. NF eggs may have had larger yolk sacs that provided more energy to the growing embryos and larvae compared to the RI eggs. Egg quality has been found to be variable, not just within one brood stock but between brood stocks and also among cohorts (Akerman et al., 1996). This ultimately may have contributed to the high variability in heat output measurements obtained for RI larvae, and the less variable results obtained from NF larvae.

2.4.3. SDA Results

Using the microcalorimeter, measurements of SDA in larval cod were obtained and values of heat output showed a consistent pattern in an increase in metabolic rate with larval age and size. For the first 30 dph, both fed and unfed larval cod exhibited increases in mean heat output on a daily basis. During this time, fed larvae had higher average heat outputs than unfed larvae with mean values of 21.7±3.9 μW for fed larvae and 12.6±4.7 μW for unfed larvae. The difference between the unfed and fed values was interpreted to be SDA. The only other study in which SDA was measured in larvae was conducted by Kiorbøe and Mohlenburg (1987). They used different individuals to determine SDA. In the present study different individuals were also used but each study was conducted with similar sized and staged larvae which limited the error due to changes in development of metabolizing tissue.

In larvae that were older than 30 dph and greater than 12 mm TL, a significant change occurred with respect to changes in metabolic rates. It was observed that fed larvae began to show lower average heat output than unfed larvae per day. Fed larvae showed a mean heat output of 18.8±9.2 μW while unfed larvae had a mean heat output of 29.7±3.6 μW. It is possible that one of the reasons for the changes in patterns of metabolic rate in larvae older than 30 dph versus younger than 30 dph was due to size restraints of the ampoules on the larvae. Ampoules in the TAM had volumes of 5ml and although larvae can fit into the ampoules, by the time they reach 30 dph they are approximately 11 mm total length, long enough that they would have trouble moving freely within the ampoule in the TAM. Restricted swimming movements would cause a decrease in total heat output for both fed and unfed larvae. Also at this stage, larvae have

well developed fins and are quickly becoming good swimmers. While fed larvae were full of *Artemia* and probably could use a large proportion of their energy from food for metabolic processes such as digestion, growth and swimming, unfed larvae may have been actively searching for food within the ampoule. Some studies have shown that starved larvae can increase their activity level prior to total starvation and it is suggested that the increase in activity is related to food search (Munk, 1995). The increased level of activity of the unfed larvae may have caused higher metabolic rates and resulted in higher heat output than the less active (digesting) fed larvae. This may have masked any measurable SDA occurring in the larger 30 dph fed larvae compared to their smaller younger counterparts at earlier stages.

Cod experienced a transformation from larva to juvenile stages at approximately 12 – 15 mm total length, or 31-40 dph in my experiments which agreed with the literature (Pedersen and Falk-Petersen, 1992). This transformation, also referred to as metamorphosis is a gradual process continuing until the cod are 40 – 50 mm total length. During this time, cod lose their finfold and form distinct fins that increase their ability to pursue and capture prey. Movement from the larval to the juvenile stage also changes the type of prey the cod are able to capture. As cod become juveniles, their alimentary tract also differentiates as gastric glands, pyloric cecae and a functional stomach form (Pedersen and Falk-Petersen, 1992). Enzyme activity increases, and the cod are better able to digest and assimilate their food (Pedersen and Falk-Petersen, 1992). All of these factors may contribute to the level of SDA that is measurable in cod. These developmental changes may also help explain differences in measurements of heat output associated with SDA in larvae older than 30 dph. Unfed larvae may have been searching

more aggressively within the TAM chamber while fed larvae may have been more sedate. In larvae older than 30 dph heat output from swimming may be greater than the heat generated from digestion and assimilation of food. In this study activity levels could not be monitored and metabolic rates could therefore not be normalized to activity levels. In Atlantic cod larvae, more than 40% of the energy budget is directed to swimming activity (Hunt von Herbing and Boutilier, 1996). Thus, it will be necessary to quantify activity in conjunction with measuring metabolic rates of larvae in the TAM, if we are to have a better grasp of the effect of SDA on metabolic rate in rapidly growing fish stages.

2.4.4. Possible Errors in SDA Determination

Results of heat output are variable. The reasons for the high variability may have to do with the unpredictable nature of a rapidly growing organism such as a larval fish as well as several aspects of TAM and its operation.

In the TAM larvae are placed into small sealed ampoules and these ampoules are lowered into the TAM. Once in the ampoules larvae are in complete darkness. Because all larval fish require some light in order to feed on motile prey, larvae could not be fed in the ampoules but were fed prior to being placed into them. Larvae were placed in the ampoules approximately one hour before any heat output recordings began. This one hour time lag between feeding and measurements of metabolic rate permitted digestion in the larvae to begin. Because SDA has never been measured in larval fish prior to the present study, no data exist on the various components of SDA (magnitude, duration and amplitude). The recordings from the TAM may not show true SDA, while it is possible that larvae completed digestion and SDA before being placed into the ampoules.

However, this is unlikely as gut residency times for food in larvae appeared to be long and food remained in the gut for several hours. Therefore, larvae were clearly still digesting food while in the TAM.

Because larvae could not be fed in the TAM, they were fed in their rearing tanks prior to each experiment. Feeding larvae in their rearing tanks minimized stress to the larvae. One disadvantage of this method, however, was that it did not allow for an exact count of the number of food items that a given larva ingested. Thus the number of food items in the gut of each larva was regulated by time of feeding and not number of prey items in the gut. This may have contributed to a variation in food concentration in the gut, and led to SDA variations. While all larvae used in the experiments had food in their guts, this determination was made qualitatively, by looking at the sampled larvae in a beaker after each run was completed.

Once ampoules were lowered in the TAM there was an initial period of equilibration that generally lasted for 30 minutes. This time was necessary for the temperature of the ampoule to reach the temperature of the water bath within the TAM. As this was completely conducted in a 10°C cold-room the time for temperature equilibration was minimized. However, some part of the SDA which characteristically increases to a peak (SDA amplitude) shortly after feeding may have been missed, but this is likely to be minimal as food remained in larval guts for a period of hours, sometimes until the end of the daily experiment.

Finally, without the use of fiber optics, there was no way to see or measure the levels of activity exhibited by the larvae within the sealed ampoules. There may have been individual variation in activity levels that also contributed to total heat output. In

some cases, the heat generated by activity may have masked other digestive processes that would have led to an increase in metabolism or heat output associated with SDA. Some or all of these factors may have contributed to the reduced differences seen between fed and unfed larval cod in cod larvae older than 30 dph.

2.4.5. Future Directions

The results of these experiments suggest that an increase in metabolism measured as heat output in the TAM may represent SDA in larval cod. A next step would be to use both respirometry and microcalorimetry to estimate the total energy budget [(ie, total heat output= aerobic metabolism (respirometry) + anaerobic metabolism (total heat output-aerobic metabolism)]. A better estimate of SDA could be recorded as well as how energy from food is directed to other metabolic processes such as activity

Other future projects could include using fiber-optic infra-red and white lights inserted into the TAM ampoules fitted with cameras (or endoscopes) to record activity and relate metabolic rates to activity levels. Microcalorimetry is a tool that permits accurate measurements of metabolic rates in small living vertebrates such as larval cod. This project showed that the TAM consistently recorded the metabolic rate as heat output generated by individual larval fish immediately after hatching and up to 40 dph. The TAM promises to be a useful instrument for future studies of small living organisms especially if modifications are made to quantify activity levels within the ampoules.

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BIOGRAPHY OF THE AUTHOR

Jessica A. Geubtner was born in York, Pennsylvania on July 7, 1976. She was raised in York and graduated from York Suburban High School in 1994. She attended The University of Delaware and graduated in 1998 with a Bachelor's degree in Biology. She entered the School of Marine Sciences graduate program at The University of Maine in the fall of 1998 to study fish physiology.

After receiving her degree, Jessica plans to spend her career researching and communicating sound marine science with the hope of ensuring a strong and stable marine environment for the future. Jessica is a candidate for the Master of Science degree in Marine Biology from The University of Maine in August, 2003.