Raft River Aquaculture Project

Final Report

DOE/ID/01757-3

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1.0 Background

Use of thermal effluents for aquaculture has received increasing interest from fish culturists for reducing production costs and from industry for productively exploiting energy rich waste streams. Many projects have investigated the commercial potential for waste heat aquaculture (McConnell and Adelman 1978; TVA 1978); however, the culture of fish directly in geothermal water is an emerging field (Oregon State University 1978).

Geothermal discharges represent a large thermal energy source for continuously maintaining the temperature of culture fluids while providing a constant source of water for year-round intensive culture of warmwater fish species.

Benefits of geothermal aquaculture include: (1) enhanced growth rates as a function of controlling water temperatures within a narrow range; (2) improved food conversion efficiency in response to accurate temperature control; and (3) the introduction of a warmwater fishery industry into the geothermal rich Pacific Northwest, currently limited to the culture of coldwater species. By directly using low-temperature resources or spent fluids from other processes, geothermal aquaculture can conserve energy, reduce the amount of waste heat released into the environment, and produce a valuable high-quality product.

The commercial potential for goethermal aquaculture was evaluated for 2 years at the Department of Energy's Raft River geothermal site in Southcentral Idaho. Common carp (Cyprinus carpio) and channel catfish (Ictalurus punctatus) were selected as culture species. Carp have been cultured in a variety of environments for thousands of years and are recognized as a prime source of high quality protein (Bardach 1972). Very hardy organisms, carp exhibit rapid growth rates, are omnivorous, and efficiently convert low grade food sources into high quality protein. Channel catfish are the most widely cultured fish in the U.S., with a highly valued flesh and a well established market. Historically limited to extensive culture, channel catfish are ideally suited for intensive geothermal culture.

2.0 Objectives

Objectives of the 2 year study included investigation of: (1) growth rates; (2) nutrition trials; (3) histological and physiological parameters; (4) bioaccumulation of heavy metals; and (5) reproductive capacity. The second year project efforts were primarily studying the effects of geothermal water on the reproductive capacity of common carp by: (1) determining the effects of geothermal water on gonadal development of common carp and; (2) determining the effects of geothermal water on common carp embryogenesis.

3.0 Introduction

Common carp (*Cyprinus carpio* L.) have been cultured in a variety of environments for thousands of years. These fish, recognized throughout the world as a prime source of high quality protein, exhibit excellent growth in a wide range of conditions (Huet 1970, Hickling 1971, Bardach et al. 1972). However, because of a poor market due to the perceived poor organoleptic quality of carp, little work has been done in the United States on the development of intensive culture methods for this fish.

Fish culture developments in the United States have instead concentrated on the rainbow trout and channel catfish. Intensive culture of the rainbow trout, especially in the Snake River Valley of southern Idaho, thrives because of a high quality water source and a constant year round water temperature. The southern United States is known for its extensive culture of the channel catfish. This area is provided with a long enough growing season to insure economic viability of culturing this warmwater species. As in other temperate areas of the world, the capability to raise fish economically in the United States is dependent primarily upon a quality water supply and water temperature.

Intensive fish culture in temperate climates has been hindered by the seasonality of water temperature. Due to the poikilothermal nature of fish, their metabolism and thus their growth is governed by water temperature. Every fish has an optimum physiological temperature at which growth, food conversion, and resistance to disease are maximized. In temperate climates, a problem of intensive fish culture has been how to regulate the water temperature at an optimum level for growth of the desired species. One way to achieve this temperature regulation is through the use of thermal effluents from steam electrical stations (SES's) or from natural (geothermal) sources.

The effects of thermal effluents from SES's on populations of fish in natural habitats have been a major concern of fish biologists. Many studies have been done to evaluate the detrimental effects of calefaction on indigenous populations of fish (Alabaster 1963, Gibbons 1974). The net result is that fish grow faster in the warmed water, and are able to avoid lethal heat plumes, as these thermal plumes become localized in the natural environment of cooler water temperatures.

An energy source related to the SES thermal effluent is geothermal discharge. In contrast to the surface calefaction of SES effluents, geothermal water is an aquifer superheated within the earth's crust. Because of the increased temperature and pressure present within the earth, geothermal water often contains supersaturated gases and high levels of dissolved solids.

The utilization of thermal effluents in aquaculture is a recent development. This concept of intensive thermal aquaculture offers great potential to fish culture, allowing the maintenance of water at an optimum growing temperature for the desired species, thus completely eliminating the seasonality of growing warmwater fish in temperate climates. Fish culture in thermal effluents is gaining in popularity, especially with a new public awareness concerning wise use of all forms of energy. However, virtually no work has been done on intensive culture of fish directly in geothermal water, nor has any work been done on evaluating the reproductive parameters of fish reared directly in geothermal water (Sea Grant 1978).

In 1978, the Department of Energy (DOE) and EG&G, Idaho, contracted the University of Idaho to assess the economic feasibility of rearing finfish directly in geothermal water. The objectives of this study included growth and disease assessment, determination of the bioaccumulation of heavy metals in the edible portion of the fish, physiological (hematological, serological, histological and oxygen uptake) evaluation, and reproductive evaluation of common carp reared directly in geothermal water. The preliminary results of this study indicated that growth was excellent in geothermal water, far exceeding that of the control in nongeothermal water. There were no clinical signs whatsoever of any infectious or noninfectious diseases present during the year-long culture period. In the physiological tests performed, there were no measurable differences noted between test fish in geothermal water and control fish in nongeothermal water. Bioaccumulation of some heavy metals (i.e., Pb, Hg, Zn) in the edible portion of the fish flesh occurred during the culture period, but did not reach levels of concern for human consumption (Beleau et al. 1979).

In the spring of 1979, the RRGS carp reared in geothermal water exhibited maturing gonads, as described by Alikunhi (1966) and Huet (1970).

During the summer of 1979, two groups of these fish were induced to spawn within a time span of seven weeks. The resultant eggs from both groups were tested for rate of embryogenesis and percent survival in three environments of differing ionic content, in triplicate: undiluted geothermal water (3500 umhos cm^{-1}), deionized intermediate test water (2250 umhos cm^{-1}), and deionized control water (1000 umhos cm^{-1}). Complete mortality of all embryos occurred between 80 and 125 hours post-fertilization, in saturated oxygen levels and constant temperatures, regardless of the ionic content of the incubating water (Beleau et al. 1979).

During the first year of culture, the common carp were reared in a constant 27 C geothermal environment. This is significantly warmer than the optimum physiological temperature (or standard environmental temperature (SET)) of 25 C reported by Beamish (1964). Although no information is available as to the effects of a constant temperature warmer than SET on the gonadal development of the common carp, Khiet (1970) reported that temperatures consistently higher than the SET occurring with natural photoperiods resulted in poor gonadal growth and reduced reproductive vaiability in the Japanese cyprinid honmoroko (Gnathologon elongatus). Since the honmoroko and common carp are both spring, asynchronous spawners (Khiet 1979, Yamamoto and Yamazaki 1961), the conclusion was drawn that there existed the possibility of an adverse effect of constant high temperatures on the reproductive capacity of the common carp. This study was initiated to evaluate the reproductive capacity of carp reared in two temperature regimens of geothermal water.

Total serum protein (TSP) levels were utilized as an indicator of gonadal maturation for female carp reared in geothermal water. Borchard (1978) reported that the TSP levels of the female rainbow trout were elevated significantly above those of the male during the process of vitellogenesis. He noted that TSP levels offer a means of prediction; an insight into the gonadal maturation of the rainbow trout. Although no work has been done on the evaluation of serum proteins together with the gonadal maturation of common carp, this fish as well as all teleosts undergo the yolk accumulation process of vitellogenesis. This process utilizes the circulatory system for the transfer of this lipophosphoprotein from the hepatic synthesis site to the ovarian absorption site. During this process of vitellogenesis, the females are expected to exhibit an elevated level of serum proteins due to the synthesis and transport of vitellogenin (Thurston 1967). Thus, this study included the utilization of total serum protein levels as an indicator of gonadal maturation.

Many female carp at the RRGS were not spawned in 1979, although they exhibited external signs of ripeness, as reported by Huet (1970) and Alikunhi (1966). Bieniarz and Epler (1976) reported that if a carp has oocytes ready to be expelled during the spawning season, but does not spawn, the mature oocytes will undergo a very slow process of resorption which may exceed two years. During this period of resorption, they note that the female is infertile. A test was designed to determine the viability of females that did not spawn in the 1979 season, compared with the viability of females that did spawn in 1979.

The ionic content of the geothermal water was tested in 1979 for effects on the incubation of carp eggs from parents reared in geothermal water. Utilizing saturated oxygen levels for optimum results (Kaur and Toor 1978) and a constant temperature, rate of embryogenesis and percent survival were determined in the two spawning groups. The ionic content, measured in conductivity (Wetzel 1975), showed no effect on the incubation parameters tested (Beleau et al. 1979). The effects of ionic content were again tested in 1980, with the conductivity of the control reduced below the 1979 control level to further test whether ionic content is responsible for the premature mortality of the carp embryos.

Gonadosomatic Indices (GSI's) have been used as an indicator of gonadal maturation of populations of fish (Kaya and Hasler 1972, Bennett and Gibbons 1975). This parameter allows the researcher to document either the gonadal development throughout the year, or document the GSI's of the males and females at maturity, allowing comparisons between populations. Other means have been utilized in evaluating the maturity of female common carp, including ovarian stages based on oocytic development (Bieniarz et al. 1978), egg weight and egg size (Bieniarz et al. 1979), spawning behavior and external signs (Shields 1957, Alikunhi 1966, Huet 1970), and GSI's (Gupta 1975). GSI's of mature male and female carp at the RRGS were taken in August, 1979, when it became apparent from the two groups spawned that summer that more information was needed concerning reproductive parameters. Rather than comparing these results with results reported in the literature, the 1979 GSI's and egg weight/body weight from RRGS were compared with the GSI's and egg weight/body weight of mature carp from the same genetic stock in Moses Lake, Washington.

The following null hypotheses were tested: (1) there is no significant difference between male and female total serum protein levels throughout the year at the RRGS; (2) rearing common carp at a constant 27 C does not affect spawning success; (3) there is no significant difference in the rate of embryogenesis or percent survival between embryos from females that spawned in 1979 and embryos from females that did not spawn; (4) there is no significant difference in the rate of embryogenesis or percent survival between the embryos from the two groups of females indicated above, due to the ionic content of geothermal water; (5) GSI's of mature common carp reared in Moses Lake will not be significantly different from the GSI's of mature common carp reared in geothermal water.

4.0 Materials and Methods

4.1 Facility Description

Geothermal Site: Geothermal water, at a temperature of 130 C, flowed from a 1525 m well under artesian pressure to two cooling ponds, where the temperature was reduced to the desired range for culture and reproduction. The geothermal water moved by gravity flow from the second cooling pond through the remainder of the aquaculture facility and was temperature regulated at 26.5 C (SD = 0.5 C). Water composition is shown in Table 1. The geothermal water was used directly for culture of the fish; no dilution was made with fresh water. Water quality parameters of the culture units are listed in Table 2. The indoor experimental culture units consisted of sixteen 113-liter (30 gal) glass tanks, each of which received a constant flow of 4 liters per minute (2.1 turnovers/hour), two 1627-liter (430 gal), and two 2384-liter (630 gal) circular tanks that received continuous flows of 26 and 38 liter/minute, respectively (Fig. 1).

4.2 Control Site

A physiological control facility was established at the University of Idaho (U of I), in Moscow, Idaho. This system utilized Moscow City water in a closed system, with water temperature controlled by ambient temperature. Fish populations, culture units, flow rates, water temperature, and diet regimes were identical to those at the Raft River Geothermal Site (RRGS).

4.3 Test Organisms

Common carp were electroshocked from Moses Lake, Washington, in September 1978. Half of the carp were randomly selected and transported to the Raft River site on October 4 and were stocked in the glass culture units

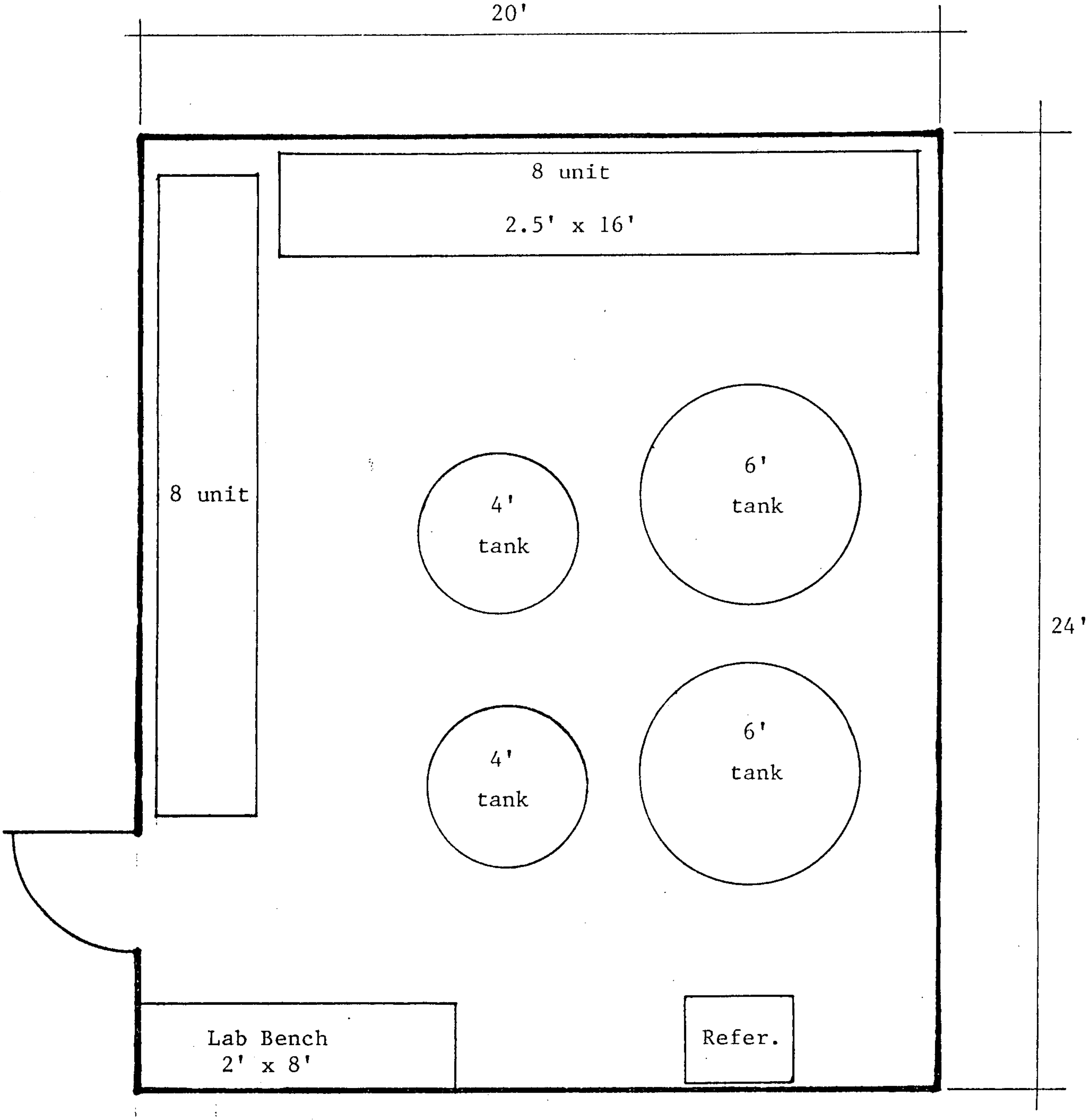

Figure 1. Layout of Raft River Geothermal Site Aquaculture Building.

Table 1. Raft River Geothermal Site supply well I.

Elements	Concentration (mg l^{-1})	Parameter	Concentration (mg l^{-1})	Chemical compounds	Concentration (mg l^{-1})
Ag	< 0.01	pH	7.05	$CaCO_3$	120
Al	< 0.1				
As	< 0.1	Conductivity (umhos cm^{-1})	3150	Br^-	< 1.5
B	0.18				
Ba	0.03			Cl^-	755
Ca	48.3	Total Dissolved Solids (TDS)	1569		
Cd	< 0.01			F^-	6.9
Co	0.01				
Cr	< 0.01	Suspended Solids	3 mg l^{-1}	H_2S	0.2
Cu	< 0.01				
Fe	0.02			I^-	0.036
Hg	71×10^{-6}	Turbidity (FTU)	<5		
K	75.0			NH_3	0.2
Li	1.48	Hardness as $CaCO_3$	120 mg l^{-1}		
Mg	0.06			NO_3^-	< 0.2
Mn	0.01				
Mo	< 0.1	Alkalinity as HCO_3^-	50.6 mg l^{-1}	Total P	0.023
Na	435.0				
Ni	0.02			$PO_4^{=}$	< 1.00
P	< 0.5	Total Gas (cc/l)	33.4		
Pb	0.02			SiO_2	145
S	.156	H_2	0.10		
Sb	< 0.05			$Si(OH)_4$	182
Se	< 0.1	He	0.03		
Si	56.6			$SO_4^{=}$	63
Sn	< 0.1	N_2	30.6		
Th	< 0.1				
Ti	< 0.05	O_2	.005		
Tl	< 0.1				
U	< 0.05	Ar	0.49		
V	< 0.1				
Zn	0.01	CO_2	1.91		
Zr	< 0.05				

Table 2. Selected water quality characteristics of culture fluids at the RRGS and U of I facilities. (Values expressed as mean with standard deviation in parentheses.)

	RRGS	U of I
Dissolved oxygen (mg/1	5.7 (0.7)	4.7 (1.4)
Total hardness (mg $CaCO_3$/1)	144.7 (6.3)	195.0 (49.8)
Total alkalinity (mg $CaCO_3$/1)	43.6 (8.6)	112.2 (40.1)
NH_3-N (mg/1)	0.5 (0.2)	0.38 (0.5)
pH (units)	8.2 (0.3)	7.5 (1.6)
Temperature (^oC)	26.6 (0.6)	26.4 (1.1)

at a mean density of 4.32 kg/m^3. The length and weight of the carp averaged 11.8 cm (SD = 1.6) and 26.7 g, respectively.

Channel catfish were obtained from Fish Breeders of Idaho in Buhl, Idaho, in October, 1978. This population of fish was split, with half going to each experimental facility. The catfish weighed an average of 0.23 g when originally stocked in the glass culture units at a density of 1.03 kg/m^3.

4.4 Nutritional Trials

Growth of the fish was assessed on three diets: (1) a commercial trout diet; (2) dried activated biomass material; and (3) a basal ration. Dietary constituents of the three rations are listed in Table 3. The biomass material, supplied by J.R. Simplot Company in slurry form, was derived from secondary waste treatment operations and consisted of a bacteriological single-cell protein. The slurry was dried in an experimental geothermal fluidized bed dryer prior to isocaloric incorporation into the biomass ration.

Both species of fish were placed on a programmed growth schedule; feeding rates were calculated by predicting the growth increase for each two-week period as follows:

a. Daily percentage weight gain = $(1 + \Delta W/100)^{1/d} - 1.0$, where ΔW is the predicted weight gain and di is the number of days in the growth period.

b. Weight gain/day = (biomass of culture unit) (daily percentage weight gain).

c. Food fed/day = (weight gain/day) (food conversion efficiency).

Table 3.

Basal I Ration (Silver Cup Mash)

Ingredient	Grams per 100 grams	Percentage of					Grams of					
		Protein	Fat	CHO	Ash	HOH	Protein	Fat	CHO	Ash	HOH	
Silver Cup Mash	42	53.1	16.8	16.4	12.2	4.6	22.30	7.06	6.89	5.12	1.93	
Gelatin	8	85	0	0	--	--	6.8	--	--	--	--	
Water	50	0	0	0	0	100	--	--	--	--	50	
Total	100	138.1	16.8	16.4	12.2	104.6	29.10	7.06	6.89	5.12	51.93	100.1

Basal II Ration (Potato Biomass Sludge)

Ingredient	Grams per 100 grams	Percentage of					Grams of					
		Protein	Fat	CHO	Ash	HOH	Protein	Fat	CHO	Ash	HOH	
Silver Cup Mash	14	53.1	16.8	16.4	12.2	4.6	7.43	2.35	2.30	1.71	0.64	
Biomass Sludge	28	37.3	1.5	16.3	43.1	4.6	10.44	0.42	4.56	12.07	1.29	
Gelatin	8	85	0	0	--	--	6.8	--	--	--	--	
Water	50	0	0	0	0	100	--	--	--	--	50	
Total	100	175.4	18.3	32.7	55.3	109.2	24.67	2.77	6.86	13.78	51.93	100.87

Table 3. Continued.

Isocaloric Comparison of Basal I and Basal II Rations

Component	Grams per 100 grams		Available calories per gram of component	Calories contributed per 100 grams of feed	
	Basal I	Basal II		Basal I	Basal II
Protein	29.10	24.67	3.9	113.49	96.21
Fat	7.06	2.77	8.0	56.48	22.16
CHO	6.89	6.86	1.6	11.02	10.98
Ash	5.12	13.80	0.0	--	--
HOH	51.93	51.93	0.0	--	--
	100.1	100.00		180.99	129.27

Feeding Rate:

Basal I = 180.99 calories/100 gms

Basal II = 129.27 x 1.4[a] = 180.99 calories/140 gms

[a] = Increase feed rate by 0.4 to feed equal calories.

Thus, the predicted increase in fish weight was utilized to determine feeding rates. Weight gains were determined every two weeks by standard inventory methods.

4.5 Bioaccumulation

Samples of the edible portion (fillets) of the catfish and carp from both the geothe-mal site and the control facility were analyzed to detect potentially harmful heavy metal accumulation. Fillets were lyophilized to remove water content, digested with 1:1 HNO_3 and deionized water for 12 hours, heated to 150 C for two hours and allowed to cool. The solutions were filtered and brought to volume (100 ml) with 5% NHO_3. Samples were analyzed directly for Ag, Cd, Co, Cu, Fe, Mn, Ni, Pb and Zn against standards prepared in 5% HNO_3. A 20 ml aliquot of each solution was modified to contain 1000 mg/ml La by the addition of 5 ml of a 5000 mg/ml La solution prepared in 5% NHO_3. Ca, K, Mg and Na were determined in lanthanized aliquots by direct comparison against standards similarly modified. Mercury was determined by the methodology of Velghe, Camp and Claeys (1978).

4.6 Physiological Studies

Monthly histological and physiological comparisons of the test and control fish were performed to detect potential subtle changes in the fish reared in geothermal water. Complete post mortem examinations were performed including hematocrit determinations, refractive index of serum proteins, and histological examination of all major organs. Metabolic rates of the control and treatment fish were also assessed each month by measuring their oxygen consumption.

4.7 Reproductive Evaluation

In 1979, the carp and catfish were examined monthly for gonadal maturation. The male and female carp exhibited gonadal development in the spring. Tests were designed to evaluate the incubatory qualities of geothermal water on common carp embryogenesis, and to evaluate the development of the gonads and eggs at the time of spawning. Ripeness of the females was determined using the following criteria: a swollen, soft abdominal region with a swollen, reddened vent; and whitish-yellow, fully rounded eggs with an eccentric nucleus (Alikunhi 1966, Huet 1980). Run One females responded to hypophysation (Clemens and Sneed 1962). Run Two females responded to natural spawning stimuli of increased water flow and submergent spawning substrate (Bardach et al. 1972, Hickling 1971).

The spawning container was a 2.0 meter diameter circular fiberglass tank with a depth of 0.6 meter. The tank contained a centrally located drain and a standpipe within a venturi pipe for adjusting the water to the desired depth. The preferred depth for spawning is just enough water to completely cover the dorsal fins of the fish (Huet 1970). The inflow of geothermal water into the spawning tank was increased to 25 liters per minute, and sprayed across the surface of the water to break surface tension and simulate rain (Wu and Chung 1964).

The spawning substrate simulated the roots of floating macrophytes. Each substrate or "kakaban" was constructed using a 1 x 2 x 12" board which supported a half skein of yarn attached at three points to the board. These kakabans were then submerged in the spawning tanks for collection of the adhesive eggs.

Nine rectangular glass tanks with a capacity of 113 liters each were used for embryo incubation for each spawning run. The incubation test evaluated rate of embryogenesis and percent survival during three prominent periods of embryogenesis: eye-up, half-hatch and button-up. The eggs were incubated in triplicate in three experimental water mixtures: (1) undiluted geothermal water with a conductivity of 3000-3500 umhos cm^{-1}; (2) deionized geothermal "control" water with an appropriate volume of undiluted geothermal water added to yield a conductivity of 1000 umhos cm^{-1}; and (3) an intermediate test water with a conductivity of 2250 umhos cm^{-1}. The partially deionized water (100 umhos cm^{-1}) was used as a control, with the conductivity based on that found in successful hatcheries in Idaho (Klontz and King 1975).

Deionization of the water chemistry was accomplished by passing water through three Culligan resin beds: a cation bed, an anion bed, and a unibed. After passage through these resin beds, the water was 99.9% free of cations, anions and solids and had a conductivity approaching 0 umhos cm^{-1}. By mixing geothermal water back in, the desired conductivity and thus the desired ionic content was obtained.

Once the test and control incubating tanks were set up, no additional water flow occurred, creating closed systems in each tank. The tanks were aerated to maintain the dissolved oxygen level at saturation (Kaur and Toor 1978). After the eggs had completed water hardening, the egg-laden kakabans were removed from the spawning tanks and randomly placed in the test tanks for incubation and embryogenesis.

Research in 1980 focused on the effects of geothermal water on the reproduction capacity of common carp. Gonadosomatic indices (GSI's) were taken in 1979 from mature male and female common carp at the geothermal site, and in 1980 from mature male and female common carp at Moses Lake, Washington, utilizing the methodology of Kaya and Hasler (1972). Mean egg weight (0.0001 g) was determined for both populations of carp at maturity.

A test was designed to determine the effects of constant 27 C geothermal water on the spawning success of common carp. The population of common carp reared in geothermal water at the RRGS was divided into two groups of approximately the same number of males and females. One group was reared in geothermal water with the temperature maintained at 27 C. The other group was reared in geothermal water with the temperature fluctuating through a seasonal cold cycle of 27 C initially to 4 C in the winter, returning to 21 C in the spring.

Female carp that spawned in 1979, fin clipped for identification, were tested for egg viability against females of the same population not spawned in 1979. Differences in the rate of embryogenesis and percent survival of embryos from the two groups of females were compared at different embryogenic periods and between waters of differing ionic content.

Evaluation: The Statistical Analysis System (SAS) was utilized to determine significant differences due to treatments of incubatory water at the different embryogenic periods, using the analysis of variance at the 0.05 level of significance. Mean egg weight was compared between the two populations with the t-test, at the 0.05 level of significance. When found significant at the 0.05 level, a coefficient of determination was assessed to the relationship between egg weight/body weight, testes weight/body weight and ovary weight/body weight.

4.7.1 Total Serum Protein

The same population of common carp used in the 1979 experiments at the Raft River Geothermal Site was used in 1980. In October, 1979, and January, March, April and May, 1980, blood samples were taken from at least five males and five females by ventral midline ventricular puncture. Sufficient blood to fill two hematocrit tubes was removed per sample, using a #18 1½ inch needle and a 5 cc disposable syringe. The two filled unheparinized hematocrit tubes were then plugged, numbered and recorded.

When all samples were taken, the hematocrit tubes were centrifuged for five minutes. Each tube was then broken at the point where the buffy layer met the serum, the serum contents drawn out on the refractometer, and the refractive index of the blood serum protein read and recorded. Water temperatures were taken at the time of each serum sample.

Evaluation: Mean serum protein levels for male and female groups per sampling were determined. Differences between male and female groups per sampling and between samples were determined by the t-test, at the .05 level of significance.

4.7.2 Histological Examination of Embryos

Samples of embryos derived from each successful spawning were collected and preserved in 10% buffered neutral formalin. The embryos were then processed and stained using standard histological techniques for examination.

5.0 Results

In 1979, two groups of common carp were induced to spawn. The first group hormone induced to spawn, obtained a mean of 47% fertilization. The second group, naturally induced to spawn, obtained a mean of 65%

fertilization. In both runs, complete mortality of the embryos occurred post hatch between 80 and 125 hours after fertilization, during the period of rapid yolk absorption.

There was no significant difference in the rate of embryogenesis ($P = .91$) or the percent survival ($P = .15$) between geothermal and non-geothermal water in the first group. The geothermal or nongeothermal treatment interaction with the eye-up or hatch period did not significantly affect the rate of embryogenesis ($P = .99$) or the percent survival (¼ = .38).

There was also no significant difference in the rate of embryogenesis ($P = .16$) or percent survival ($P = .09$) between geothermal and nongeothermal water in the second group. The geothermal or nongeothermal treatment interaction with the eye-up or hatch period did not significantly affect the rate of embryogenesis ($P = .08$) or the percent survival ($P = .95$).

In comparing the two groups, there was no significant difference in the rate of embryogenesis ($P = .09$) or the percent survival ($P = .95$) due to the treatment of geothermal or nongeothermal water. Rate of embryogenesis was not significantly different ($P = .38$) between runs whatsoever, however, percent survival was significantly higher in the second group at fertilization and eye-up and lower at hatch.

In 1980, two groups of common carp reared in geothermal water with a cold annual temperature cycle were spawned by natural induction methods. The first group of common carp that spawned was comprised of the females that spawned in 1979. The second group, comprised of females that were not spawned in 1979, spawned 24 hours later. Percent survival and rate of embryogenesis are graphed over time and temperature in Figure 2. Between 80 and 170 hours post fertilization, complete mortality (<0.01% survival) of the embryos of both groups occurred during the period of rapid yolk absorption.

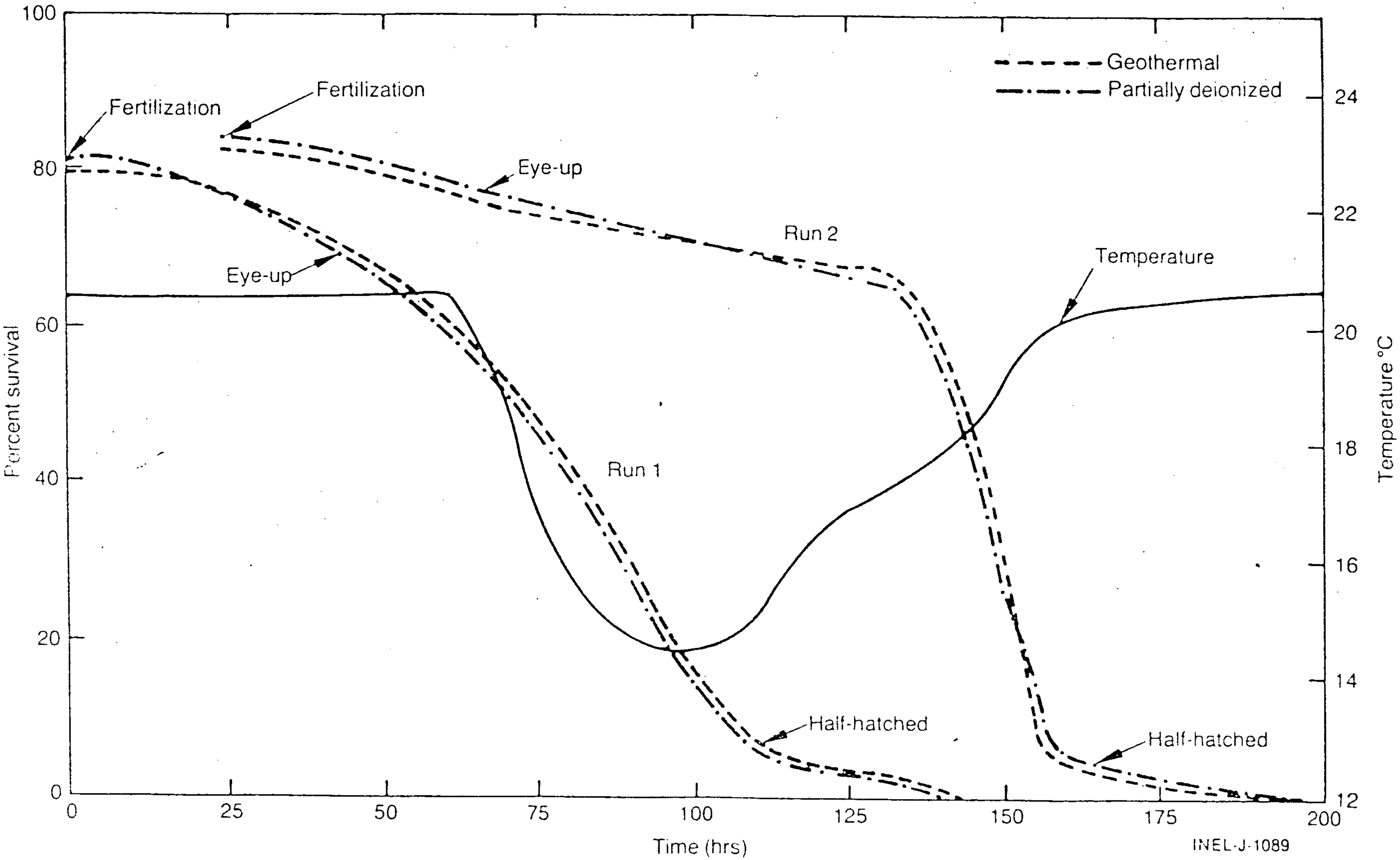

Figure 2. Carp embryogenesis at the Raft River Geothermal Site.

There was no significant difference in the rate of embryogenesis (P = .94) or the percent survival (P = .80) between geothermal and non-geothermal water in the first group. The geothermal or nongeothermal treatment interaction with the fertilization, eye-up or hatch period did not significantly affect the rate of embryogenesis (P = .99) or percent survival (P = .80).

There was also no significant difference in the rate of embryogenesis (P = .57) or percent survival (P = .77) between geothermal and nongeothermal water in the second group. The geothermal or nongeothermal treatment interaction with the fertilization, eye-up or hatch period did not significantly affect the rate of embryogenesis (P = .84) or percent survival (P = .98).

In comparing the two groups, there was no significant difference in the rate of embryogenesis (P = .83) or percent survival (P = .99) due to treatment of geothermal or nongeothermal water. However, the rate of embryogenesis was extended significantly in the second group (P = .001) during the period prior to hatch. The percent survival was also significantly higher in the second group (P = .01) during the period of eye-up.

The experiment testing spawning success of common carp reared in constant 27^{o} geothermal water could not be tested due to the mortality of the entire control in February, 1980 by a rapid increased in water temperature, exceeding 41 C.

Gonadosomatic Indices (GSI's) were taken from the RRGS common carp males and females in August, 1979. The males exhibited a significant relationship between testes weight and body weight (P = .013) with an R-square value of 0.71 (Figure 3). The females exhibited a significant relationship between ovary weight and body weight (P = .0003) with an R-square value of 0.52 (Figure 4). However, there was no significant

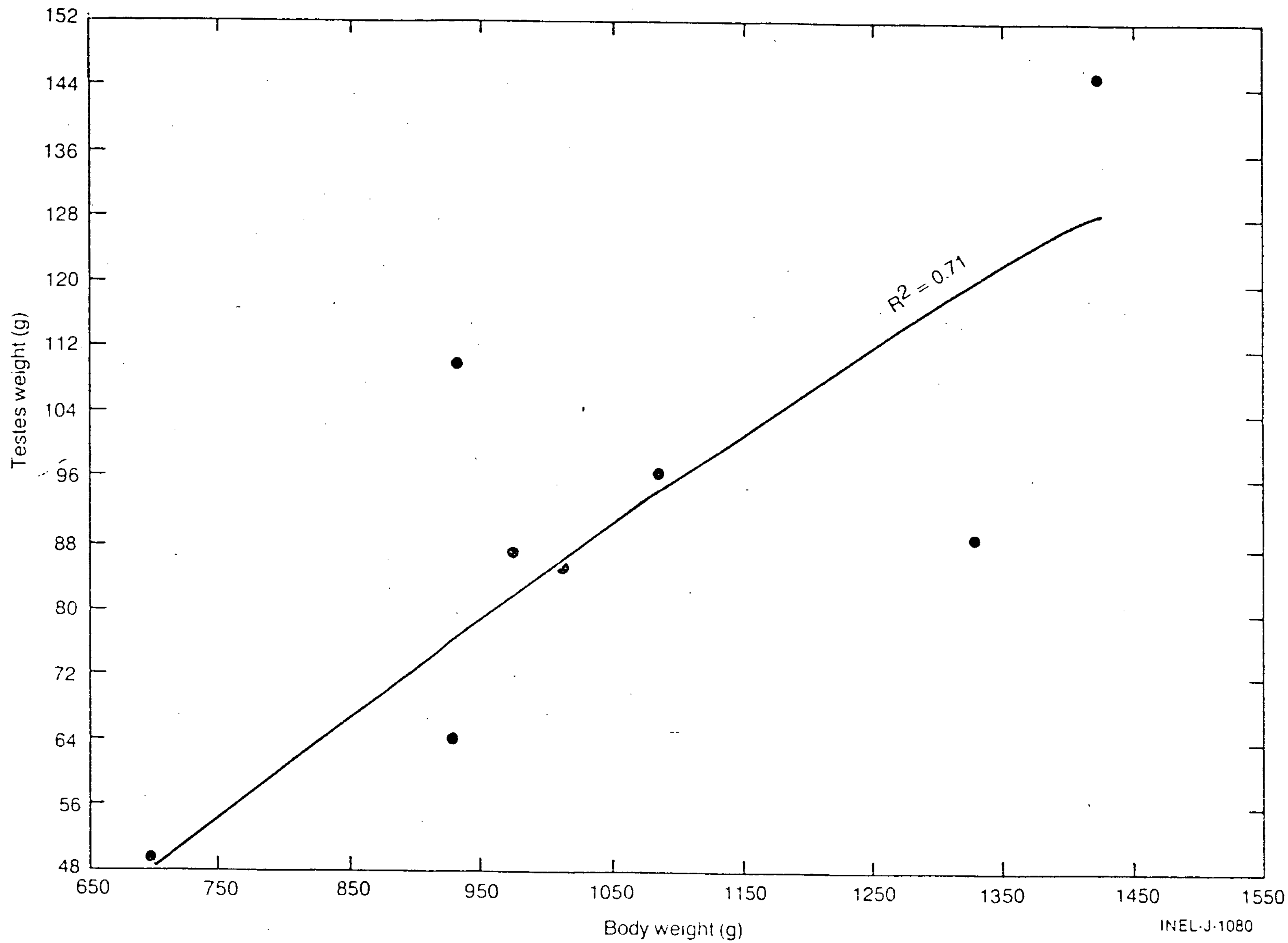

Figure 3. Gonadosomatic index of Raft Geothermal Site male carp.

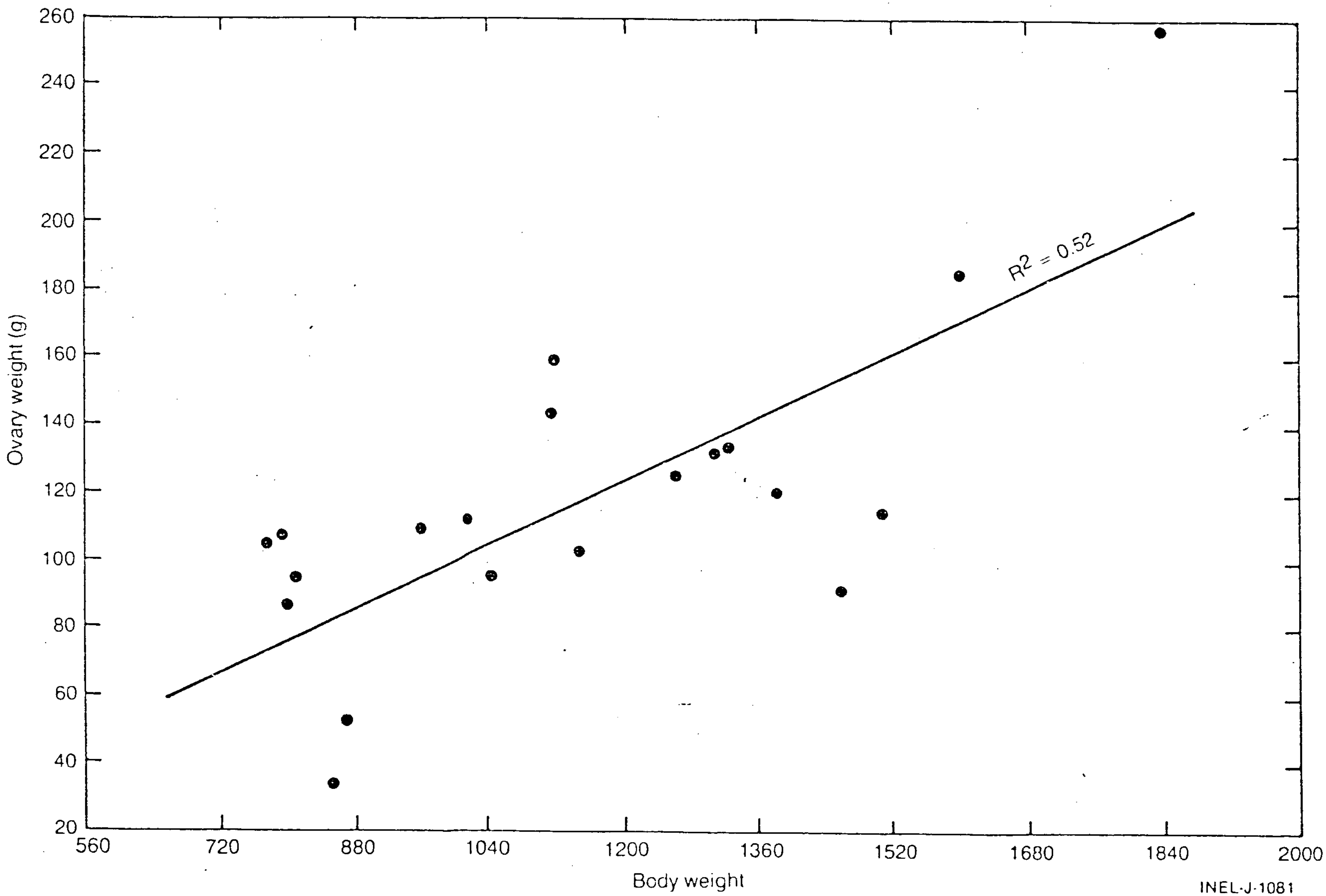

Figure 4. Gonadosomatic index of Raft River Geothermal Site female carp.

relationship (P = .15) between egg weight and body weight (Figure 5).

Gonadosomatic Indices were taken from the Moses Lake common carp males and females just prior to spawning on May 2, 1980. The males exhibited a significant relationship between testes weight and body weight (P = .0001) with an R-square value of 0.78 (Figure 6). The females exhibited a significant relationship between ovary weight and body weight (P = .0001) with an R-square value of 0.92 (Figure 7). There was also a significant relationship between egg weight and body weight (P = .0001) with an R-square value of 0.56 (Figure 8).

A comparison of GSI's was made between mature common carp from RRGS and Moses Lake. The regression slopes between RRGS males and Moses Lake males were not significantly different (P = .63), indicating a straight-line relationship between the two groups (Figure 9). However, the regression slopes between RRGS females and Moses Lake females were marginally significantly different (P = .015), indicating that there were two somewhat distinct groups to which regression lines could be applied (Figure 10). There was no significant difference (t = 1.86, 42 d.f.) between the mean egg weight from the RRGS females and the mean egg weight from the Moses Lake females (Figure 11).

Total Serum Protein (TSP) levels were taken from both the male and female common carp brood stock throughout the year at RRGS. There were no significant differences between male and female TSP levels at the RRGS in October (t = 0.367, 12 d.f.), January (t = 0.949, 19 d.f.), March (t = 0.75, 12 d.f.), April (t = 0.932, 12 d.f.) or May (t = 0.169, 9 d.f.).

The TSP levels for both males and females were combined for each sampling period. This combined sample mean was compared between months (Figure 12). There was no significant difference in the mean TSP levels between October and January (t = 1.232, 39 d.f.). However, there was a

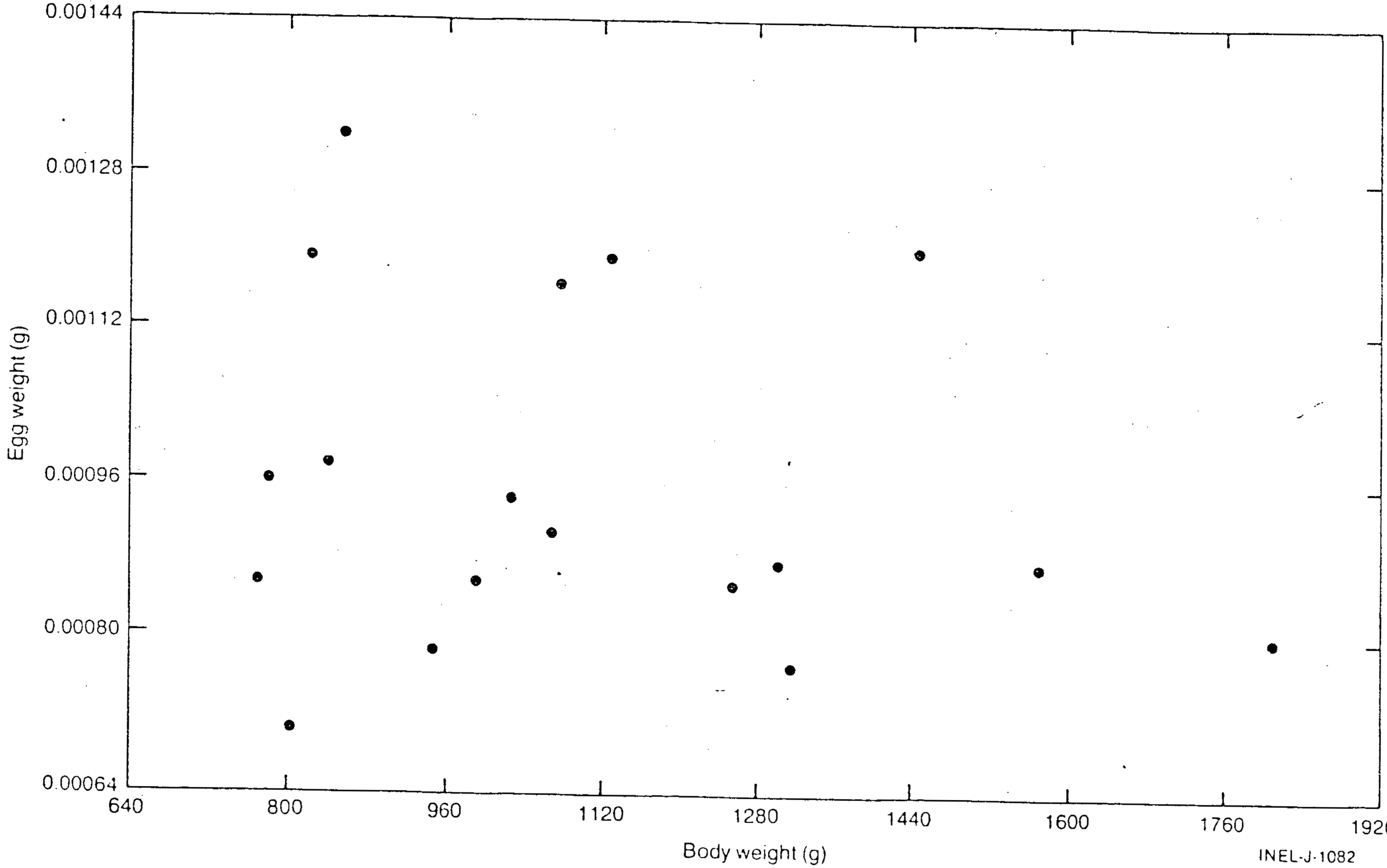

Figure 5. Regression of Raft River Geothermal Site carp egg weight on total body weight.

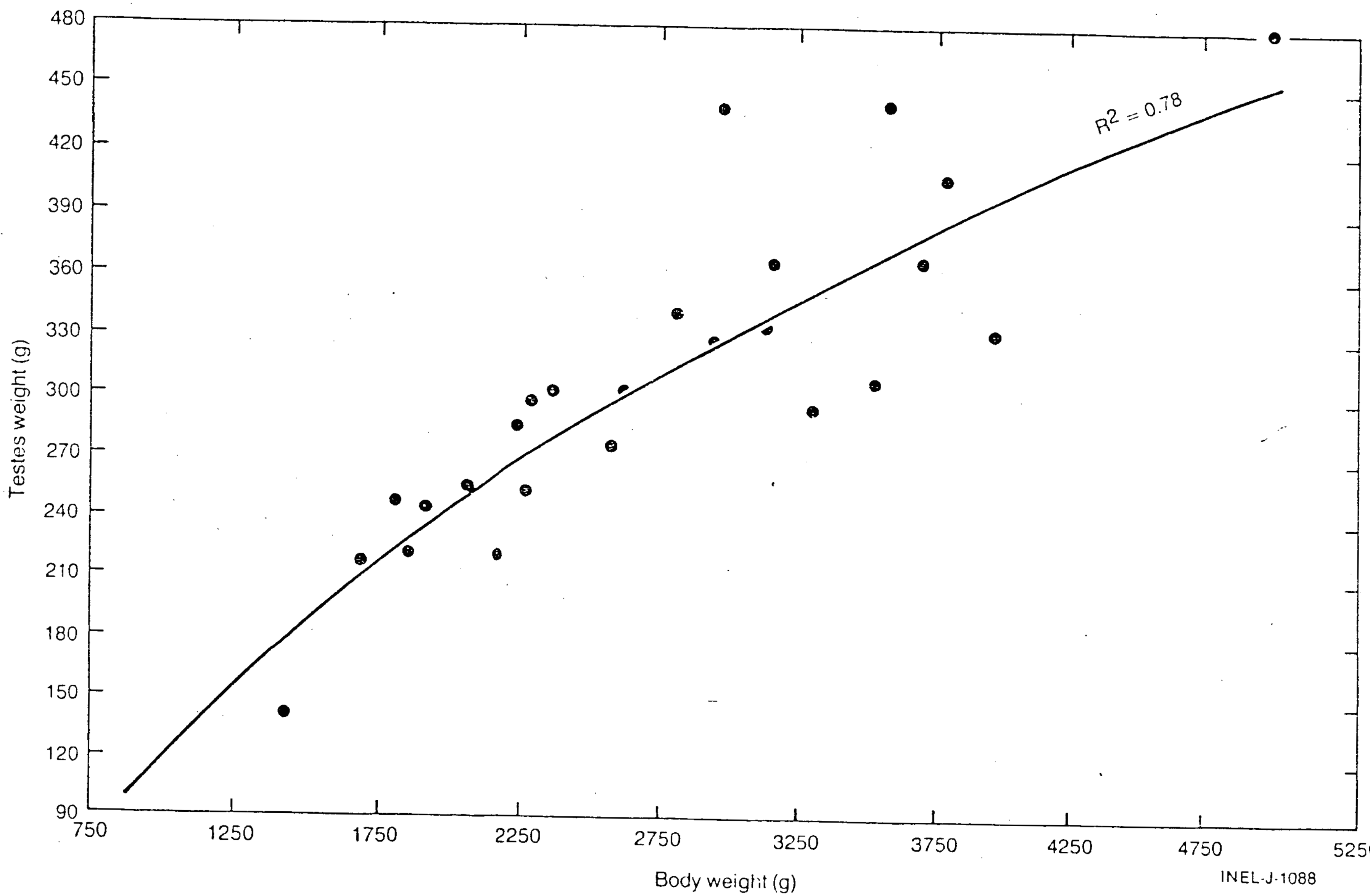

Figure 6. Gonadosomatic index of Moses Lake male carp.

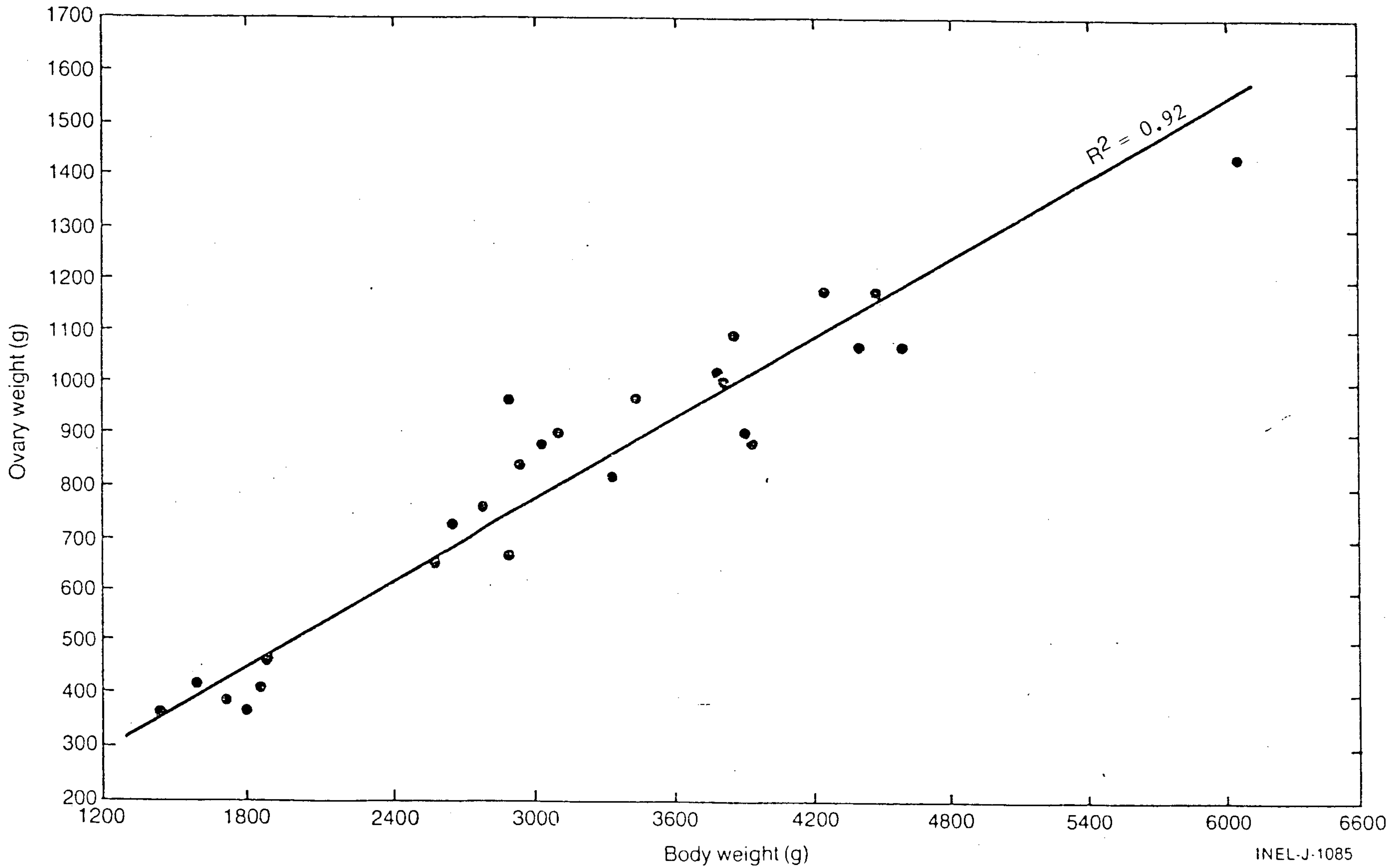

Figure 7. Gonadosomatic index of Moses Lake female carp.

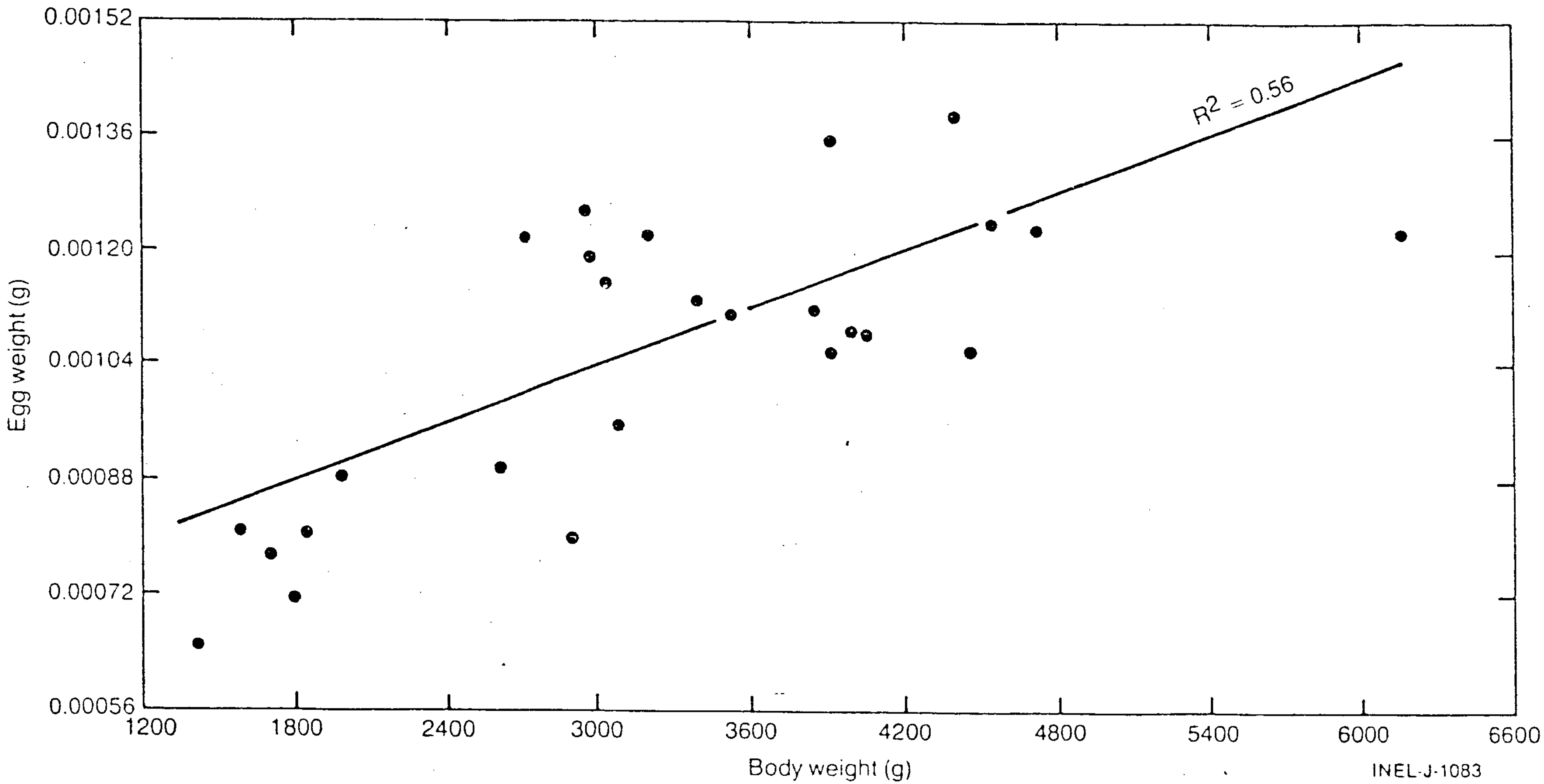

Figure 8. Regression of Moses Lake carp egg weight on total body weight.

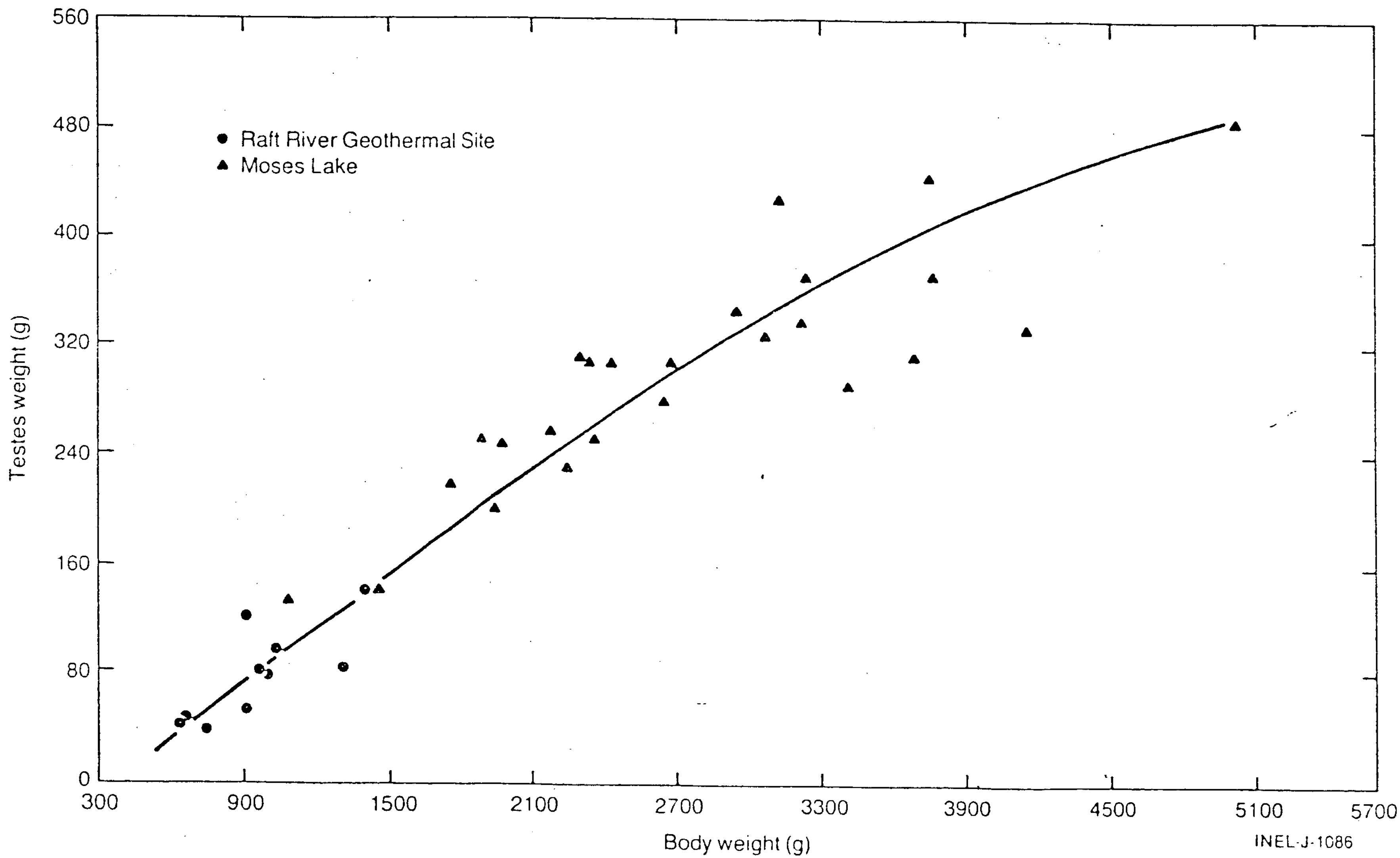

Figure 9. Gonadosomatic index of male carp.

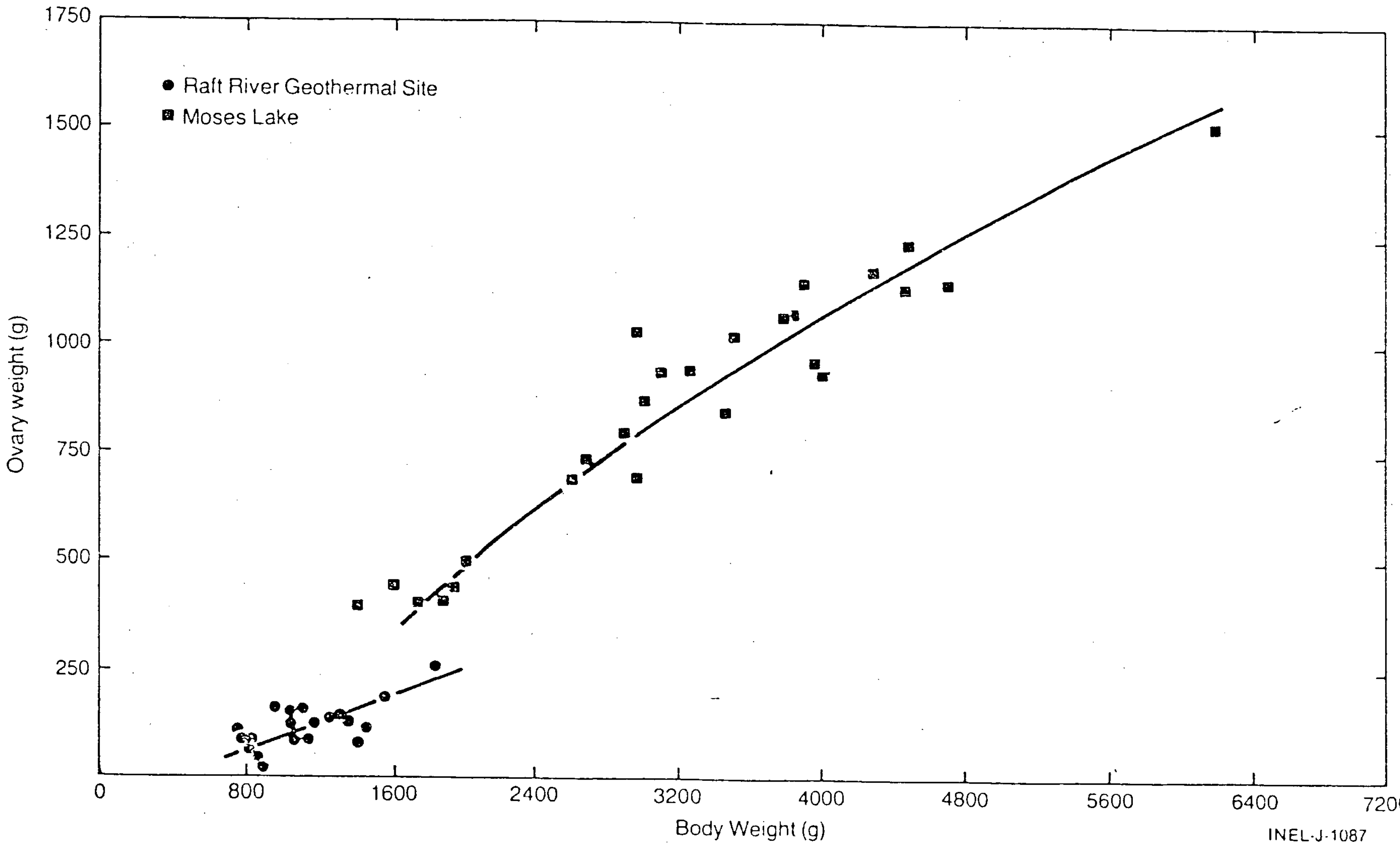

Figure 10. Gonadosomatic index of female carp.

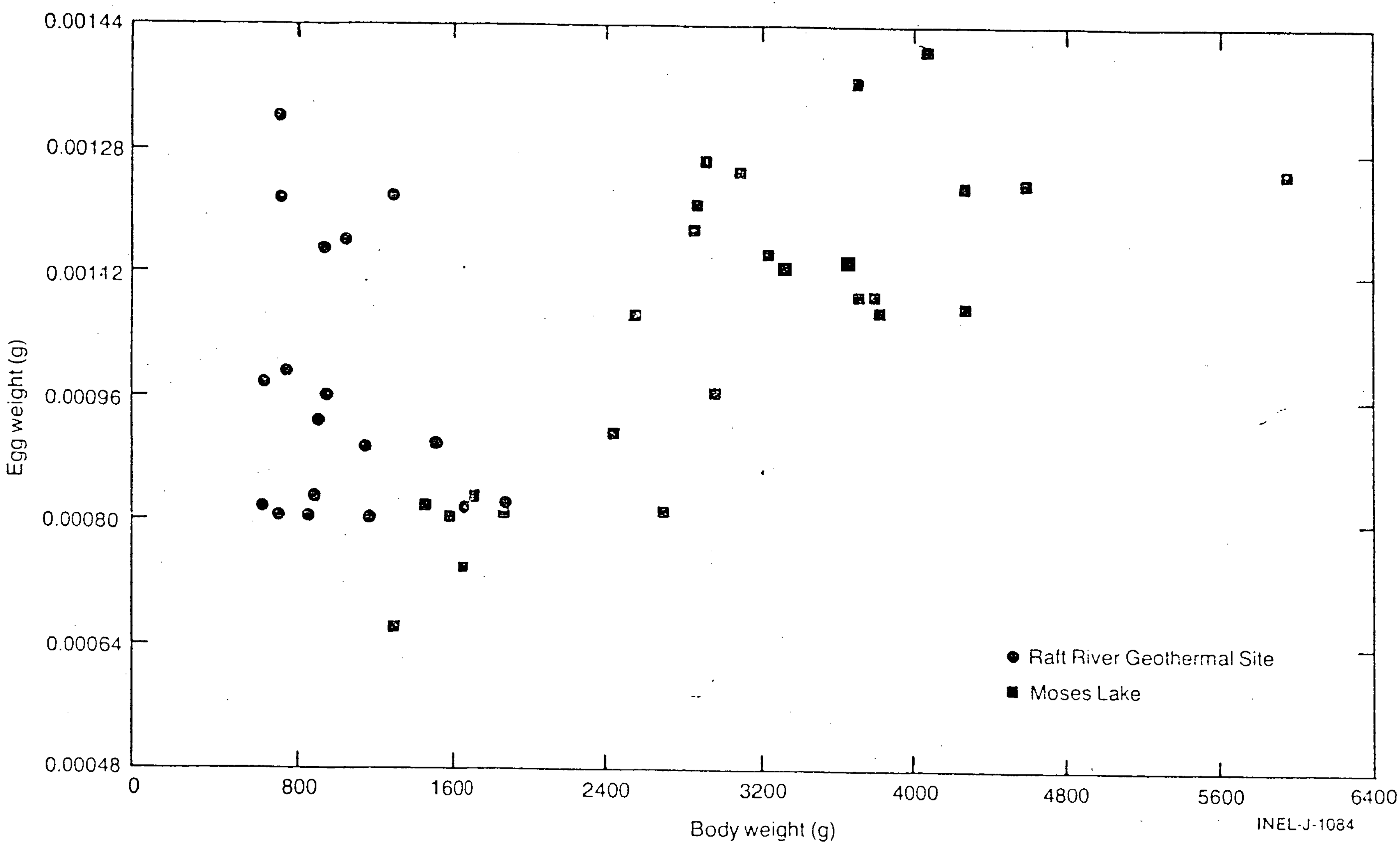

Figure 11. Regression of carp egg weight on total body weight.

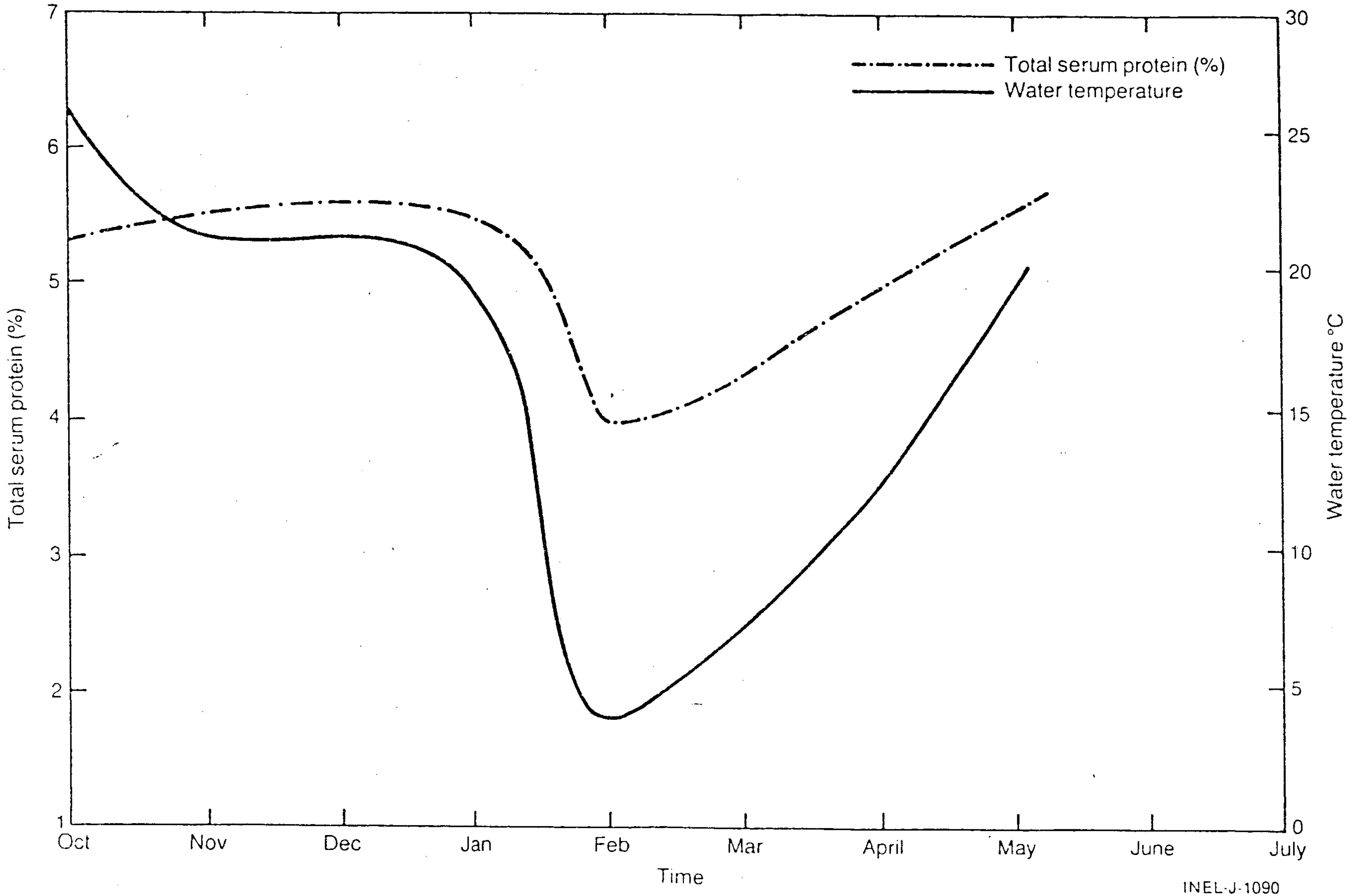

Figure 12. Carp blood serum protein at the Raft River Geothermal Site.

significant decrease in the mean TSP levels between January and March (t = 5.113, 33 d.f.), followed by a significant increase in mean TSP levels between March and April (t = 5.856, 26 d.f.), with another significant increase between April and May (t = 4.046, 23 d.f.).

Total Serum Protein levels were compared between male and female common carp blood stock in Moses Lake just prior to spawning. The mean male TSP level was significantly higher than the mean female level (t = 4.53, 48 d.f.).

Histological examination of sample embryos from all the successful spawnings revealed no apparent pathology. No abnormal development was evident histologically through the developmental stages examined.

6.0 Discussion

In 1979, two groups of common carp reared in constant 27 C geothermal water were spawned seven weeks apart. The first group was hormone induced to spawn; the second group was naturally induced. The results indicated no significant differences in either the first or second group's rate of embryogenesis or percent survival due to the ionic content of geothermal water.

However, when comparing the two groups, the first group exhibited significantly higher survivals at fertilization and eye-up, and significantly lower survival at hatch. Group one was induced to spawn by hypophysation techniques, possibly hurrying the release of immature oocytes (Pickford et al. 1957) and resulting in poorer fertilization and eye-up. In the second group, the females spawned in response to a natural increase in hormonal activity, resulting in more complete oocytic development and higher levels of fertilization and eye-up.

The technique for evaluating percent survival at hatch was changed from determining survival at the beginning of hatch in the first group to a more accurate determination of survival at approximately half-hatch, utilized in the second group. Since this was the period of greatest mortality in both groups the percent survival was significantly lower in group two, evaluated for survival at a much later time in the embryogenic development than was group one.

In 1980, the two groups of common carp were induced to spawn by natural means. Both groups obtained mean fertilization rates of 80-83%. In both groups, complete mortality of the embryos occurred (<0.01% survival) during and post hatch, between 80 and 170 hours post fertilization, during the period of rapid yolk absorption.

The spawning results indicate that the ionic content of geothermal water during the incubatory period did not significantly affect rate of embryogenesis or percent survival in the first or second run. However, there were significant differences between runs in rate of embryogenesis and percent survival. Bieniarz and Epler (1976) reported that if a carp has oocytes ready to be expelled during the spawning season but does not spawn, the mature oocytes will undergo a very slow process of resorption which may exceed two years, rendering the fish infertile during that period. Although the females that were not spawned in 1979 took an additional twenty-four hours to spawn, the resultant embryos had a significantly higher percent survival at eye-up than the embryos resulting from females that had spawned in 1979. This result indicates that the oocytes retained by the females during the first spawning season never reached final oocytic development and release from the follicle cells, and did not start the process of resorption.

The 24 hour delay in the spawning of the second run may have been significant in attaining a higher percent survival at eye-up. When checking the females in the first run prior to spawning, one particular female exhibited all esternal signs (Huet 1970, Alikunhi 1966) of ripeness. This female was placed in a spawning tank with the three other less ripe females that had spawned in 1979, along with six freeflowing males. Predictably, the ripest female was spawning early the next morning, and so were the other three females, apparently in response to the aggressive courtship behavior of the males and the active spawning of the ripe female. This may have hurried the release of the oocytes of the less mature females (Pickford and Atz 1957), resulting in poorer survival during the earlier stages of incubation.

The five females in the second run, none of which showed external signs of complete ripeness, took about forty hours of courtship behavior before they simultaneously spawned with six males. This longer period of courtship allowed a longer hormonal response in the females (de Vlaming 1974), which allowed a more gradual reinitiation of meiosis and release of the oocytes from the follicle cells. This could account for the greater percent survival of the second run, noted especially at eye-up.

The 24 hour delay between the spawning of the first and second run gave rise to an interesting test that was not originally anticipated. The question arises, does the greatest mortality of embryos occur after a specific exposure time to the incubating water, or is the mortality more a function of the embryonic stage? The result is important: in both the first and second run, the highest percent mortality occurred at a particular embryonic stage, irrespective of the fact that the second run took 26 hours longer to reach this stage.

The rate of embryogenesis for the second run was significantly longer than that of the first run during the period prior to hatching. Although the test was designed to compare the rate of embryogenesis between the two groups of females, this test lost its validity when the assumed constant temperature became a variable due to the cold shock resulting from a snow storm and its staggered effect on the two spawning runs (Figure 2). Although this difference in the rate of embryogenesis could be explained by the difference in female groups, it is much more plausible to apply this difference to the cold shock the eyed eggs received in the second run (Fish and Burrows 1939). While it is ture that the first run also received this cold shock, it is significant to note that they received it at a later time in their embryonic development.

The eggs in group two received the cold shock during the period of eye-up. The embryos of run one received the cold shock after the initiation of hatching. This period of hatch and post-hatch has been shown to be the period of greatest mortality. By delaying this period of mortality during eye-up, group two exhibited a longer rate of embryogenesis, shown in Figure 2.

The additional length of time the second run required to hatch is put into perspective by Swee and McCrimmon (1966), who reported that wild populations of common carp eggs took from 50-144 hours for half of the eggs to hatch, depending on the temperature. Carp are eurythermic fish, and carp eggs are able to withstand a wider range of thermal exposures than eggs of stenothermic fish. However, Frank (1974) reported that common carp go through two developmental stages that are particularly sensitive to thermal shock: cleavage and blastopore-closing stages. Both of these stages occurred well before the cold shock affected either the first or second group. The conclusion is drawn that the cold shock had little impact on the mortality of the embryos.

The optimum physiological temperature (of SET) reported for common carp is 25 C (Beamish 1964). Thus the environmental conditions facing the common carp during the first year in geothermal water was comprised of temperatures consistently higher than SET, with naturally occurring photoperiods. Khiet (1970) reported that an environment with a consistently higher than normal SET, and natural day lengths throughout the year resulted in poor gonadal growth and reproductive capacity of the Japanese cyprinid honmoroko (Gnathologon elongatus). Although no information is available as to the gonadal response of the common carp to the environmental conditions indicated above, both cyprinids honmoroko and common carp are spring, asynchronous spawners (Khiet 1970, Yamamoto and Yamazaki 1961). The control for the experiment designed to test the effects of two temperature regimens on reproductive capacity - was killed in February, 1980. However, the test fish were left intact and were spawned. The results, as indicated above, were that 100% of the embryos produced from the females reared in the cold annual temperature regimen dies before swim-up. Although this test did not have a control, the results are important because they were negative, indicating that even with an annual cold cycle following that in Potholes Reservoir, Washington, the females could not produce viable offspring in geothermal water.

The possibility of nitrogen supersaturation and subsequent gas-bubble disease was a concern. The only data on dissolved gas analyses were taken at the well-head (Table 1), and indicated some validity for the concern. Nitrogen saturation was reported at a mean of about 180%, significantly above the 120% tolerable limit of adult carp (Miller 1974). If this level of saturation remained in solution from the well-head to the aquaculture facility, the fish would have shown some clinical sign of gas-bubble disease throughout the last two years of culture. A closer look at the water system shows that the well-head water was sprayed into the air in both cooling ponds before artesian flow brought it to the facility, eliminating the pressure that originally supersaturated the nitrogen. The dissolved oxygen level increase from .005 mg l^{-1} at the well-head (Table 1) to 4.5-5.5 mg l^{-1} at the aquaculture facility, indicating a thorough mixing and spraying of the water in the cooling ponds. Although yolk-sac fry are more susceptible to gas-bubble disease than are adults (Wood 1968), no clinical signs of gas-bubble disease have ever been observed in any yolk-sac fry sampled at the RRGS. This observation, together with the method and volume of water sprayed into the air for cooling, indicates that nitrogen supersaturation was not a problem at the RRGS aquaculture facility.

The results of the GSI's from the RRGS and Moses Lake indicate that egg weight and testes weight/body weight were not significantly different between the two populations at maturity (Figures 9 and 11). However, the ovary weight/body weight of the RRGS females was significantly lower than that of its Moses Lake counterpart (Figure 10). This is in spite of the equal mean ovum weight of the two populations of fish sampled (Figure 11). This apparent discrepancy is explained by the fact that the RRGS females were first year spawners when sampled in 1979, and the females sampled at Moses Lake represented age classes from two to approximately fifteen years. The qualitative evaluations of the gameters (i.e., freeflowing milt, shape of oocyte and nucleus positioned within the oocyte) have all been positive. The conclusion is drawn then, that gonadogenesis was complete prior to spawning.

With such a hardy, prolific, adaptable fish as the common carp, what could be causing such complete mortality at this particular embryonic stage of development? Several possibilities have been ruled out by this study: ionic content of the geothermal water for incubation, completeness of gonadal development of the males and females, and temperature effects on gonadal and embryogenic development. Other possibilities have been ruled out with further insight into the design of the RRGS aquaculture system: photoperiod effects on gonadal development, and gas-bubble disease affecting the eggs or yolk-sac fry.

Under the doubtful contention that either the yarn or the wood comprising the kakabans contained toxic substances, eggs were left adhered to the spawning tanks with controls of deionized water set up in buckets. These eggs followed the identical pattern of mortality as their counterparts on kakabans in the glass incubating aquaria.

There are two possibilities which were not tested during the course of this study, and should be the focus of future research: the effects of diet on the production of viable gametes, and bioaccumulation of heavy metals in the oocytes.

The common carp were reared completely on a diet of Silver Cup trout feed, with no supplemental natural feed provided. Although this diet is sufficient to rear actively spawning rainbow trout in the hatchery environment, the fact that the diet is designed for carnivorous fish may be significant when feeding it as a sole food source to the omnivorous carp. Few diets have been tested as a sole source of food for carp, and none were found to have been tested with the goal of proper and complete development of the gametes (Chervinski et al. 1968, Hepher and Chervinski 1965).

In geothermal wells containing high levels of dissolved solids, the presence of heavy metals may be pronounced. Although no metal by itself exceeded tolerable limits at the RRGS (EPA 1976), the bioaccumulation work in 1979 showed light bioaccumulation of lead, mercury and zinc. This analysis focused only on heavy metal accumulation in the edible portion of the fish flesh (Beleau et al. 1979). Visceral organs that also accumulate metals and organic compounds were not tested. Concentrations of mercury by fish have exceeded 25,000 times the amount of mercury in the surrounding water; and chronic tests of mercury on three generations of brook trout showed reproductive impairment (McKim 1976). In the embryogenic development of the common carp hatchling, the greatest mortality occurred during the rapid absorption of the yolk-sac. Any toxic metal bioaccumulated in the lipids of the yolk would have its greatest impact on the functions of the animal during this period of rapid yolk absorption.

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