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THERMOREGULATORY METABOLISM IN MALLARD DUCKS (*ANAS PLATYRHYNCHOS*) EXPOSED TO CRUDE OIL AND DISPERSANT

by

GABRIELLE LAMBERT

Presented to the School of Graduate Studies and Research of the University of Ottawa in partial fulfillment of the requirements for the degree of Master of Science

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ABSTRACT

THERMOREGULATORY METABOLISM IN MALLARD DUCKS (ANAS PLATYRHYNCHOS) EXPOSED TO CRUDE OIL AND DISPERSANT

Gabrielle Lambert

Effects of external contact with oil and/or dispersant on mallard ducks (Anas platyrhynchos) were studied under controlled laboratory conditions. Groups of ducks were exposed to either a 50 µm oil slick of Prudhoe Bay Crude Oil, to a 50 µm oil slick of oil dispersed with Corexit 9527 (in a 30:1, oil: dispersant ratio), or to Corexit 9527 alone, in seawater.

After preliminary testing to optimize methodology, oxygen consumption and CO_2 production were measured as an index of metabolism in response to contamination with oil and/or dispersant. Metabolism was calculated over a 3 hour period using an open-flow metabolic chamber kept at approximately - 12°C, and Beckman oxygen and CO_2 analyzers. Each bird was used as its own control. Metabolic rates were calculated on pre-exposure runs to establish basic metabolic rate (BMR); on day of exposure to determine immediate effects of the specific pollutant; and on post-exposure runs to follow their recovery over a two week period.

The average percent increase in metabolic rate was calculated for each treatment group. External contact with oil resulted in an increase in metabolic rate on the day of exposure, followed by a further increase over the next two weeks. This may have been caused partly by ingestion of oil during preening. External exposure to dispersant for a period of one hour did not result in an increase in metabolism although it was noticed to cause a progressive waterlogging and sinking of the ducks. The wetting effect lasted over the two-week recovery period but still had no effect on metabolism.

Combination of oil + dispersant showed to be quite stressful to ducks on the day of exposure. Compounding waterlogging and sinking due to dispersant with damage to the plumage caused by oil, the metabolic rate increase calculated on the day of exposure was more than the sum of effects of oil and of dispersant separately. This "synergistic" effect seen on the day of exposure however decreased during the recovery period where the increase in metabolism in ducks exposed to oil + dispersant became almost identical to that seen in ducks exposed to oil alone.

A consideration resulting from this study, applicable to oil spill mitigation measures, is that when facing the decision of dispersant use to control an oil spill. In areas of shallow water (small pools or estuaries), use of a dispersant might not lead to an increase in the metabolic stress caused by oil but it might expose birds to waterlogging and

sinking and eventually to drowning. In areas physically removed from shortlines, or where there is no seabirds activity, the use of a dispersant is innocuous to birds, and may be recommended since the dispersant will mix with oil and remove it from the surface.

RESUME

EFFETS DU CONTACT EXTERNE AVEC DU PETROLE ET/OU DU DISPERSANT SUR LE METABOLISME DES CANARDS MALLARDS (ANAS PLATYRHYNCHOS)

Gabrielle Lambert

Cette étude porte sur les effets du contact externe avec du pétrole et/ou du dispersant sur les canards mallards (Anas platyrhynchos) en conditions contrôlées de laboratoire. Les canards ont été exposés, soit à une nappe d'environ 50 µm d'épaisseur de pétrole brut de Prudhoe Bay, soit à une nappe de 50 µm d'épaisseur de pétrole dispersée à l'aide de Corexit 9527 (dans une proportion de 30:1, huile:dispersant), soit à du Corexit 9527 seul, dans un réservoir d'eau salée.

Après une série de tests visant à optimaliser la méthode utilisée, le métabolisme des canards a été mesuré, utilisant comme indice la consommation d'oxygène et la production de CO_2. Ainsi les variations du métabolisme des canards, en réponse à la contamination avec l'un ou l'autre des polluants, ont pu être calculées et comparées entre différents traitements.

Le métabolisme a été calculé sur une période de 3 heures à l'aide d'une chambre métabolique à flot continu, gardée à environ -12°C. L'analyse de l'air sortant de cette chambre métabolique a été faite à l'aide d'analyseurs d'oxygène et de CO_2 de Beckman. Le métabolisme de base de chaque oiseau a d'abord été calculé pour servir de point de référence individuel, afin d'évaluer l'effet de la contamination par le pétrole et/ou le dispersant sur le métabolisme des oiseaux. Le métabolisme de chaque canard a été calculé: avant le contact avec le polluant, ce qui a servi à déterminer son métabolisme de base; immédiatement après la contamination par le pétrole et/ou le dispersant, pour mesurer l'effet immédiat du polluant; et à deux reprises au cours des semaines suivantes pour déterminer les effets à plus long terme. La moyenne de l'augmentation du métabolisme a été alors calculée pour chaque groupe. Le contact externe des oiseaux avec le pétrole a amené une augmentation du métabolisme le jour même de la contamination, suivie d'une autre augmentation maintenue au cours des deux semaines suivantes. Une partie de cet accroissement peut être due à l'ingestion de pétrole au cours du nettoyage du plumage.

Le contact externe avec un dispersant durant une période d'une heure n'a pas entraîné d'augmentation du métabolisme, quoiqu'il ait causé un mouillage progressif du plumage, jusqu'à ce que l'oiseau sombre. Cet effet de mouillage a persisté durant les deux semaines de récupération, bien qu'il

n'y eut aucun effet sur le métabolisme.

Le mélange pétrole + dispersant s'est avéré très stressant pour les canards le jour même de leur contamination. Le dommage physique causé à leur plumage par le pétrole s'est ajouté au mouillage résultant de la présence d'un dispersant. L'augmentation du taux métabolique calculée au moment du contact avec le pétrole + dispersant a été supérieure à la somme des effets causés par le pétrole et le dispersant séparément. Ce synergisme, entre les deux polluants, observé le jour de la contamination, a disparu au cours des deux semaines subséquentes jusqu'à ce que le taux métabolique devienne identique à celui des canards exposés au pétrole seulement.

Une considération en particulier ressort de cette étude et devrait être évaluée sérieusement face à la décision d'utiliser ou non un dispersant dans le cas d'un déversement de pétrole en milieu marin. Lorsqu'une nappe de pétrole se répand en eau peu profonde (près d'un estuaire ou de marais), l'usage d'un dispersant, quoiqu'il n'entraîne pas de stress métabolique, peut par contre causer un mouillage sérieux et même la noyade des oiseaux.

Dans le cas d'un déversement de pétrole en milieu marin géographiquement isolé de la côte, ou dans un endroit où l'on retrouve très peu d'oiseaux, l'utilisation d'un dispersant est alors innoffensive envers les oiseaux. On pourrait alors en recommander l'utilisation, puisque le dispersant se mélangera avec le pétrole etentraînera sa dispersion, nettoyant ainsi la surface de l'eau.

Chapter 1

INTRODUCTION

1.1.0 Crude oil

Since the 1900's, crude oil production has increased around the world by more than 150 - fold, to a projected yearly production of over 3 billion tons in 1980 (Korte & Boedefeld, 1978), with the greatest portion of this increase occurring in the last 20 years. Use of petroleum is generally concentrated in major industrial areas situated along seacoasts or beside rivers that empty into coastal waters (Wilson & Hunt, 1975). This rapid increase in demand and utilization of petroleum and petrochemical products has resulted in a steadily increasing level of petroleum contamination of marine and estuarine waters. With the introduction of supertankers and increased exploitation of offshore deposits around the world, problems of oil pollution of marine habitats can only be expected to continue (Anderson et al., 1974). The National Academy of Science, however, seems very hopeful that new methods of loading, deballasting and bilge pumping will prevent an increase and even lead to a decrease of the present level of petroleum contamination in marine environments (in preparation, 1982).

1.1.1 Sources of petroleum contamination

Wilson and Hunt (1975) have estimated that over 6 million metric tons of petroleum are introduced into the sea each year from various sources. The most spectacular are, without doubt, major oil spills that result from marine accidents involving barges, ships dragging their anchor across submarine pipelines in bays, or offshore well blow-outs. Interestingly enough, such accidents contribute to no more than 3 to 5% of the total input of oil into the sea (Hyland & Schneider, 1977).

Major contributors, accounting for as high as 45 to 50% of the total influx of oil into the environment, are land-based discharges from inadequate waste-oil disposal, sewage and run-offs. This includes operation of motor vehicles, lubricants, cutting and hydraulic oils, oil used in heating systems, coolants, solvents, etc. "Normal" marine operational discharges from shore facilities and terminal operations, including deballasting, tank flushing and washing, bilge pumping and dry-docking, account for about 30% of total input of oil at sea.

Another less significant contributor of oil pollution is the operation of and discharge from oil refineries and petrochemical plants. As a result of increasingly restrictive laws and regulations, refinery contributions have been steadily decreasing to less than 5% of total dumping of oil into the sea. Because of our extensive use of petroleum and petroche-

mical products, the atmosphere has progressively become saturated with hydrocarbons, resulting in atmospheric fall-out as dustfall or in rain, accounting for as much as 10% of the total oil at the sea. Natural seepage from offshore deposits also contributes significantly to about 10% of entry of oil into the sea and is the only truly uncontrollable agent of oil pollution (Hyland & Schneider, 1977).

1.1.2 Composition and fate of crude oil in the marine environment

Crude oil is a complex mixture of organic compounds, mostly hydrocarbons (50 to 98%), of which the more volatile and soluble elements are very toxic to marine organisms (Bourne, 1976). Hydrocarbons can be subdivised into 3 classes: aliphatics or straight-chained compounds; alicyclics in which all carbons are arranged in a ring; and aromatics which contain at least one benzene ring. This last group includes the most toxic compounds such as benzene, toluene, xylene, naphthalene and phenanthrene. Crude oil also contains traces of various compounds of nitrogen, sulphur and oxygen as well as metallic compounds of vanadium, nickel and mercury (Korte & Boedefeld, 1978).

When assessing ecotoxicological effects of a pollutant such as petroleum, not only direct and indirect adverse consequences of the chemical under review will have to be taken into consideration but also any effect of its degradation

products. When oil reaches the marine environment, it immediately undergoes weathering processes that alter its physical and chemical nature. Spreading of oil over the surface until it forms a thin continuous layer or oil slick will increase its surface exposed to air, water, sunlight and therefore will enhance greatly other weathering processes that will follow: evaporation of volatile components, which itself is very sensitive to environmental factors such as salinity and temperature (Laughlin et al., 1979); dissolution or disintegration and dispersal of oil into the water column; emulsification which is the mixing of water into a non-miscible oil resulting in a stable emulsion known as "chocolate-mousse"; adsorption of oil to any material present; agglomeration or compaction of the remaining oil components into tar balls and their sinking to the bottom or accumulation on shores; biological degradation of the remaining oil by microorganisms (bacteria, yeasts, fungi), zooplankton or invertebrates (copepods, barnacles, mussels, etc.) through ingestion and excretion of oil present in the form of minute droplets; and finally oxidation of crude oil through thermal or photochemical processes which also contributes to the modification of oil at sea and which is obviously dependent on climatic conditions (Clark & Brown, 1977).

1.1.3 Effects of petroleum on the marine biota

Numerous extensive and detailed studies have been published on the effects of petroleum on a wide variety of organisms, from bacteria, algae and plankton to fish, birds and mammals; at lethal and sublethal levels; on short and long term; in cold and warm environments and under various other conditions, as well as the total ecological impact of the presence of petroleum in marine and estuarine environments.

The most obvious impact of petroleum is its acute toxicity to a wide range of aquatic organisms. This is easily observed and quantified, but may represent only a fraction of the total impact of petroleum on the biota. Much harder to assess would be direct chronic effects such as delayed toxicity, modification of behavior leading to reduced feeding success, decreased resistance exposing animals to infections, predation and other stresses, interference with reproduction processes and success and retardation in growth or development setting the animal "out-of-tune" with its environment. An animal may also suffer indirect effects of petroleum resulting from a reduction or pollution of its food supply or decreased food value through incorporation of oil and oil products into the marine food chain.

In an even broader scope, we can see that reduced reproduction or survival of a species at any level of the food chain can lead to a reduction of species diversity and an unbalanced predator-prey relationship. Bioaccumulation of oil

along the food chain eventually affects organisms which were never exposed to the original pollution, thus impacting the whole biological community (Ricklefs, 1974).

1.2.0 Clean-up of oil spills

Strict legislated regulations have been imposed on waste oil disposal over the last decade to reduce negligent dumping of petroleum and petroleum products at sea. Simultaneously, considerable efforts have been made in the field of oil spill clean-up to try to minimize damage resulting from an accidental spill.

1.2.1 Physical containment and recovery of oil

Many modern methods of containment and recovery of oil have been devised and tested: floating booms and barriers which physically restrain spreading of an oil slick; sorbent booms, made of porous materials such as straw or synthetic products which combine containment and recovery; bubble barrier, producing a rising curtain of bubbles against which the slick cannot spread; vacuum-type suction equipment to pick up oil from the water surface (Fingas et al., 1979). All these different methods of physical containment and recovery share one common limitation which is their availability at the site of an oil spill immediately after an accident, independently of the time of day, season or weather conditions. They also

imply the availability of trained and qualified manpower and adequate material everywhere and at all times where there is as much as a threat of an accidental spill.

The perfect application of such methods is therefore unrealistic and this is certainly what has led researchers into other or additional approaches, such as the use of a chemical agent which could be added to oil to facilitate its clean-up or removal from the water surface.

1.2.2 Oil spill dispersants

Oil spill dispersants were brought out on the market about 20 years ago, promising to be the all-time solution to the ecological problems resulting from accidental spills at sea or near shores. These new chemicals made use of synthetic surface active agents, developed during the 1950's to replace classical soaps in detergents and known for their property to emulsify oil into water, therefore "removing" oil from the water surface. They were given the hopeful name of "oil spill removers". As more and more got to be known on their toxicity to a wide range of organisms, it became clear that these removers did not magically remove oil but actually pushed it into the water column. Their name then changed to "solvent-emulsifiers", "synthetic detergents" and finally "oil spill dispersants".

The basic composition of a dispersant is a mixture of: a surfactant (contraction of surface active agent) , the

molecular structure of which contains both a hydrophilic group and a lipophilic group, normally a hydrocarbon chain which acts as primary emulsifier; an organic solvent which serves as carrier and enables the surfactant to mix with oil to form an emulsion, and a stabilizer which permits maximum dispersion of oil and prevents coalescence of oil back at the surface of water (Butler et al., 1974). Because of its amphiphatic (both hydrophilic and lipophilic) nature, a dispersant locates itself at the oil-water interface, as shown Fig. 1. A surfactant reduces the interfacial tension between two non-miscible liquids and thereby aids in forming of oil-in-water emulsions and in generating of finely dispersed droplets (Canevari, 1977).

The advantage justifying using a dispersant to handle an oil spill in obviously that it removes oil from the water surface by dispersing it into the water column. This eliminates fire hazard, air pollution from volatilization of light petroleum hydrocarbons and immediate threat of acute oiling to waterbirds and to other air-water interface inhabiting species. It also accomplishes a great aesthetic improvement by removing oil from sight. It also prevents formation of the previously mentioned "chocolate-mousse" and tar balls which are particularly difficult to deal with when they accumulate on shores and beaches (Canevari, 1975). The use of a dispersant also seems to reduce adsorption of oil to objects such as plants and animals in intertidal areas, beaches, islands (Exxon,

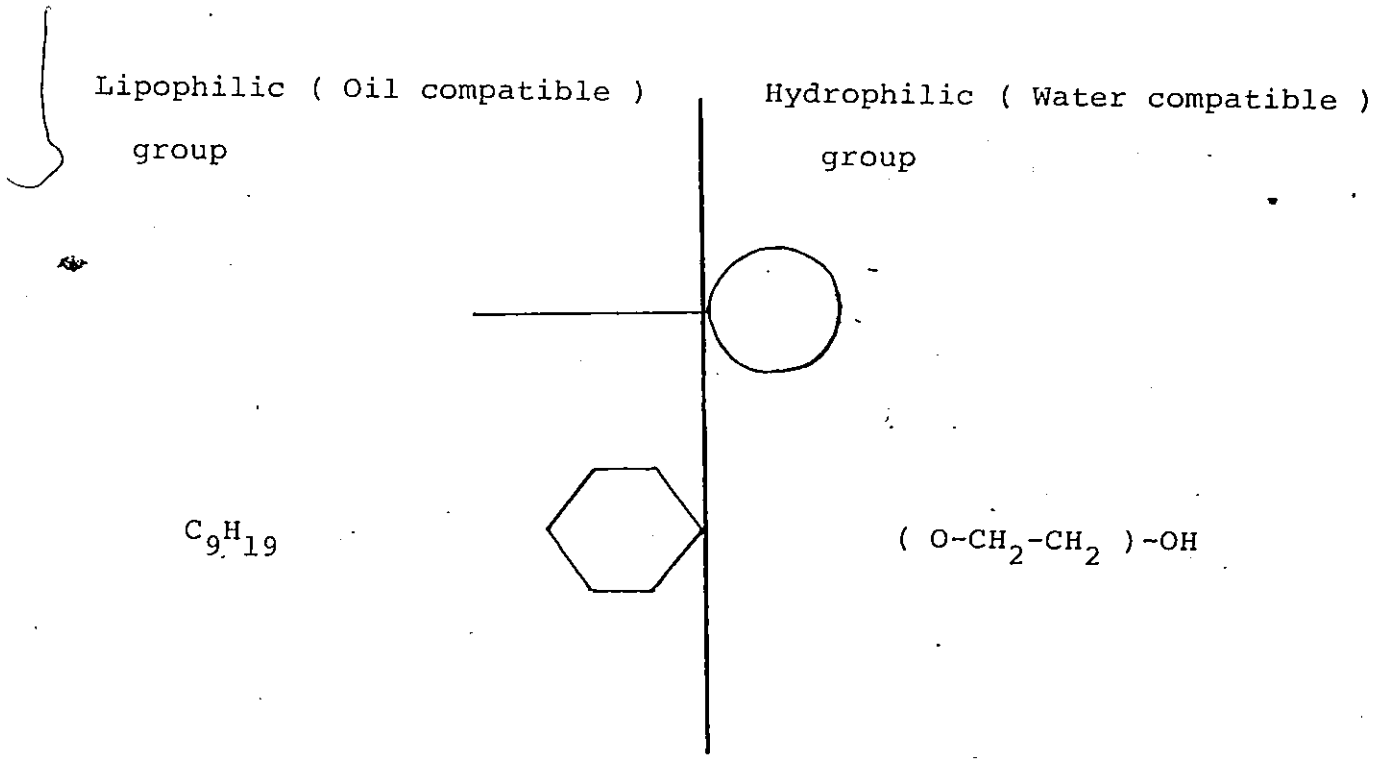


Fig.1. Schematic representation of a typical surfactant: nonyl phenolethyne oxide (from Canevari, 1977)

1978). A dispersant also increases the surface area of spilled oil, permitting more rapid weathering and biological alteration (Wells & Keizer, 1975).

On the other hand, using a dispersant on an oil slick causes the formation of tiny oil droplets of about 1 micron diameter (Canevari, 1977) which greatly increases the total surface area of oil in contact with water. This accelerates diffusion of petroleum hydrocarbons into the water column and their concentration rapidly increases to 10 times or more their previous level (Wells & Keizer, 1975; MacKay & Leinonen, 1977; Trudel, 1978) thus placing oil into greater potential contact with biological systems.

The application of a chemical dispersant also means that more potentially toxic xenobiotic compounds are added to those already present (Trudel, 1978). All solvents such as those used in the composition of a dispersant contain aromatic hydrocarbons. The higher the proportion, the more effective (but also the more toxic) the solvent (Butler et al., 1974). Therefore, the specific toxicity of oil can be greatly increased by addition of a dispersant and affect species that would normally not have been affected by floating oil.

Consequently, there is a much greater short-term biological impact in intertidal areas, shallow estuaries or wetlands than that seen with oil alone (Albers, 1979). It should be emphasized though that this increased environmental toxicity of oil when treated with a dispersant is due not only

to addition of a potentially toxic dispersant to oil already present but also to the fact that a dispersant substantially increases the concentration of oil in the water column. Since the toxicity of oil to most organisms is dependent on its concentration, this "dispersion" of oil into the water column logically results in an increase in environmental toxicity, proportional to the increase in concentration.

The effects of dispersant and dispersed oil on the marine biota have been reported for years with special emphasis on marine invertebrates and fish which are obviously the most likely to come in contact with any significant quantity of these pollutants.

1.3.0 Toxicity of oil and dispersants to bird

Oil from natural seepage through parts of the ocean floor has already been known since the 15th and 16th centuries to represent a threat to seabirds but it is only since the beginning on this century that man has added to this pollution by spilling considerable amounts of oil at sea especially near shores.

Already in 1907, release of two million gallons of crude oil into the waters around the Isles of Scilly led to high mortality among birds in the vicinity of the spill (Holmes & Cronshaw, 1977) . Numerous other reports on effects of oil spills on birds were published during the period between First and Second World Wars. But it was not until oil pollu-

tion reached a serious level that a general public awarness of this problem developed and led to publication of more complete records of mortality among seabird populations (Holmes & Cronshaw, 1977). Thousands of seabirds die each year presumably as a direct result of contamination with oil. The most vulnerable species are sea ducks (scoters, eiders, etc.), alcidae (auks and relatives) , laridae (gulls and terns), loons, grebes, skimmers, cormorants (Gochfeld, 1979).

The vulnerability of a particular species to oil contamination is strongly correlated with the proportion of time they spend on water and also dependent on their behavior. Colonial birds which congregate in flocks in offshore waters or at breeding or wintering grounds more frequently come in contact with oil. Migratory birds might be exposed to oil while migrating near heavily trafficked shipping lanes (Croxall, 1977).

The behavior of marine birds may affect their vulnerability to contamination by oil slicks. For example, while feeding, diving ducks do not appear to avoid oil and consequently often become covered with oil when they break the surface through an oil slick (Szaro, 1977).

Seabird mortality resulting from contamination with oil has been shown to continue for several weeks after a spill (Holmes & Cronshaw, 1977; King et al., 1979). The cause of mortality is either an acute external and physical contamination leading to a rapid death of the bird or a long-term sys-

temic contamination resulting directly or indirectly from ingestion of petroleum.

1.3.1 Effects on plumage

The watertight plumage of seabirds is dependent on constituent feathers in which a system of interlocking barbs provides a strongly water-repellent surface (Rijke, 1970; Clark, 1978). This system is arranged in such a way that there is a gradient of water repellency from the base to the tip of a feather so that a drop of water placed on it tends to run off towards its tip (Clark, 1978). For a long time, it was thought that the monolayer of preen gland wax which is spread on the feathers during preening gave the plumage its water repellency. This theory was dispelled by several studies showing that even in completely soaked and waterlogged birds, the wax coating of feathers remained intact (Clark, 1978) . Waterproofness of the plumage is dependent on its feather structure and secretion and spreading of preen gland wax helps in maintaining this physical structure but does not function as a waterproof coating, as shown by experiments in which the gland was extirpated but waterproofness not affected (Rijke, 1970). A layer of air trapped between feathers aids a bird not only in maintaining buoyancy, but also provides a warm and dry insulation, even in cold water (Rijke, 1970).

Upon contamination with oil, feather barbules adhere to each other (Croxall, 1977); even small amounts of oil on

flight feathers may prevent them from sliding easily over one another as wings change shape during flight, thus impairing active flight (Holmes & Cronshaw, 1977). This matting of feathers also destroys waterproofing of the plumage and allows water both to saturate contour feathers and to penetrate down feathers (Szaro, 1977). When the entrapped air between feathers is eliminated and replaced by water, the effective body weight of a bird may be increased substantially leading to waterlogging and a further loss of buoyancy (Holmes & Cronshaw, 1977). This added burden, compounded with impaired or inefficient flight of contaminated birds may adversely affect their ability to forage and contributes to their physical exhaustion. This may account for the lean and emaciated condition of so many beached birds. Many others do not succeed in reaching land and drown at sea (Vermeer & Vermeer, 1975).

In less extreme instances, matting of feathers and elimination of entrapped air between them leads to an increased thermal conductance of the plumage, proportional to the amount of oil contaminating it (McEwan & Koelink, 1973). This rapid breakdown of insulation results in a high rate of heat loss which needs to be compensated by an increased energy metabolism (McEwan & Koelink, 1973; Hartung, 1967). A heavily oiled bird is exposed to the same temperature stress at 15^{o}C as a normal bird is at -20^{o}C (Hartung, 1967). To offset this loss of thermal insulation and consequent increased metabolism, a moderately oiled bird would have to double its dietary intake

(Hartung, 1967), yet many authors have observed that oiled birds generally do not increase their foraging activities but appear to stop feeding and prefer to rest on shore where they are virtually isolated from their food supply (Hawkes, 1961; Erickson, 1963).

Therefore, energy to maintain normal temperature has to be drawn from body fat reserves which are rapidly mobilized (within 48 to 72 hours). Since oiling prevents effective feeding because of resulting loss of buoyancy, these reserves cannot be replaced and muscular energy reserves soon have to be mobilized, rapidly leading to atrophy of pectoral muscles and severe emaciation (Croxall, 1977).

As a result, birds are dangerously exposed to chilling and starvation and show symptoms of general physiological stress, such as reduction of blood corpuscules, reduced resistance to infections, environmental stressors (such as weather or predation) , disruption of endocrine balance usually associated with enlarged adrenals, fatty degeneration of the liver, reduced ability to break down toxins, kidney changes restricting elimination of waste products (Croxall, 1977). These indirect effects of contamination of birds with oil can eventually lead to deaths over a period of weeks after the original exposure.

1.3.2 Systemic effects of petroleum

Birds may ingest significant quantities of oil when trying to preen oil off their feathers to restore waterproofness of their plumage. They may preen up to 50% of the oil off their feathers within a week or so (Hartung, 1963). To demonstrate this, birds were oiled and after six hours of active preening, their bill, tongue and throat were shown to be covered with a black film of oil (Hartung, 1963). Gross examination of their digestive tract also revealed the presence of brownish colored mucous in both oesophagus and proventriculus (Austin-Smith, 1968); normally yellow lining of the gizzard showed an oily coating that made it brown or black and lining of the intestinal tract also showed traces of carbon particles. Use of radiolabelled iodine in oil has also confirmed this by appearing within 24 to 36 hours in the bird's faeces (Hartung, 1963).

Birds may also ingest considerable quantities of oil through ingestion of heavily contaminated marine organisms or through water that contains oil droplets in suspension (Holmes & Cronshaw, 1977).

Ingestion of oil has been repeatedly reported to cause a wide range of deleterious effects on birds from reduced body weight, stunted growth, spleenic atrophy, liver hyperplasia and retardation of feather development in young birds (Szaro, 1977; Miller et al., 1978) to lipid pneumonia, severe gastrointestinal irritation, diarrhoea, fatty infiltration and

liver degeneration, acinar atrophy of the pancreas, toxic nephrosis, hyperplasia of adrenal cortical tissue, which is a general stress adaptation syndrome, inhibition of acetylcholinesterase activity leading to uncoordination, ataxia and tremors in adult birds (Hartung & Hunt, 1966).

In laboratory experiments, where they had free access to food, birds showed persistent hyperphagia but without any increase in body weight even though they ate up to 15% more food (Holmes et al., 1978b). This seems to be a compensatory reflex in response to impaired intestinal absorption of essential amino acids and possibly glucose (Holmes et al., 1978b; Crocker et al., 1974; Miller et al., 1978).

Seabirds have evolved complex osmoregulatory mechanisms which enable them to make use of seawater without incurring any excessive osmotic water loss (Schmidt-Nielsen et al., 1957). When seabirds are continuously maintained on hypertonic water, an increased level of Na^+ in plasma stimulates the release of corticosterone (Crocker & Holmes, 1971) which in turn triggers an enhancement of water and Na^+ uptake from ingested seawater across the small intestine mucosa. This increased rate of water and Na^+ transport brings about certain structural, developmental and enzymatic changes in nasal gland tissues, which then acts as an extrarenal organ, thus augmenting the limited salt excretory capability of kidneys and helping seabirds to maintain an osmotic balance in a hyperosmotic environment. This is done through an

increased ATPase activity in nasal glands which results in discharge of highly concentrated excretory fluid via the anterior nasal cavity (Crocker & Holmes, 1971).

The full development of this extrarenal excretory mechanism therefore would seem to be dependent upon absorptive properties of the intestinal mucosa (Crocker & Holmes, 1971) since an increased rate of intestinal absorption of H_2O and Na^+ is essential for the development of excretory functions of nasal glands. Ingestion of oil has repeatedly been shown to interfere with these adaptive responses of nasal glands to seawater (Holmes & Cronshaw, 1977; Holmes et al., 1978b; Crocker et al., 1974). This is understandable since ingestion of oil has been shown to inhibit ion-dependent ATPase activity in different organisms (Wong & Englehardt, 1981; Peakall et al., 1980). After ingestion of oil, a close examination of the mucosal epithelium of the small intestine where ion absorption normally takes place reveals changes in organization of the villi with mucosal epithelial cells frayed out or flattened at the tip (Holmes & Cronshaw, 1977). This results in inhibition or at least severe attenuation of sodium and water transfer across the intestinal mucosa. Without a proper signal for its activation, nasal glands ATPase activity decreases, leading to a seriously impaired electrolyte balance, to dehydration and eventually to death of the bird (Crocker & Holmes, 1971).

1.3.3 Effects on reproduction

Effects of sublethal low level oil pollution may be more deleterious to bird populations over a long term than the spectacular kills resulting from oil spills (Stickel & Dieter, 1979). Although survival of individual birds might not be seriously threatened, exposure to oil through contact or ingestion might result in significantly impaired reproduction. Alcid populations which already have a low reproductive rate and are heavily concentrated on nesting grounds are especially vulnerable to this longer term impact of the presence of oil in the environment (Vermeer & Vermeer, 1975).

As previously mentioned, oil ingestion affects growth and development in young birds and may also result in delayed maturity (Croxall, 1977) in both females and males through impaired development of ovaries or impaired spermatogenesis. Oil also seems to impair vitellogenesis and subsequent differentiation of ovarian follicles in females as well as storage and viability of spermatozoa in males (Holmes et al., 1978a). As a result, mature birds display less reproductive behaviour and lay considerably fewer eggs (Holmes et al., 1978a; Hartung, 1965). A single small dose of crude oil can completely inhibit egg laying for over a week (Hartung, 1965). A close examination of an ovarian mass after ingestion of oil reveals a large number of atretic follicles (Holmes et al., 1978a), which explains the resulting impaired oviposition. The few eggs that are laid show an irregular pattern of yolk

deposition, a reduction in size of the yolk sphere and a reduced eggshell thickness (Grau et al., 1977). A lot of them are not fertilized and those that are have a very low hatchability and viability.

Birds exposed to sublethal quantities of oil during incubation can also transfer some oil from their feathers to their eggs when returning to their nests from feeding at sea (Albers & Szaro, 1978; Albers, 1980). It has been shown extensively that even minute quantities of oil (microliters) applied externally on the eggshell surface of fertile eggs of various species can kill the developing embryos or seriously reduce the chick's chance of survival by producing smaller, weaker or deformed chicks (Albers, 1978; Albers & Szaro, 1978).

This is clearly due to the toxic nature of oil rather than blockage of normal respiratory gas exchange through shell pores, as was previously speculated. This was demonstrated, using propylene glycol, a highly viscous blocking agent which covered the same amount of surface area as oil but did not reduce the normal hatching success (Albers, 1977).

The aromatic fraction of oil has been shown to be the toxic component responsible for embryolethality (Szaro et al., 1978). Sensitivity of eggs to oil is dose-dependent and stage-sensitive; the earlier in its development that an egg is exposed, the higher its probability of either embryonic death or deformation, especially if application corresponds to a

time of rapid growth of the chorioallantoic membrane (Hoffman, 1978).

Embryotoxicity and teratogenesis have also been shown to result from contamination of eggs with petroleum. Embryos from contaminated eggs display reduced weight, crown-rump length, bill length and generally stunted growth. The most common abnormalities observed include deformed bills, incomplete ossification of wing or foot bones and reduction in size of liver lobes. Exceptionally, embryos will show a reduction in number of ribs, abnormal cervical vertebrae, spina bifida or incomplete ossification of the skull (Hoffman, 1978). Teratogenesis is further enhanced when metals such as nickel, vanadium or mercury, which are found as trace components in crude oil, are added to the experimental oil applied on eggshell surface (Hoffman, 1978).

Oil spills therefore probably pose the greatest threat to seabird population during development and reproductive phases of their life cycle (Eastin & Hoffman, 1978).

1.3.4 Oil pollution as an environmental stressor

Seabirds which winter along the east coast of Canada lead a precarious existence. Severe and prolonged winter conditions, coupled with a limited food supply, result in a considerable hardship for many birds. This is an important factor since most chronic oil pollution and large-scale oiling incidents occur in winter and early spring when environmental con-

ditions are already at their most difficult for birds (Croxall, 1977). In such cases, even an insignificant amount of oil contamination may trigger a series of events leading to extreme emaciation and eventually death of the birds.

It has been shown in laboratory experiment and confirmed with field observations that oil contamination may lead to death of a seabird when its effects are synergistically combined with other environmental stressors such as cold temperature, water salinity, intraspecific competion or predation, parasitism, food shortages, etc. (Austin-Smith, 1968; Holmes et al., 1978a; Hartung, 1965; Levy, 1980). This might also explain why some species may be more or less vulnerable to oiling during certain phases of their life cycle, depending on whether they migrate to warmer climates or inhabit a freshwater environment during their breeding season. The less stressful the environmental conditions, the more likely a bird will be able to tolerate the effects of oiling.

This has been shown clearly in various studies where ingestion of petroleum or petroleum-contaminated food is considered as a non-specific stressor. Birds are then more vulnerable to adrenocortical exhaustion if already exposed to other stressors such as hyperosmotic drinking water or persistant cold temperature (Holmes et al., 1978c). It was concluded that when two or more stressors were imposed simultaneously, their effects on bird mortally become additive and often synergistic. A series of non-specific stressors may

cause interrenal tissues to be stimulated further until they can no longer respond to a persistent and increasing release of corticotropin. This then leads to a state od adrenocortical insufficiency and eventually death (Holmes et al., 1978b). Results of these laboratory experiments have been confirmed by field observations where an extremely minute oiling may lead to death of seabirds when its effects are combined with severe environmental conditions (Levy, 1980).

1.3.5 External contact with dispersant

The only available information on the effects of external contact of dispersants on birds deals with detergents rather than oil dispersants as such. This is however relevant to review because of the similarity in chemical composition of the two products.

Various observers have reported that birds swimming in waste-waters containing detergents lost their buoyancy and became waterlogged (Clark & Gregory, 1971; Nero, 1964; Nero & Taylor, 1968; Choules et al., 1978). Detergents clearly seem to produce hydrophilic feathers (Clark & Gregory, 1971) and thus induce wetting of the plumage and subsequent loss of buoyancy (Choules et al. , 1978).

Birds swimming in a detergent-water mixture (with no other pollutant) were observed to become waterlogged until two-thirds to three-quarters of their bodies were submerged,

but when they were removed from the water, their plumage was wet but not structurally damaged (Choules et al. , 1978). This has always been and still is the frustration on the bird rescue teams that try to clean up oiled birds after an accidental oil spill. Whatever the method of cleaning used (normally some formulation of detergent), the bird's plumage loses its water repellent property and as soon as placed on water, birds rapidly become waterlogged and sink. Many attempts of rescue and rehabilitation of oiled birds have therefore lead to an almost total failure (Austin-Smith, 1968). Recent workers, however, are very hopeful that the more modern cleansing methods now available will yield more satisfying results.

As soon as they are out of the water, birds engage in desperate preening of their soaked plumage with stropping motions of their bills, drawing feathers of their belly and flanks, strip by strip, through their bill, thus squeezing out excess water (Nero, 1964).

The wetting of a bird's plumage inevitably results in a loss of its insulative properties as well. The considerable heat loss that follows exposes the bird to serious chilling that could lead to its death (Choules et al. , 1978). Birds seen swimming in water contaminated with detergent are clearly uneasy in water and seem anxious to get back to land (Nero & Taylor, 1968). In a study where birds were forced to remain in water, they shivered, trod water and were

obviously chilled. Their body temperature dropped more than 10°C from normal and their survival time was inversely dependent on water temperature (Choules et al. , 1978).

When a mixture of crude oil and dispersant was applied on incubating fertile eggs, the hatching success was greatly reduced to even lower levels than with the same quantity of oil alone (Albers, 1979). A dispersant may speed up the lethal effect of crude oil on a developping embryo. The significance of this will have to be tested to see if incubating adults exposed to oil and dispersant actually will transfer oil and dispersant as a mixture to their eggs when returning to their nests.

1.4.0 Objectives

All information on effects of oil alone on birds is currently very incomplete. Because of the major changes in chemical composition, behaviour, and fate of oil at sea when a dispersant is used, it seems reasonable to expect modifications in the effects of oil on birds when combined with a dispersant.

The purpose of this study is therefore to develop an experimental method that can be used to compare the effect of oil, dispersant and oil plus dispersant on the metabolic rate of birds under controlled laboratory conditions. This method can then be used to test whether the use of a dispersant to handle an oil spill will modify the effects of oil on the metabolism of birds.

The organisms used for the present study were wild strain mallard ducks (Anas platyrhynchos). Although not true seabirds, mallard ducks do occasionally frequent marine environments. However, mallards of any age group are readily available year-round from game farms, which is a considerable advantage over seabirds that are only accessible during a very short period and normally in quite small numbers. Mallards also stand captivity very well with limited and very well documented requirements for space, shelter, food and water. Many studies have already been conducted on mallard ducks, thus providing a good basic knowledge of the bird's behaviour and physiology (metabolic rates, hormonal state, daily and seasonal rhythms of activity, etc.). Therefore, it serves as a good experimental model for initial studies on seabirds (Holmes & Cronshaw, 1977). The findings from this research could be extented to true seabirds.

Prudhoe Bay Crude Oil (PBCO) was chosen for these experiments because it is a representative of arctic oils. Similarly, the dispersant Corexit 9527, manufactured by Exxon, was selected for this project because its use is rapidly spreading in the Canadian environment. Being formulated as a concentrate, Corexit 9527 does not require any mechanical mixing as most dispersants do and this interesting property makes it a very good candidate for aerial spraying in remote areas such as the Arctic or along northern coasts. This is, for instance the dispersant tested in the major Baffin Island Oil Spill (BIOS) project in 1981.

Chapter 2

EFFECTS OF OIL AND/OR DISPERSANT ON THE WETTING OF PLUMAGE

2.1.0 Materials and Methods

The experimental work for this project was carried out at the Mount Desert Island Biological Laboratory in Bar Harbor, Maine. Mallard ducks were purchased from Whistling Wings, Hanover, Illinois and flown in cardboard boxes to Maine. All birds were full-grown and in full plumage. In the laboratory, they were kept in groups of 3 or 4, in 1 ½ m X 2 ½ m pens made of 1 ½ m high metal fencing, with a thick bedding of dry hay (Fig. 2A). They had free access to a 1 ½ m diameter and 25 cm deep plastic wading pool of fresh water (Fig. 2B) and were fed commercial breeder's poultry pellets (Blue Seal) ad libitum throughout the experiments. Temperature in the animal room was kept at around 15°C year-round. Light periodicity was that of daylight.

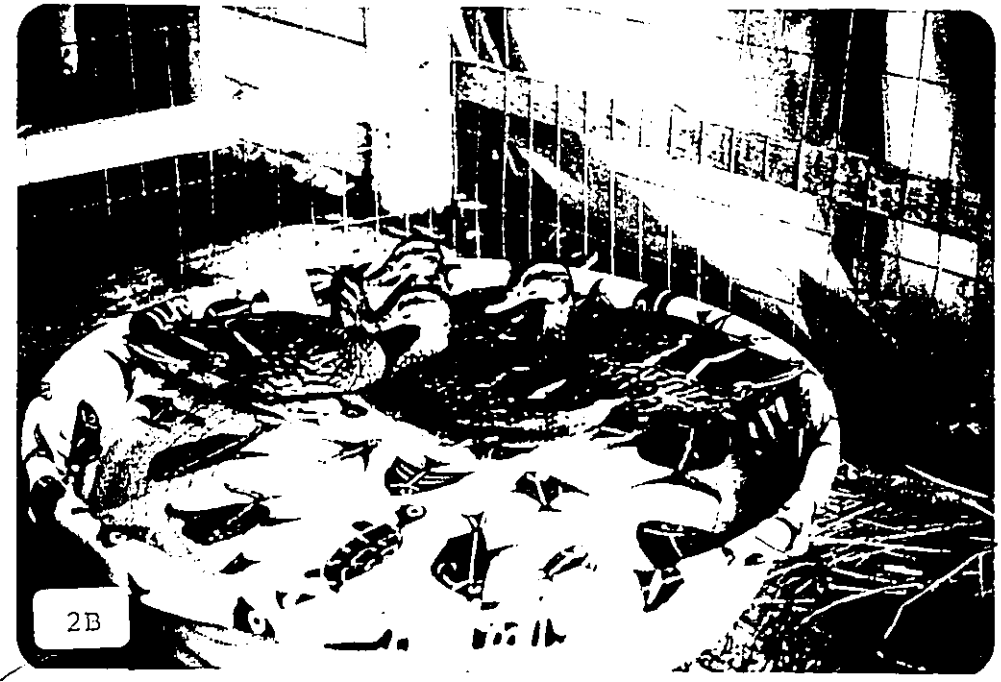
Plastic tanks measuring 50 cm X 40 cm X 30 cm were used for exposure of ducks to oil or dispersant. Each tank was surrounded and covered by metal fencing, forcing birds to remain on water during the whole one-hour exposure perior. These tanks were filled with approximately 60 liters of seawater for every experiment, rinsed and cleaned after each oil and/or dispersant treatment. Prudhoe Bay Crude Oil (PBCO) exposure was prepared by spreading 12 ml of oil on the water surface to simulate a 50 µm thick oil slick (Fig. 3A, 3B, 4A, 4B). It was then left to evaporate under a fan for for a period of one hour.

FIGURE 2:

A: Wire pens with thick bedding of hay where mallards were maintained in groups of 3 or 4.

B: Wading pool of freshwater to which mallards had free access.

2A



2B

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FIGURE 3:

A: Prudhoe Bay Crude Oil after being poured on the water surface.

B: Oil-water mixture after spreading with a glass rod.

3A

3B

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FIGURE 4:

A: A simulated 50 μm oil slick.

B: Oil left to evaporate under a fan for one hour.

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FIGURE 5:

A: Effect of one drop of dispersant-seawater mixture (1:2); it breaks up the oil slick instantaneously.

B: Oil slick broken into small droplets after stirring dispersant with a glass rod. The oil almost disappears from the water surface.

5A

5B

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Dispersant, Corexit 9527, was diluted in seawater (Corexit : seawater 1:2). Then 1.2 ml of the resulting solution was added to seawater in a swimming tank and stirred with a glass rod (to a final concentration of approx. 6×10^{-6} ml dispersant/ liter of seawater).

For the oil + dispersant mixture, PBCO was first prepared exactly as described above, and after a one-hour evaporation period, 1.2 ml of the Corexit/seawater mixture was added (Fig. 5A) and stirred until the surface oil was broken into small droplets and has almost completely disappeared from the surface (Fig. 5B). This gave a 30:1 (oil: dispersant) ratio which corresponds to application procedures recommended by Environmental Emergency Branch of the Environmental Protection Service, Environment Canada, for use in case of an oil spill.

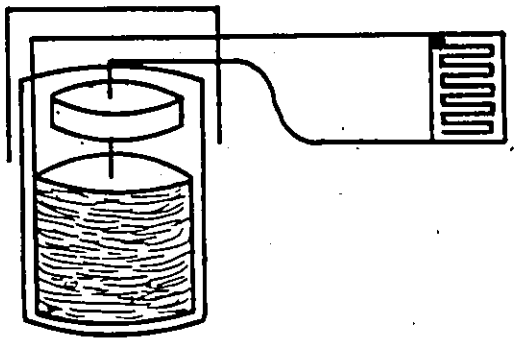
2.1.1 Experiment one

A temperature-sensitive mini-transmitter was modified to become wetness-sensitive. The transmitter (Model V, manufactured by Mini-Mitter Co., Indianapolis) was contained in an air-tight plastic capsule, 19 mm long and 12 mm in diameter. Operating on a MS-13 Mallory battery, its signal could be received on a standard AM radio kept at proximity. The modification required for our experiments involved opening its electrical circuit to insert a moisture-sensitive chip (Fig. 6A, 6B). As long as the chip was dry, the transmitter, with its open circuit would remain silent. But as soon as

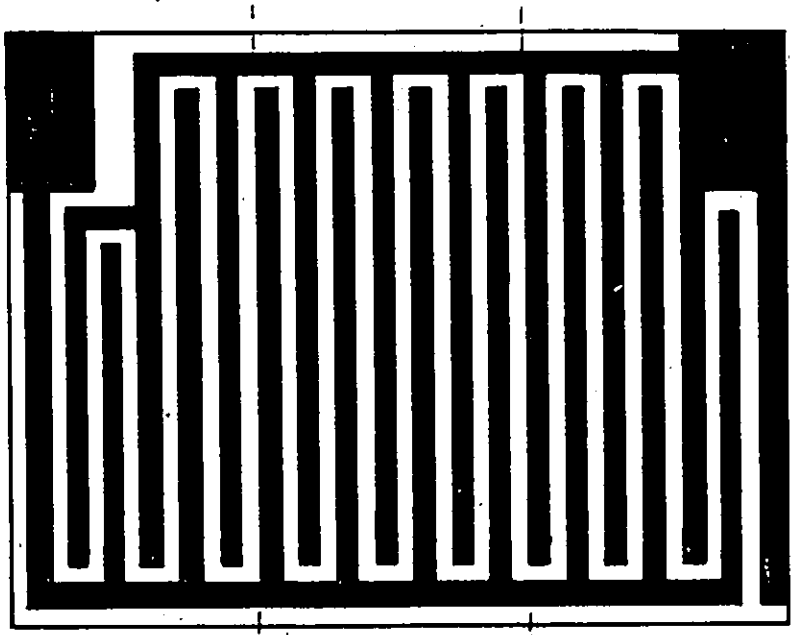
FIGURE 6:

A: Moisture-sensitive chip inserted in the electrical circuit of the mini- transmitter.

B: Moisture-sentive chip, consisting of a grid where water closes the circuit, causinf the transmitter to beep.
The dotted lines show where the chip was cut to reduce it to one-third its original size.



6A



6B

water would bridge the gap and thus close the circuit on the chip, the transmitter would emit its regular "beep", indicating its being wet.

The chip was small enough (15 mm X 10 mm) to be attached to the birds skin under its feathers, preferably on the breast which is where exposure to oil and/or dispersant was most likely expected to affect a swimming duck. Wires running from the chip to the transmitter itself could be hidden under the bird's feathers along breast and shoulder, to the back where a small harness held the transmitter (Fig. 7A, 7B). This harness was made of cotton and was attached with three straps forming a loop around the duck's neck and under its wings (Fig. 7C).

For exposure to water, a duck was contained in a swimming tank and a small AM radio was attached to wire fencing around it, with a spiral coil antenna over the tank to pick up any signal emitted by the transmitter attached to the duck's back (Fig. 8). The lenght of time required for water to penetrate the plumage and for the transmitter to "beep" could be calculated and compared between treatments with oil alone, dispersant or oil + dispersant.

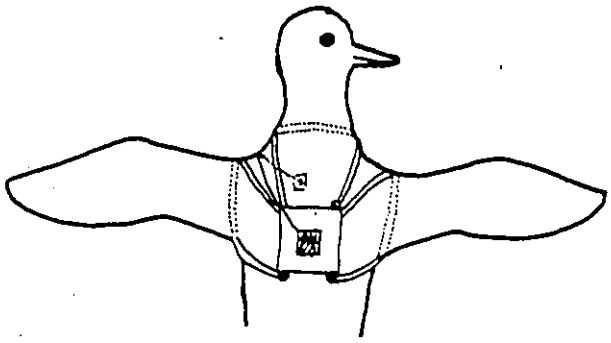
2.1.2 Experiment two

Since behaviour of the captive ducks interfered with the experiment, an anesthetic drug was called for to calm the birds and reduce their struggling. Pentobarbitol was used

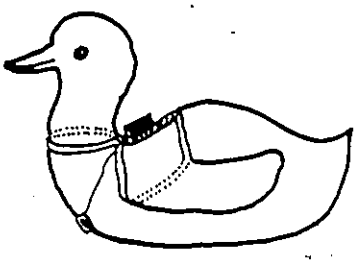
FIGURE 7:

A & B: Moisture-sensitive chip attached to the duck's belly. The wires run from the chip, along the breast and shoulder, to the back where the transmitter is held by a harness.

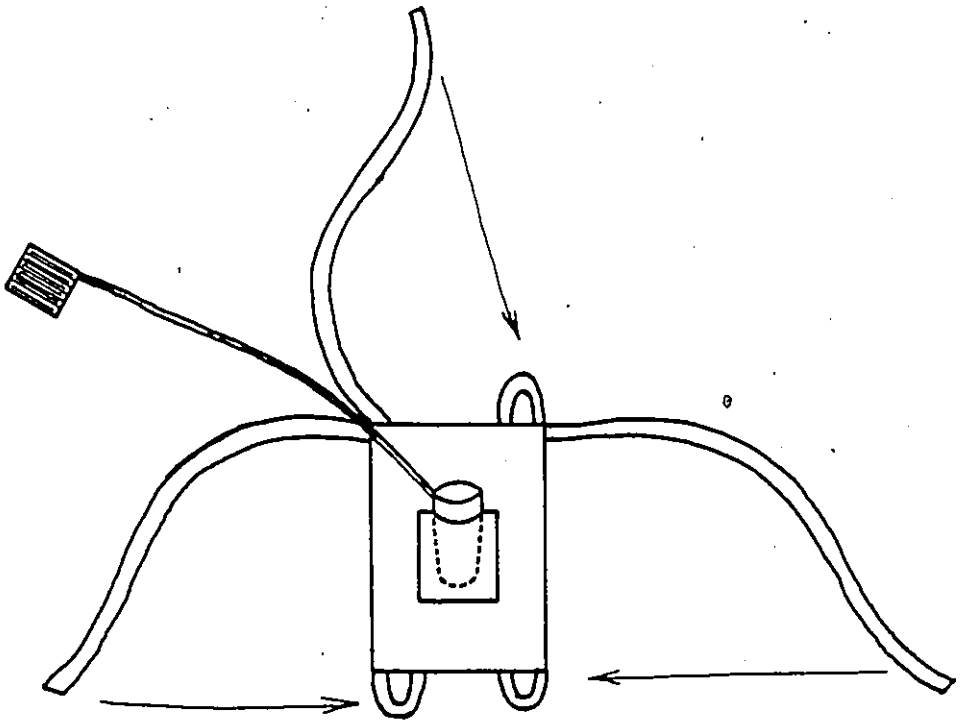
C: Harness made of cotton and attached to the bird with three straps, forming loops around the neck and under the wings.



7A



7B



7C

FIGURE 8:

A small AM radio, attached to the wire of the fencing around the swimming tank and with a spiral coil antenna over the tank, picked up the signal emitted by the transmitter on the duck's back.

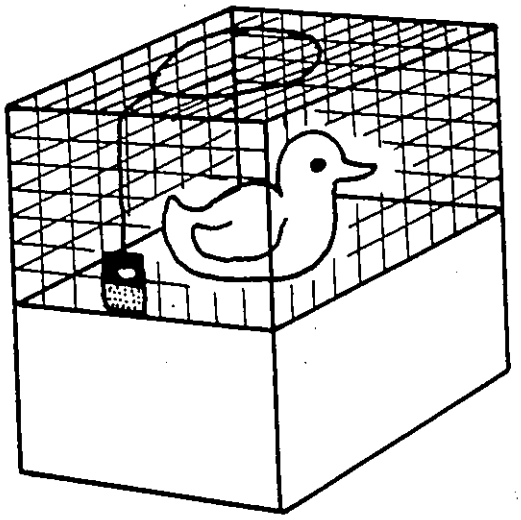


Figure 8.

initially to produce temporary relaxation and a more docile behaviour in the ducks. Doses used were similar to those recommended for mammals. Pentobarbitol was diluted to a concentration of 1 M and later mixed in a 1:1 ratio with 0.9% saline solution in hope that it would retain its calming effect but reduce twitching.

In later studies, ketamine hydrochloride replaced pentobarbitol because of its milder effect on ducks and its wider safety range. Used at a recommended dosage of .02 mg/gm of body weight, it allowed for a finer control of its effects on ducks. Another procedure tested to prevent ducks from pulling the chip off their skin was to suture it to their skin, using procaine as a local anesthetic.

Since ducks reacted to the attachment of a chip to their skin, it seemed that a smaller chip (one-third the size of the previous one) might be more easily accepted by ducks and also better concealed in their plumage. This involved cutting the chips into three smaller ones and resoldering the transmitter wires on the two opposite grids of the smaller chips (Fig. 6B).

2.2.0 Results

2.2.1 Experiment one

No results were obtained from these first experiments described on page 32, since ducks consistently succeeded in pulling the moisture-sensitive chip off their skin with their bill. Several types of glue were used: crazy glue, silicone glue, epoxy glue and rubber cement. An attempt was also made to restrict birds for a longer period of time while holding the chip in place to allow the glue to dry. Some ducks did indeed accept a chip long enough to expose them to water but none lasted longer than 20 minutes, which was too short a period for detecting any effect of the contaminant used.

2.2.2 Experiment two

Results of the experiments using pentobarbitol as described on page 34, are summarized in Table 1. Bird #1, given 0.2 ml of pure pentobarbitol (1M), did not show any effect of the drug on its motor activities and quickly pulled the chip off its skin. The intramuscular injection of 0.5 ml of pure pentobarbitol caused bird #2 to twitch violently for about one hour but was not sufficient to put it to sleep. Birds #3 and #4, given a 1 ml dose of pure pentobarbitol, quickly fell asleep and died within one hour. One of their carcasses was used to test how efficient the wetness-detection system was. The carcass was

Table 1. Variations in the effects of different pentobarbitol doses on the behaviour of ducks.

Duck #	Body Weight (kg)	Dose (ml → ml/kg)		Formulation	Results
1	1.1	0.2	0.18	pure	No response to the drug
2	1.25	0.5	0.4	pure	Twitched violently but never fell asleep
3	1.25	1	0.8	pure	Quickly fell asleep & died within one hour
4	1.15	1	0.87	pure	Quickly fell asleep & died within one hour
5	1.1	1	0.91	1:1 *	Intravenously: no effect, very mild twitching
6	1.8	1	0.56	1:1 *	Intramuscularly: no effect, very mild twitching

* 1:1 Pentobarbitol 1M : 0.9% saline solution

floated by hand on water and later attached in upright position. It took 2 ½ hours (150 minutes) for the transmitter to start emitting its "beep" signal, indicating that water had finally penetrated through the plumage.

One ml of pentobarbitol 1M / 0.9 % saline solution (1:1) injected intravenously in bird #5 and interstitially in bird #6, hardly produced any effect beyond a mild twitching. These birds were no more receptive to attachment of a moisture-sensitive chip to their skin than non-anesthetized birds. Suturing a chip to its skin did indeed prevent bird #7 from pulling it off, but this time the wires came off the chip.

Ketamine hydrochloride was much gentler on ducks than pentobarbitol. Intramuscular injection of this drug resulted in a rapid relaxation of the birds without any twitching. The ducks were soon ataxic and when floated on water, lost their balance and coordination, therefore tilting upside-down, head under water. This drug also interfered with birds' reflex to stop breathing when immersed and a few birds almost drowned. To prevent this, their head had to be held out of water for as long as the effect of ketamine lasted. Ducks also went into contortions that relaxed only when the effect of the drug decreased (Fig. 9). Results of these experiments are summarized in Table 2. Of eight birds used in this set of experiments, birds #10 and #11 pulled the chip off their skin before any significant result could be obtained (at 20 and 30 minutes respectively). Bird #15 pulled the chip off its

FIGURE 9:

Duck under the effect of the drug ketamine hydrochloride. The bird went into contortions that relaxed only when the effect of the drug decreased.

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Table 2. Variations in response of ducks to different doses of ketamine hydrochloride

Duck #	B.W. (kg)	Dose (ml → ml/kg BW)	Treatment	Time (min)	Results
8	1.2	0.2 → 0.17	dispersant (concentrated)	0	Transmitter beeped immediately
9	1.35	0.25 → 0.185	water	4	Transmitter beeped
10	1.15	0 → 0	water	20	Pulled the chip off
11	1.4	0.25 → 0.18	water	30	Pulled the chip off
12	1.25	0.25 → 0.2	dispersant (diluted)	45	Transmitter beeped
13	1.3	0.6 → 0.46	oil	60	No response
14	1.15	0.35 → 0.23	water	60	No response
15	1.2	0.22 → 0.18	water	90	Pulled the chip off

skin after 90 minutes. Since the transmitter was still silent, this indicates that water had not yet penetrated its plumage. This result can be used with those from birds #13 and #14 in which the plumage was still dry after one hour of exposure, as indicated by the lack of response from the transmitter when the ducks were taken out of the water. Transmitters on birds #8, #9 and #12 exposed respectively to concentrated dispersant (1:10, dispersant:seawater), water alone and diluted dispersant (1:30, dispersant:seawater) beeped to indicate wetness at 0,4 and 45 minutes.

2.3.0 Discussion

2.3.1 Experiment one

The "mechanics" of these experiments , as described in pages 32, 33 and 34 , were tested under various conditions and functioned very well. Transmitters "beeped" as soon as the chip was in contact with water or even when held between moist fingers. Harnesses held perfectly well on duck's backs. After about 1 or 2 hours of struggling to remove it, ducks finally accepted and ignored it.

The "biotic" part however was much less successful. The behaviour of birds greatly interfered with the experiments. A small area of skin was stripped of even its smallest down feathers to allow better contact and adhesion between the chip and skin. Ducks reacted very strongly to the chip attached

to their skin and wires, however thin, hidden under their feathers from their breast to their back. They consistently succeeded pulling wires until the chip eventually came off their skin, either before or soon after being transferred to a swimming tank, as shown in the results on pages 38 and 39.

It is therefore clearly necessary to modify the experiment to either acquire a greater control on the ducks' behaviour or improve the equipment to render it less vulnerable to ducks' persistent struggling and pulling.

2.3.2 Experiment two

The anesthetic pentobarbitol used on ducks proved to be inadequate for these experiments. It produced its desired effect only within a very narrow dosage range. As seen on table 1 on page 39, a very low dose produced twitching which is less than desirable when one has to attach a minuscule chip to a duck's skin, while too high a dose rapidly killed birds and therefore interfered with experiments in which the bird's recovery from contamination with a pollutant was to be followed for one or two weeks. Even when diluted with 0.9% saline solution, pentobarbitol dosing remained difficult to evaluate for each bird relative to its weight to obtain an adequate effect. At its best, it still produced too much twitching for the purpose of these experiments.

Ketamine hydrochloride was a much milder and more adequate anesthetic for ducks. Much gentler in its effects, it

was also more effective and predictable than pentobarbitol at similar doses. This allowed for proper attachment of a moisture-sensitive chip to the birds' breast and also prevented ducks from pulling the chip off at least for the duration of an experiment. On the other hand, it also interfered with the birds' normal swimming activity. To prevent drowning, they had to be held, head up, otherwise they tilted their head under water, the whole bird being covered with oil instead of the normal plumage contamination pattern (Fig. 10) where oil from the surface stains mostly breast and wing feathers. The only motions birds went through were those imposed on them while being held on water and moved around by hand, which is obviously not typical of birds' natural behaviour.

Suturing of a chip to a duck's skin was a limited success. The chip did indeed remain attached to its skin in spite of the duck's tenacious tug-of-war with the wires but the solder bonds holding these wires to the chip were very weak on such small sized chips and broke from too much tension on the wires.

From results summarized in Table 2, it would be tempting to draw general conclusions such as: it seems that a dispersant causes the plumage to wet faster than water alone; or, oil does not seem to affect the impermeability of a duck's plumage.

But upon closer examination, one could question the validity of such conclusions if we consider the importance of

FIGURE 10:

Mallard swimming on the water surface where a 50 µm oil slick was spread. After only a few minutes, most of the oil is picked up by the bird at the level of the breast and wings.

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variables such as disruption of the plumage by gluing or suturing a chip in place, or behavioral response of ducks to the presence of a foreign object on their skin or to captivity.

These results are better understood and certainly closer to reality when we relate the quick response of a transmitter to the behaviour of birds which, while trying to escape from a swimming tank, slide against the sides and thus, ruffle the plumage on their belly and breast, opening a way to infiltration of water to lower layers. A lack of response in other birds, whether exposed to water alone or water + oil, may be explained by a relative calm and apathy of those birds while swimming on the water surface.

Modifications of the earlier method, including the use of smaller chips and calming drugs, although a slight improvement, do not eliminate such factors as disruption of the plumage during implantation of the chip or the bias introduced by modifying birds' behaviour when hand-floating and moving them around artificially while anesthetized.

Successive tests on the various experimental methods used in this study have shown that it is very difficult to monitor the rapidity of plumage wetting in ducks since the experimental procedure itself involves the manipulation and consequent disturbance of the plumage.

Although one of the major problems in these experiments was the method used for attaching the moisture-sensitive device to the ducks' skin and preventing them from pulling it off,

experiments where the chip did stay in place yielded inadequate results. The use of drugs modified the behaviour of the ducks to such an extent that they were exposed to drowning, were impaired in their normal reactions to stimuli and even in their ability to swim. Every addition or modification of the method created experimental conditions that were further and further away from the natural situation the experiments were attempting to simulate. The results obtained under these conditions would merely be a vague approximation of what could happen to the birds in their natural environment. Every aspect of the natural exposure of a bird to an oil slick was modified: the physical conditions (size of pool, thickness of oil slick, weathering, wave action); the actual encounter of the bird with the oil (angle and speed of contact, movements of wings); the behaviour and reactions of the birds to the oil (avoidance reaction or quick escape from the oil slick, or preening reaction and even swimming patterns), inhibited by the forced experimental conditions and the use of soporific drugs.

It was therefore decided to abandon this first approach and concentrate on the possible effects of oil and/or dispersant on the metabolism of the birds.

Chapter 3

EFFECTS OF OIL AND/OR DISPERSANT ON THE METABOLISM OF DUCKS

3.1.0 Experimental Design Study

An elaborate methodology had to be developped in order to adequately measure the metabolic rate in ducks exposed to a pollutant. Equipment had to be designed and oftentimes modified to adapt it to the requirements and limitations of experimental procedures. A method also had to be developped and progressively improved to control or monitor variables that affected and compromised the validity of results. This was done by a series of separate studies, the results of each indicating the methodological improvements required for subsequent ones.

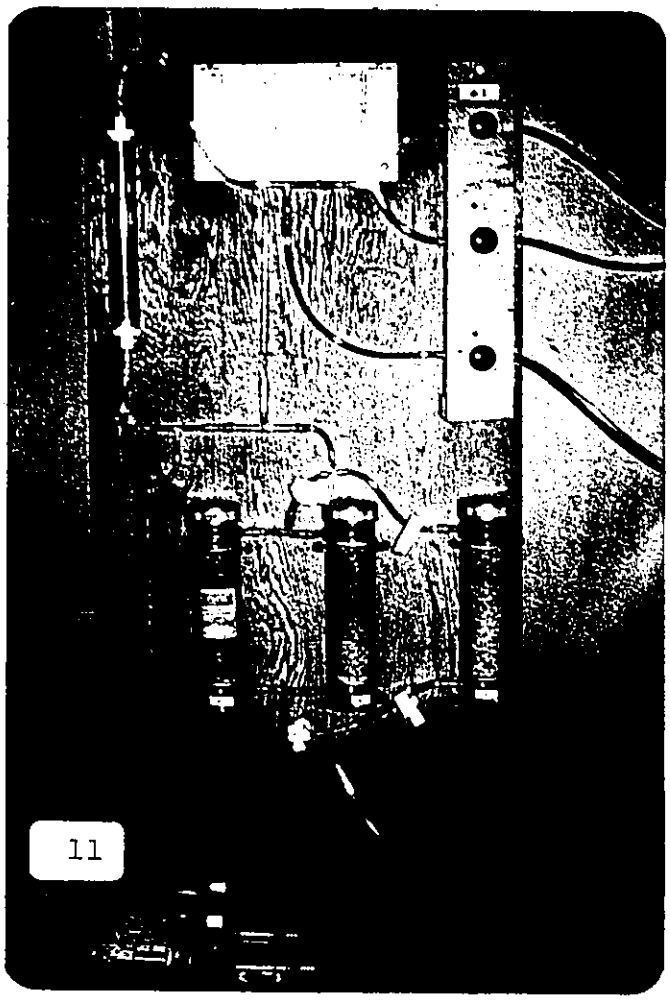
3.1.1.0 Materials and Methods

3.1.1.1 Study one

A metabolic chamber system was designed to measure the metabolism of birds at three different temperatures, -20°, 0° and 20°C. A compressor was used to draw air from the room, at room temperature and ambient humidity, dry it through drierite columns and pump it through the system. A manifold panel made of three needle-valves distributed the air-flow among three metabolic chamber (Fig. 11). The metabolic chambers could be

FIGURE 11:

System used to pump and dry the air. The air was pumped through a compressor and dried through drierite columns. A selection panel made of three needle-valves distributed the air-flow among the three metabolic chambers.



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used simultaneously or one at a time. A flowmeter and a manometer between drierite columns and needle-valves allowed for monitoring of the total flow through the manifold panel and the back-pressure resulting from selective shutting of one or two valves.

Each of the three metabolic chambers consisted of a 40 cm X 30 cm X 20 cm clear plexiglass box with an air-tight fitting door (Fig. 12A). A bird sat in the box on a grid raised by 2 cm from the bottom of the box (Fig. 12B). The air inlet and outlet were in opposite upper corners of the box. Thermal probes, also in the upper corner of chambers, were connected to a Yellowspring Telethermometer. This allowed for constant monitoring of temperature in metabolic chambers.

One chamber used for measurements of metabolism at room temperature (approx. + 20°C) was darkened with opaque plastic. The other two chambers, for measurements at approximately 0° and -20°C, were contained inside a thermostatically controlled incubator (Fig. 12A), where temperature control was achieved manually. The air-flow to each chamber was monitored through a flowmeter attached to the side of the metabolic chamber and maintained at 2.5 liters/minute. A set of three 3-way valves directed out-coming air either to be vented out or pushed towards oxygen and CO_2 analyzers (Fig. 12C). Here again, a manometer between metabolic chamber and valve corresponding to each one of the three chambers was used to monitor back-pressure and therefore resistance of the air-flow

FIGURE 12:

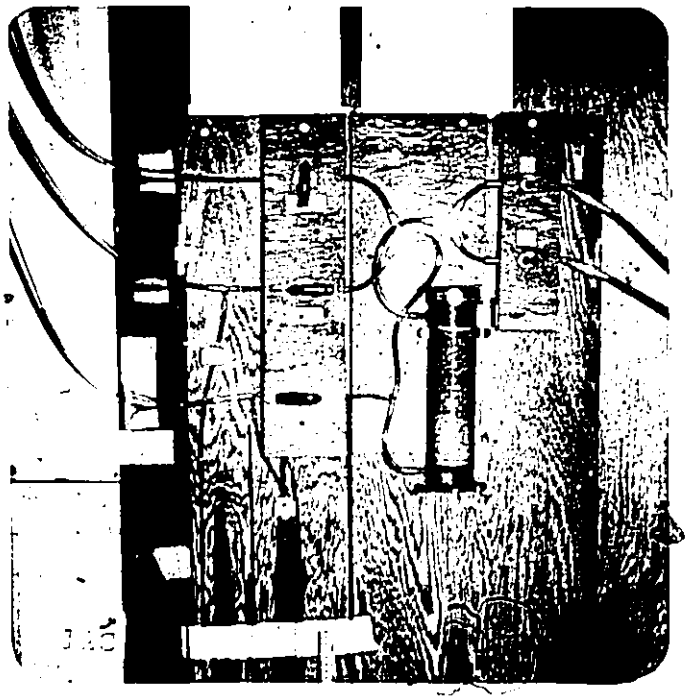
A: Metabolic chamber located inside a thermostatically controlled incubator. The air coming into the chamber was cooled through a copper coil, surrounded by ice, in a foam-chest located under the metabolic box in the incubator.

B: Metabolic chamber consisting of clear plexiglass with an air-tight fitting door. The bird sat on a grid about 2 cm off the bottom of the box.

C: Set of three 3-way valves directing the air from the three metabolic chambers to either be vented out or pushed towards the analyzers. The air to be analyzed was dried through a drierite column.

12A

12B



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going through for analysis. This back-pressure also reflected pressure build-up inside the metabolic chamber to which the ducks were directly submitted. Air to be analyzed was dried through a drierite column (Fig. 12C) to remove moisture from inside the chamber (from the duck's wet plumage, excretion and respiratory moisture) to avoid condensation in gas analyzers. After drying, a fraction of air, controlled again by needle-valves and monitored through two flow-meters was analyzed, 750 cc/min in a Beckman Model 864 Infrared (CO_2) analyzer and 250 cc/min in a Beckman Model 755 Paramagnetic Oxygen analyzer (Fig. 13). A 2 µm millipore filter was inserted in the air-flow just before the two analyzers to act as particulate filter.

Oxygen and CO_2 analyzers were calibrated daily using known gas mixtures as points of reference. The oxygen analyzer was easily calibrated using atmospheric air, dried through a drierite column and in which oxygen content is known and stable at 20.93%. A fine adjustment could be made by comparing readings on each of four ranges (11-21%, 16-21%, 19-21%, 20-21%) until all four simultaneously showed 20.93%. For calibration of the CO_2 analyzer, dry nitrogen (100% N_2) was used as a "zero standard gas". Custom-prepared specific gas mixtures of 0.41% and 2.26% CO_2 in air were used as standard points for calibration of the 0.0 to 0.5% and 0.0 to 2.5% ranges respectively.

Ducks were assigned randomly to one of the four treatment groups (PBCO, Corexit 9527 or PBCO + Corexit). Each

FIGURE 13:

A Beckman Paramagnetic Oxygen analyzer and a Beckman Infrared CO_2 analyzer, used to analyze the gas sample.


BECKMAN
13

bird was fasted for approximately 12 hours before the experiment and so, was expected to be in post-absorptive state by the time of metabolic measurement. The exposure period to either seawater alone or seawater + pollutant lasted one hour after which the bird was transferred to the first metabolic chamber.

Metabolic measurements at each temperature involved first a 45 minutes stabilization period during which a bird was left to adapt to the conditions of confinement in a metabolic chamber and temperature changes. Its metabolic rates were obtained by measuring the exchange of respiratory gases. Oxygen and CO_2 readings were taken every 3 minutes for 60 minutes. These were used to calculate oxygen consumption, CO_2 production and respiratory quotient ($RQ = CO_2/O_2$). Metabolic rates were then calculated using the formula of Romijn and Lokhorst (1966):

$$kcal = 3.871 \text{ (liters } O_2 \text{)} + 1.194 \text{ (liters } CO_2 \text{)}$$

These results were then transformed to kilocalories per kilogram of body weight per day, thus eliminating any difference in metabolism due to the size of birds. The average metabolic rate for each temperature was calculated from at least 20 readings. Results of each experiment were then compiled into three metabolic rates corresponding to the three temperatures. From these, a graph could be drawn and a slope calculated. Regression coefficients were also calculated to check the validity of these slopes.

Each bird was used as its own control. Its metabolism was first calculated without any exposure to water or a pollutant (dry run) to establish its basic metabolic rate. It was then exposed once only to water contaminated with oil and/or dispersant. After a few days, its metabolism was calculated again to follow its "recovery" from contamination.

3.1.1.2 Study two

Methods applied here were basically identical to those in the previous experiments, with a few slight modifications to the equipment. The lower part of the incubators was sealed with foam boards and masking tape, to prevent heat from penetrating the incubator every time the door was opened. Door fittings on the metabolic chambers were modified to facilitate opening and closing of boxes. These improvements greatly reduced the length of time an incubator door had to be opened and the subsequent warming-up of the metabolic chamber.

The two millipore filters were removed to reduce resistance along the path of air being analyzed in hope that the drierite column with its granules and two felt pads would act as a particulate filter. This reduced pressure build-up in the chamber by two-thirds. To help maintain this low pressure, outgoing tubes were checked regularly to prevent icing-up and blockage.

The 16 ducks used in these experiments were divided among four treatment groups: control (exposed to seawater

alone), oil, dispersant and oil + dispersant. Their metabolism was calculated once before exposure (pre-exposure run), immediately after exposure (exposure run), and again 7 to 9 days after exposure (post-exposure run). These three results were plotted on the same graph to allow for comparisons.

3.1.1.3 Study three

Only one metabolic chamber was used for this experiment thus reducing handling of birds from one chamber to the next. The chamber was contained in one of the thermostatically controlled incubator. This allowed for manual control of temperature, from room temperature to cold (approx. 0°C) and very cold (approx. -20°C) temperatures required for this experiment. Use of only one chamber instead of three also allowed the airflow to go through the analyzers at all times, without switching on and off as was previously required for sampling from three chambers alternately. A small light was installed in the incubator to reduce stress imposed on birds from captivity in a totally dark environment.

The tube bringing air from the compressor to the metabolic chamber divided into two parallel lines and then joined again before the chamber (Fig. 14). One of these was kept at ambient temperature to deliver warm air directly from compressor to chamber, when operating the metabolic system at room temperature. The other, used when operating at colder temperatures,

FIGURE 14:

System used to control air flow temperature. The air tube divided into two parallel lines and joined again before the metabolic chamber. One of these was used when operating the system at room temperature. The other was used when the air had to be cooled before entering the metabolic chamber, to help maintain its low temperature.

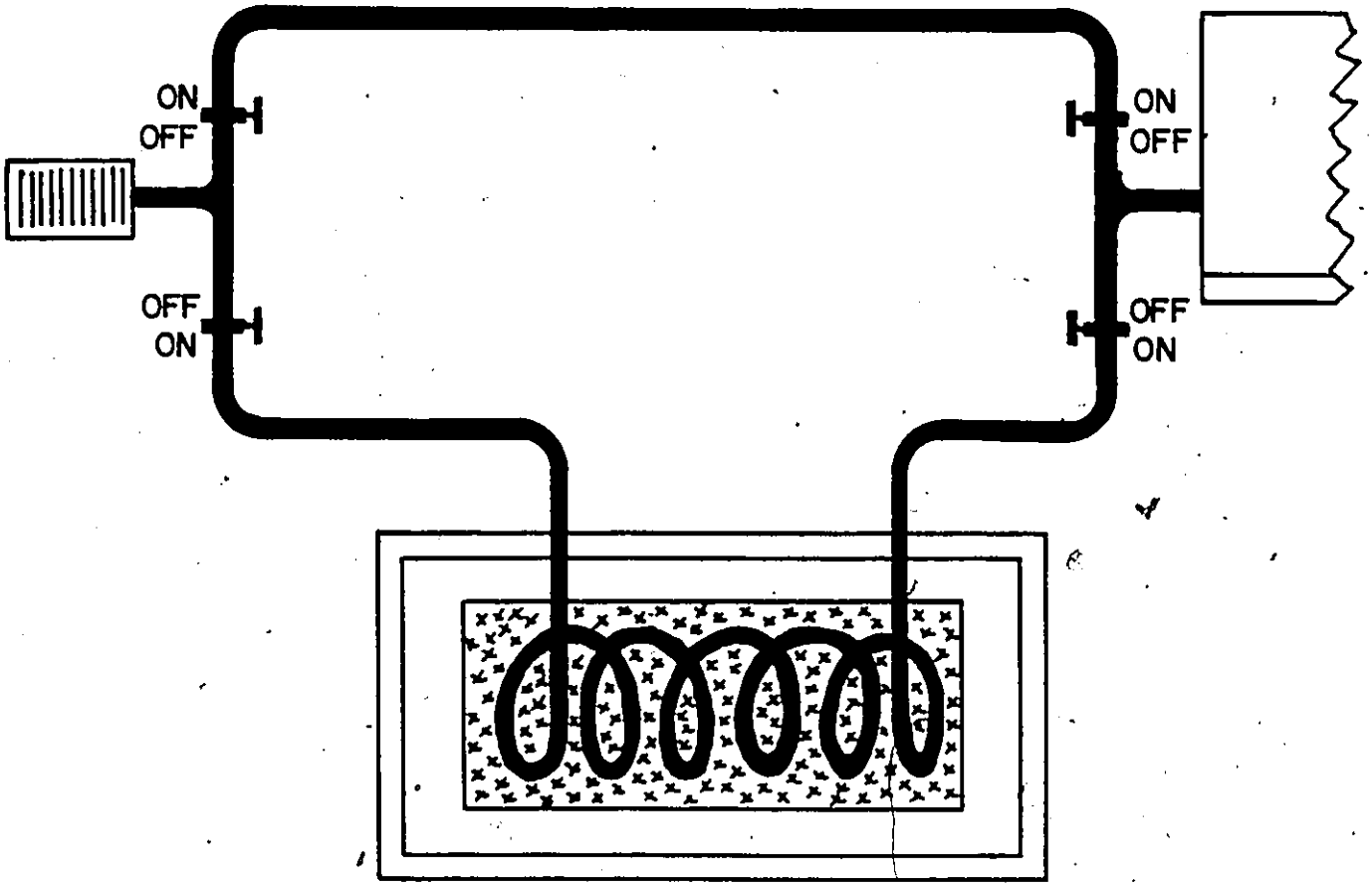


Figure 14.

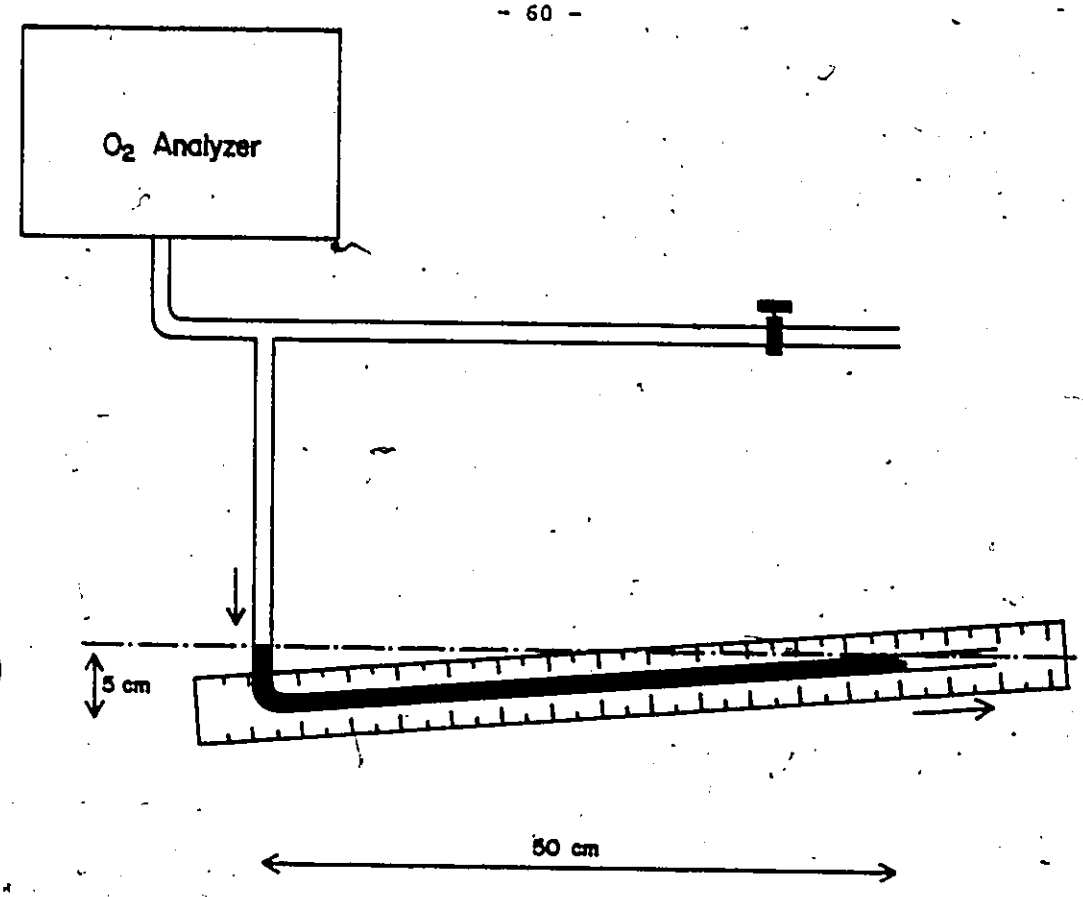
went through long copper coils surrounded by ice before delivering cold air to the metabolic chamber. Selection for either one of these two modes was simply done by opening or shutting two clamps to divert the airflow through one or the other parallel line.

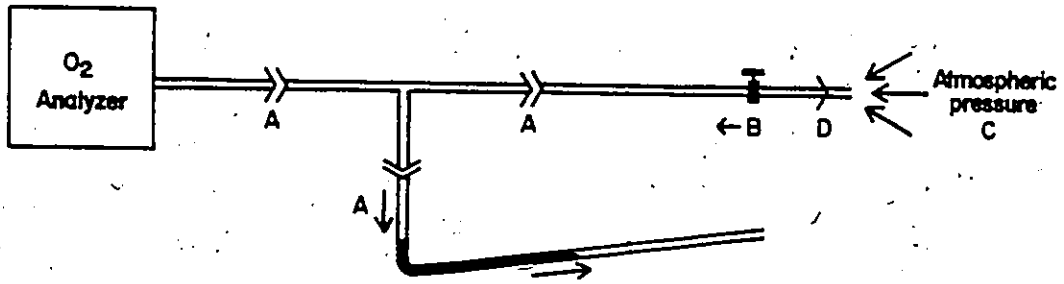
Hourly recordings of barometric pressure obtained from the local U.S. Coast Guard radio station were used in a simple calculation to correct oxygen analyzer readouts, thus compensating for any fluctuations of pressure. Back-pressure building up inside the analyzer could also greatly vary according to the pressure in in-coming airflow and valve aperture. Control of airflow coming into the analyzer alone was not sufficient to prevent such variations since a same flow would result from different combinations of pressure and valve aperture.

A "back-pressure regulator" was therefore designed to control and monitor pressure inside the analyzer. A needle-valve was installed between oxygen analyzer and venting outlet and an open-end manometer between analyzer and valve (Fig. 15A, 15B). This allowed for prevention of "back-pressure" build-up and maintenance of constant pressure in the analyzer. Barometer pressure variations, which are easily compensated for were then the only variables still affecting the accuracy of oxygen readouts.

FIGURE 15:

A & B: Design of back-pressure regulator.
A needle-valve between the oxygen analyzer and venting outlet allows for the control of the venting and a manometer between the analyzer and needle-valve allows for the monitoring of the pressure inside the oxygen analyzer and for the prevention of variations which would affect the accuracy of the readings.





$A \geq B + C$ $\quad$ $A = B + C + D$

Figure 15.

3.1.1.4 Study four

Metabolic chambers were redesigned to ensure a more diffuse flow pattern, air mixing and, therefore, a more even temperature throughout the box. Air inlets were relocated from upper left corners to two different points on the bottom of the box, under the grid. With air outlets still in upper right corners, air had to circulate from under the grid, around the bird, up to the outlet thus producing a much more efficient mixing and consequently temperature diffusion (Fig. 16A).

To ensure a better monitoring of temperature, three telethermometer probes, located at upper, middle and lower levels of the box were used instead of only one in upper right corner (Fig. 16B).

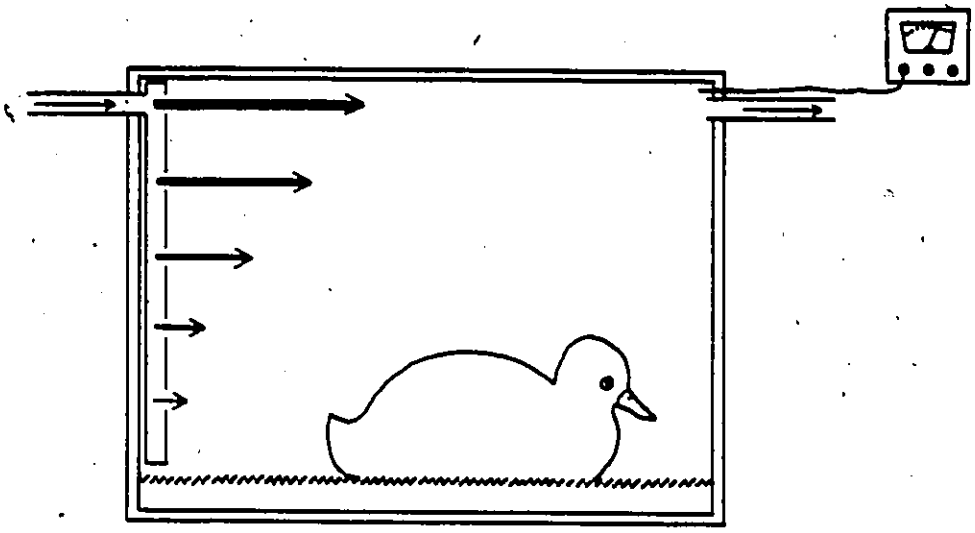
3.1.1.5 Study five

The purpose of this study was to evaluate the time required for a duck's metabolism to reach a point of stability after being transferred to a metabolic chamber and the consistency of the metabolic readings thereafter. In this way, it could be detected if a bird went through alternate periods of activity and rest or if its metabolism remained fairly stable after the acclimation period. Results from these tests were obviously to influence the method used and especially timing of data gathering in subsequent experiments.

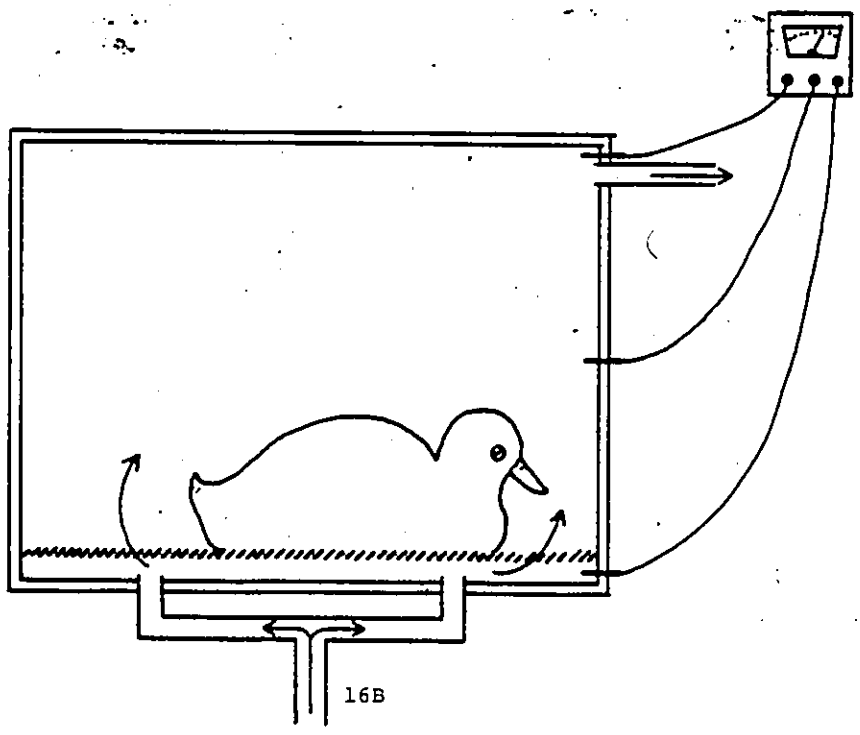
FIGURE 16:

A: Airflow pattern in metabolic box, as previously designed.

B: Airflow pattern in redesigned metabolic box.



16A



16B

One duck was exposed to water for one hour and then transferred to a metabolic chamber at room temperature, where its metabolism was monitored every 10 minutes for over 6 hours without any change in temperature. A second bird was handled in the same way with the only difference that temperature in the metabolic chamber was lowered to approximately 0°C. Here again, metabolism was monitored similarly for about 6 hours to follow the progression and evaluate the timing of a bird's acclimation to cold temperature, time required to detect its response and stability of readings thereafter.

3.1.1.6 Study six

This set of experiments was carried out in the same way and using the same equipment as previous one, with a few modifications in its timing. Ducks were now fasted for a minimum of 24 hours before exposure to either water or water + pollutant. As soon as a bird was transferred to the metabolic chamber, its metabolism was monitored closely to see it reached a point of stability. This lasted a minimum of one hour but often longer, until it reached a stable plateau. From that point, the metabolic rate was recorded every 2 minutes for a minimum of 30 minutes and up to one hour.

Results

3.1.2.1 Study one

Using the method described on page 49, it was very difficult to maintain a stable temperature in each of the metabolic chambers. A wide variation and scattering of temperatures is therefore seen in the results, instead of the +20°, 0° and - 20°C as originally planned. Results (Fig. 17) show quite a variety of responses, from a very slightly positive slope (bird #16), to a lack of response (bird #17 /oil), to a slightly negative slope (bird #17 and #19). The most intriguing results are those (bird #18, #20 and #21) where the duck's responses differed between two consecutive drops in temperature. A first drop caused the expected increase in metabolic rate with decreasing temperature but a second one resulted in a decrease in metabolic rate instead of a further increase, as expected. It is also puzzling that the same bird (#17) showed such a different response when exposed to oil. In fact, where a greater effect of cold temperatures was expected due to the contamination with oil, it showed no response at all to decreasing temperatures (Fig. 17B).

3.1.2.2. Study two

With the modifications described on page 56, results obtained from these experiments were much more consistent than the previous ones (Fig. 18, 19, 20 and 21). Using the slope from each drop of temperature (Table 3),

FIGURE 17: Variation in metabolism of 6 ducks exposed to three different temperatures.

A: Bird #16

B: Bird #17 (with and without oil)

C: Bird #18

D: Bird #19

E: Bird #20

F: Bird #21

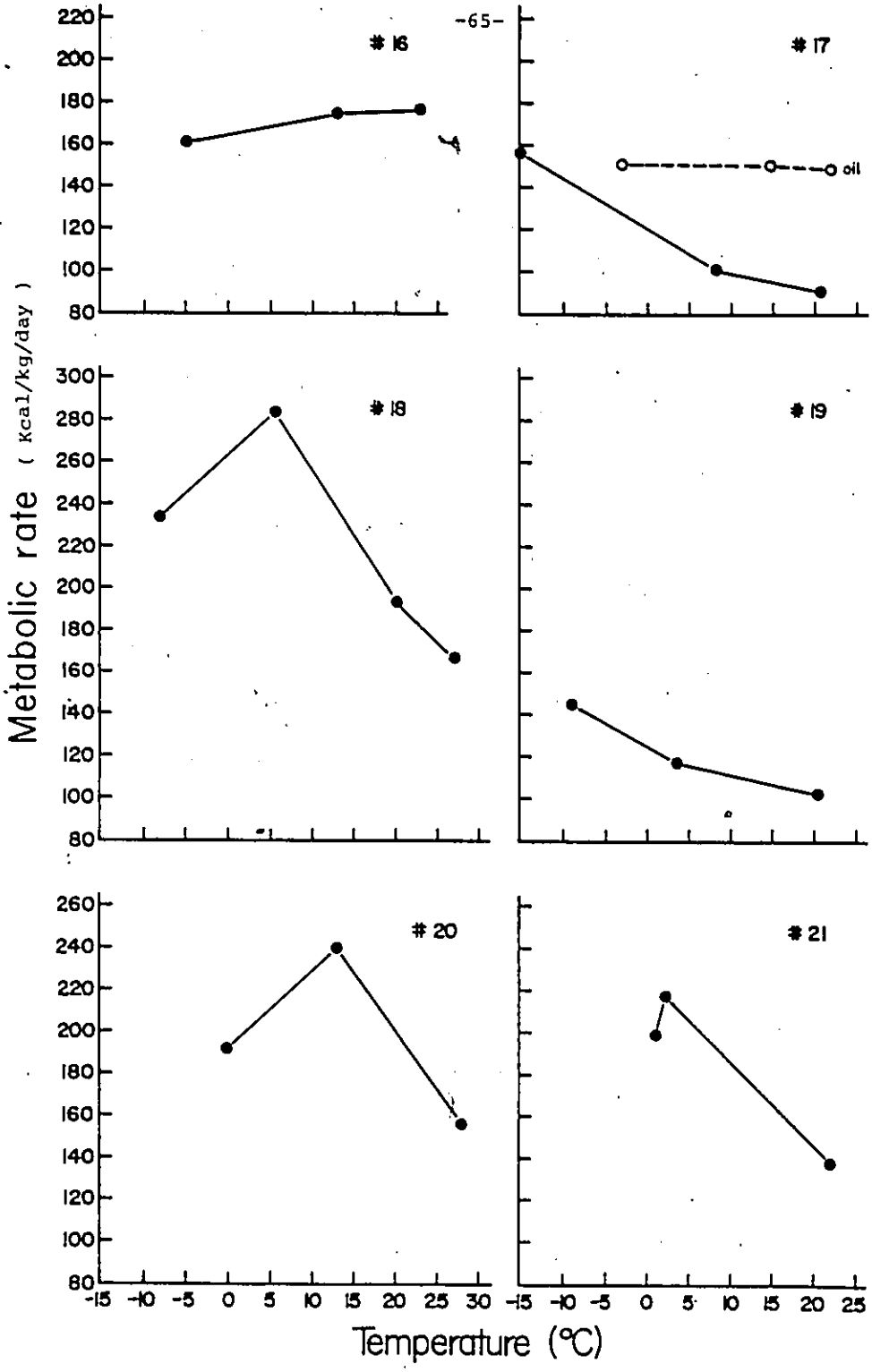

#16
#17
oil
#18
#19
#20
#21
Metabolic rate (kcal/kg/day)
Temperature (°C)

FIGURE 18: Metabolic rate as a function of temperature in ducks exposed to three different temperatures.

A: Bird #22

B: Bird #23

C: Bird #24

D: Bird #25

LEGEND

▲ pre-exposure

● exposure

■ post-exposure

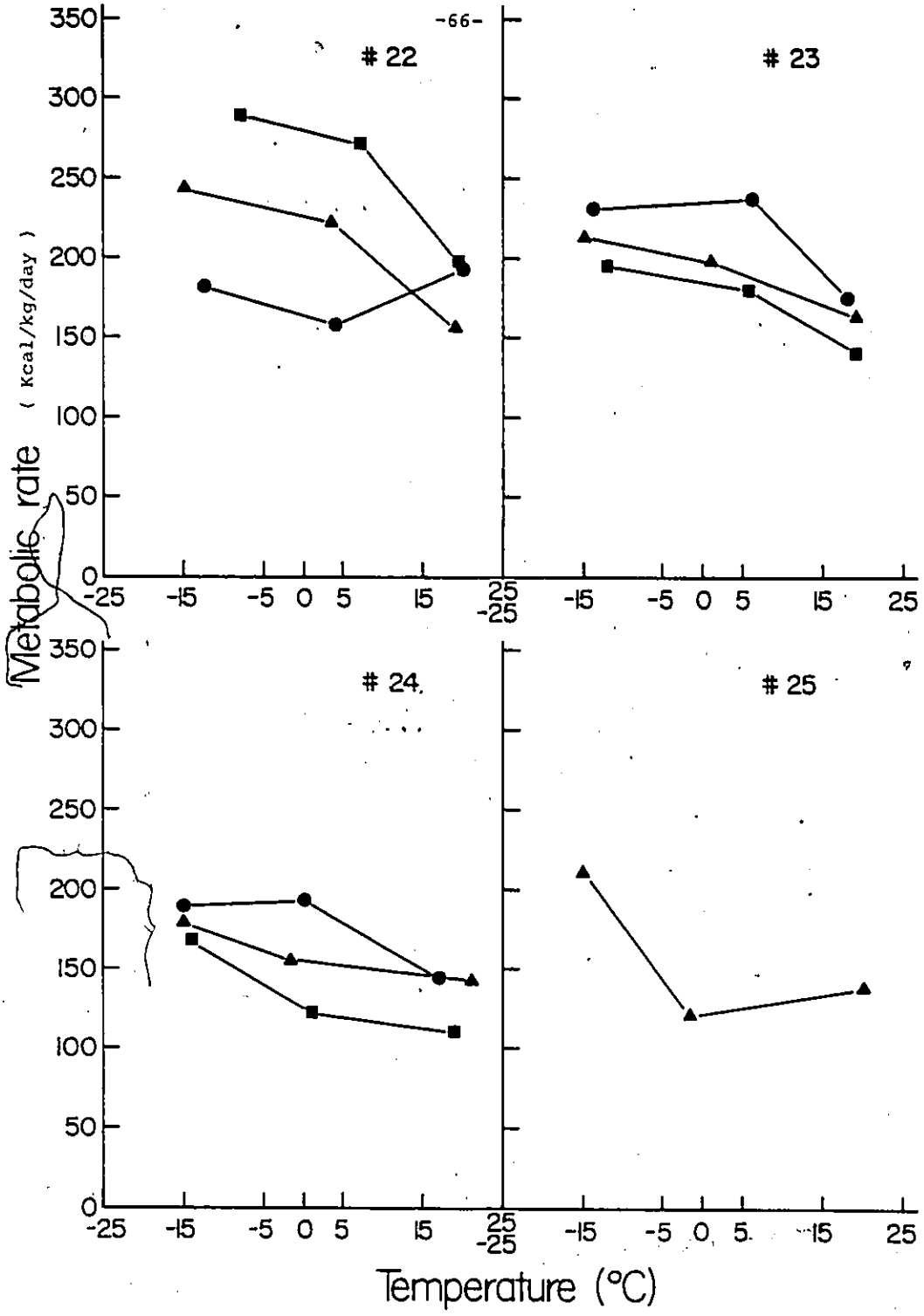

#22
#23
#24
#25
Metabolic rate (Kcal/kg/day)
Temperature (°C)
350
300
250
200
150
100
50
0
-25
-15
-5
0
5
15
25

FIGURE 19: Metabolic rate as a function of temperature in ducks treated with PBCO and then exposed to three different temperatures.

A: Bird #26

B: Bird #27

C: Bird #28

D: Bird #29

LEGEND

▲ 1st pre-exposure

△ 2nd pre-exposure

● exposure

■ post-exposure

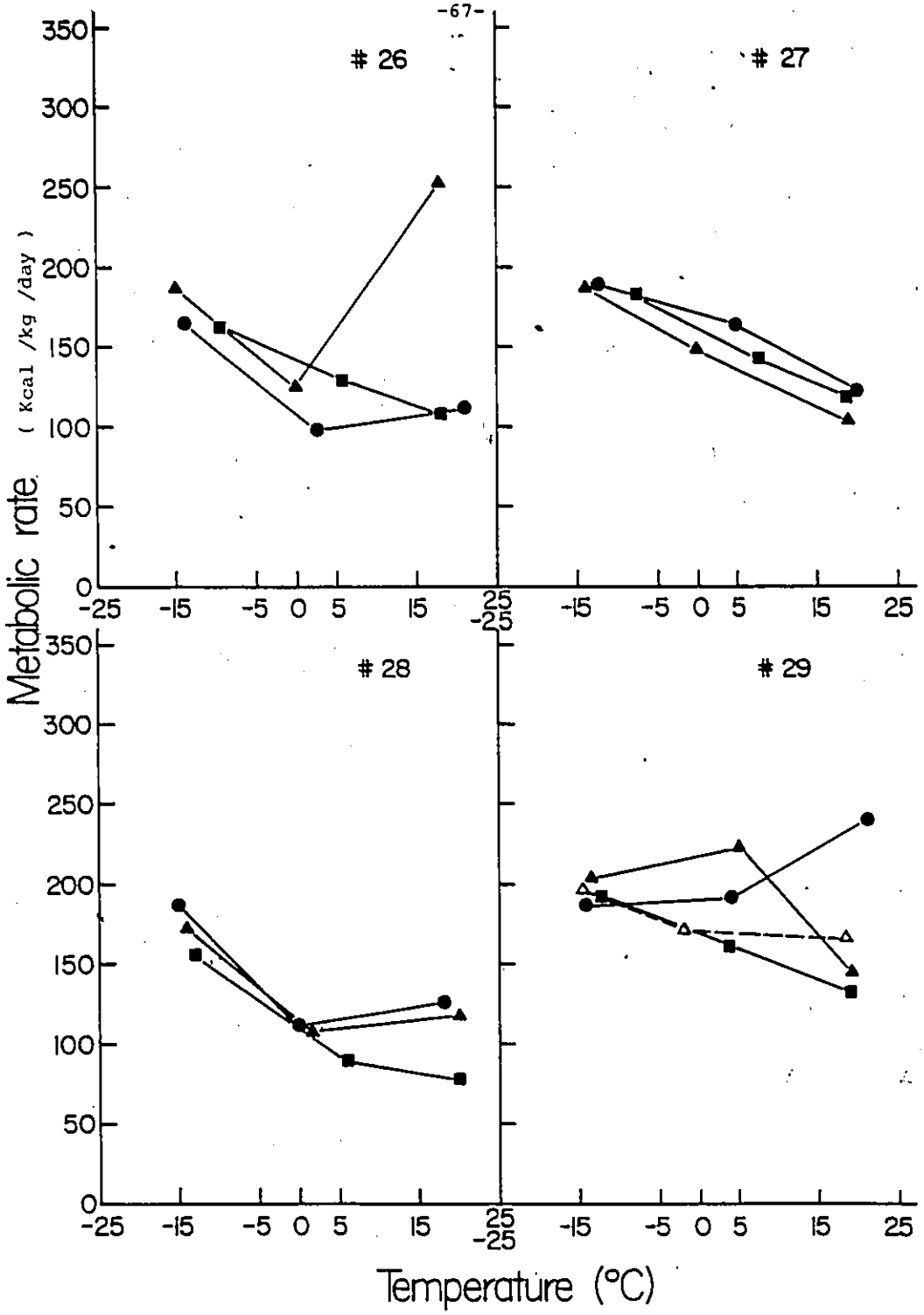

Metabolic rate (Kcal /kg /day)
26
27
28
29
350
300
250
200
150
100
50
0
-25
-15
-5
0
5
15
25
Temperature (°C)

FIGURE 20: Metabolic rate as a function of temperature in ducks treated with Corexit 9527 and then exposed to three different temperatures.

A: Bird #30

B: Bird #31

C: Bird #32

D: Bird #33

LEGEND

▲ pre-exposure

● exposure

■ post-exposure

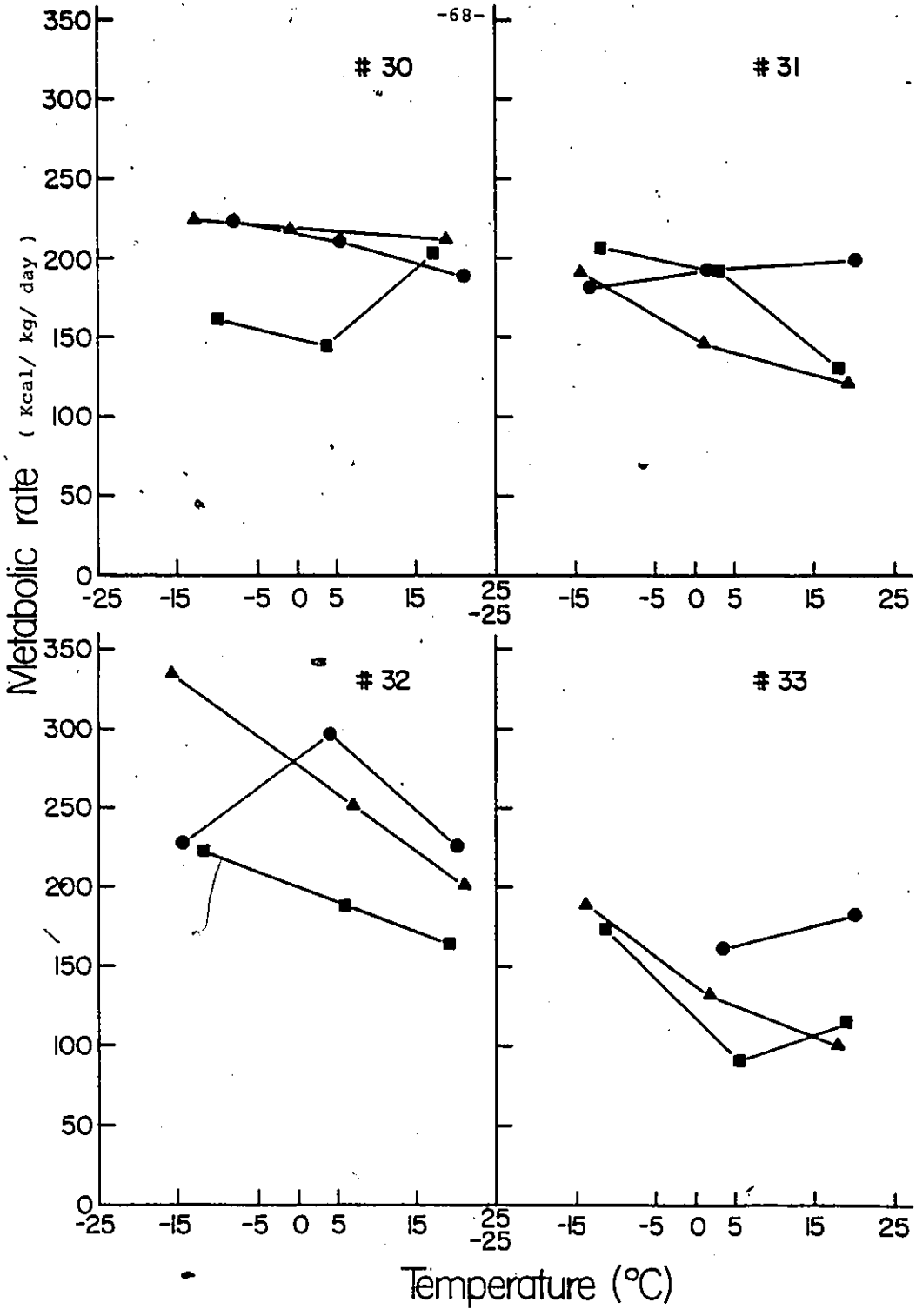

#30
#31
#32
#33
Metabolic rate (Kcal/ kg/ day)
Temperature (°C)
350
300
250
200
150
100
50
0
-25
-15
-5
0
5
15
25

FIGURE 21: Metabolic rate as a function of temperature in ducks treated with PBCO + Corexit 9527 and then exposed to three different temperatures.

A: Bird #34

B: Bird #35

C: Bird #36

D: Bird #37

LEGEND

▲ 1st pre-exposure

Δ 2nd pre-exposure

● exposure

■ post-exposure

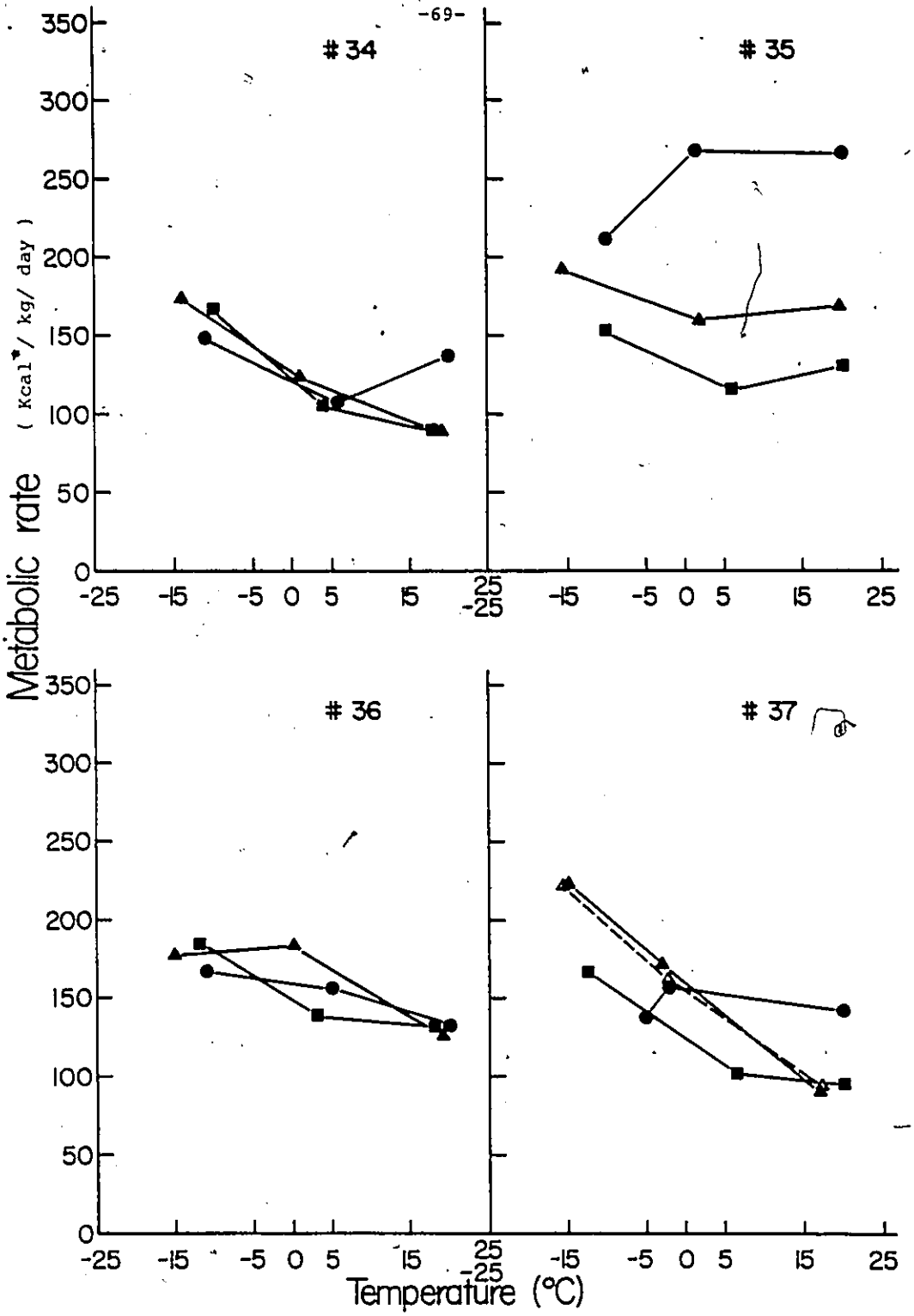

#34
#35
#36
#37
Metabolic rate (Kcal / kg/ day)
Temperature (°C)
350
300
250
200
150
100
50
0
-25
-15
-5
0
5
15
25

Table 3. Slopes calculated from the metabolic rates of ducks at different temperatures for each of the pre-, exposure, and post-exposure runs

			SLOPES ($\Delta MR / \Delta T^{\circ}$)						
Group	Bird #	Run	$-15^{\circ} \rightarrow 0^{\circ}$	$0^{\circ} \rightarrow +15^{\circ}$	Group	Bird #	Run	$-15^{\circ} \rightarrow 0^{\circ}$	$0^{\circ} \rightarrow +15^{\circ}$
CONTROL	22	pre	- 1.0	- 4.3	OIL (pmco)	26	pre	- 4.1	+ 7.0
		exp	- 1.4	+ 2.3			exp	- 4.0	+ 0.6
		post	- 1.1	- 6.0			post	- 2.0	- 1.7
	23	pre	- 0.9	- 1.9		27	pre	- 2.8	- 2.3
		exp	+ 0.3	- 5.2			exp	- 1.2	- 2.7
		post	- 0.8	- 2.9			post	- 2.6	- 2.0
	24	pre	- 1.7	- 0.5		28	pre	- 4.1	+ 0.2
		exp	+ 0.3	- 2.7			exp	- 5.0	+ 0.9
		post	- 2.9	- 0.6			post	- 3.4	- 0.8
	25	pre	- 6.5	+ 0.8		29	pre (1)	+ 1.0	- 5.5
							pre (2)	- 2.0	- 0.2
							exp	+ 0.2	+ 2.9
							post	- 1.9	- 1.8

Table 3. (cont'd)

			SLOPES (Δ MR / Δ T^{o})						
Group	Bird #	Run	$-15^{o} \rightarrow 0^{o}$	$0^{o} \rightarrow +15^{o}$	Group	Bird #	Run	$-15^{o} \rightarrow 0^{o}$	$0^{o} \rightarrow +15^{o}$
DISPERSANT (COREXIT 9527)	30	pre	- 0.4	- 0.4	DISPERSANT OIL	34	pre	- 3.4	- 1.9
		exp	- 0.1	- 1.3			exp	- 2.4	+ 2.1
		post	- 1.2	+ 4.2			post	- 4.3	- 1.2
	31	pre	- 2.9	- 1.3		35	pre	- 1.8	+ 0.5
		exp	+ 0.8	+ 0.3			exp	+ 4.9	+ 0.1
		post	- 0.9	- 4.2			post	- 2.3	+ 1.1
	32	pre	- 3.7	- 3.6		36	pre	+ 0.4	- 3.0
		exp	+ 3.7	- 4.4			exp	- 0.7	- 1.5
		post	- 1.8	- 1.9			post	- 3.0	- 0.5
	33	pre	- 3.7	- 1.9		37	pre (1)	- 4.4	- 4.5
		exp	+ 0.2	+ 1.3			pre (2)	- 4.1	- 4.0
		post	- 5.0	+ 1.9			exp	+ 2.7	- 0.9
							post	- 3.4	- 0.4

can see that in 69 out of 94 cases, a drop in temperature resulted in an increased metabolic rate. In 10 cases, there was no obvious response, while in 15 others, ducks reacted by a decrease in metabolic rate, in opposition with the expected response. Of these 15 cases, 4 only corresponded to the first drop in temperature (from approx. +20^{o} to 0^{o}C) while the other 11 corresponded to the second drop in temperature (from approx. 0^{o} to approx. -15^{o}C). This difference was important for finding what had interfered with the expected response of birds to cold.

A comparison between pre-exposure, exposure and post-exposure metabolic rates from the same bird yielded confusing results. A non-parametric statistical analysis showed that 9 times out of 15, exposure of birds to water (either alone or with oil and/or dispersant) increased their metabolic rate but it decreased in the remaining 6 times. In 11 cases out of 15, post-exposure metabolic rates, 8 days after exposure to water or water + oil and/or dispersant, turned out to be lower than both earlier runs. Of the four exceptions to this, one of the post-exposure average metabolic rate (bird #22) was higher than both earlier runs (pre-exposure and on day of exposure). The post-exposure metabolic rate of another (bird #27) was higher than pre-exposure but lower than exposure. Yet another (bird #26) was the exact opposite, with its post-exposure metabolic rate lower than pre-exposure but higher than exposure. Finally, a last one (bird #31) showed

almost equal exposure and post-exposure metabolic rates and both were higher than the pre-exposure rate.

In summary, metabolic rates measured during the pre-exposure runs were as often higher as they were lower than metabolic rates obtained at the time of exposure, while metabolic rates of post-exposure runs were almost consistently lower than results from both previous ones.

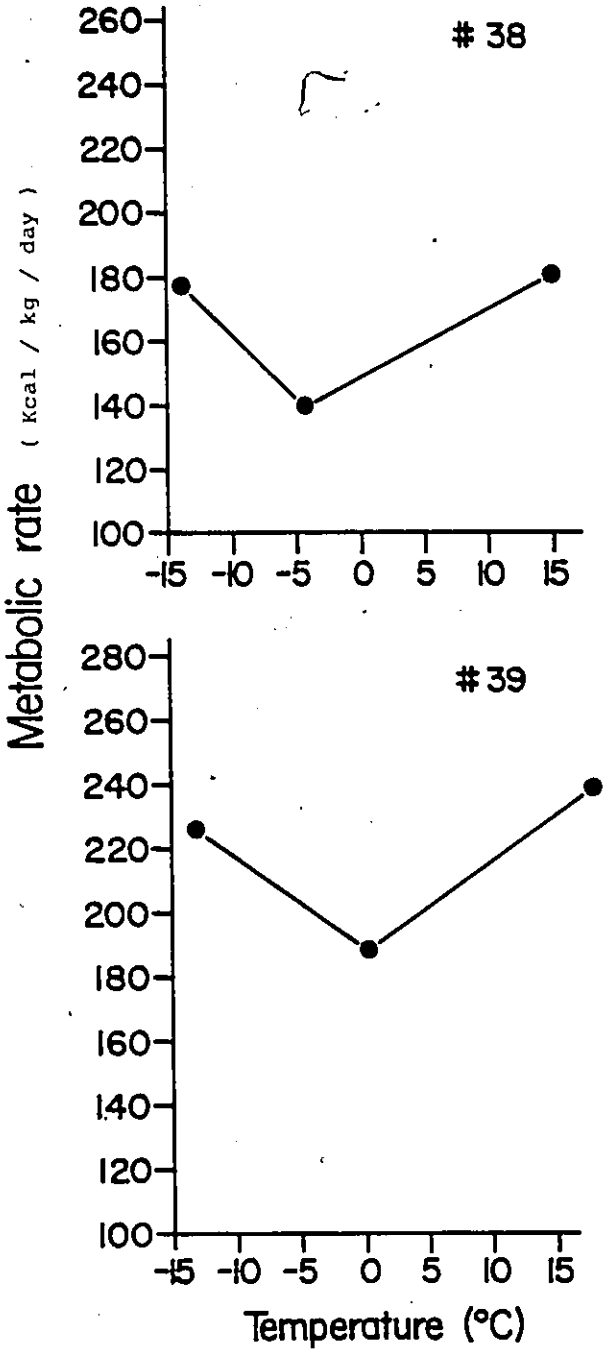
3.1.2.3 Study three

Figures 22, 23, 24 & 25 summarize results from experiments involving only one metabolic chamber and a better control of back-pressure in the oxygen analyzer as described on pages 56 to 60. Metabolism of two of the 8 birds (birds #44 and #45) was measured more than once to check the consistency of these results. Even when the same bird was used more than once, its response differed greatly from one time to the next. The incubator sometimes required a long time to cool to desired temperature. Moreover, it was discovered, with a telethermometer placed at the bottom of the metabolic box, that there was a marked difference in temperature between the top of the box, where temperature was monitored and the bottom where the bird actually sat. There could be as much as a 30°C difference, with top telethermometer indicating -11°C and bottom thermometer +18°C. A duck therefore could create a warm environment around itself by lying down in a corner of the box, tightly pressed against the walls.

FIGURE 22: Metabolic response of ducks exposed to decreasing temperatures using only one metabolic chamber.

A: Bird #38

B: Bird #39


#38
#39
Metabolic rate (Kcal / kg / day)
260
240
220
200
180
160
140
120
100
280
-15 -10 -5 0 5 10 15
Temperature (°C)

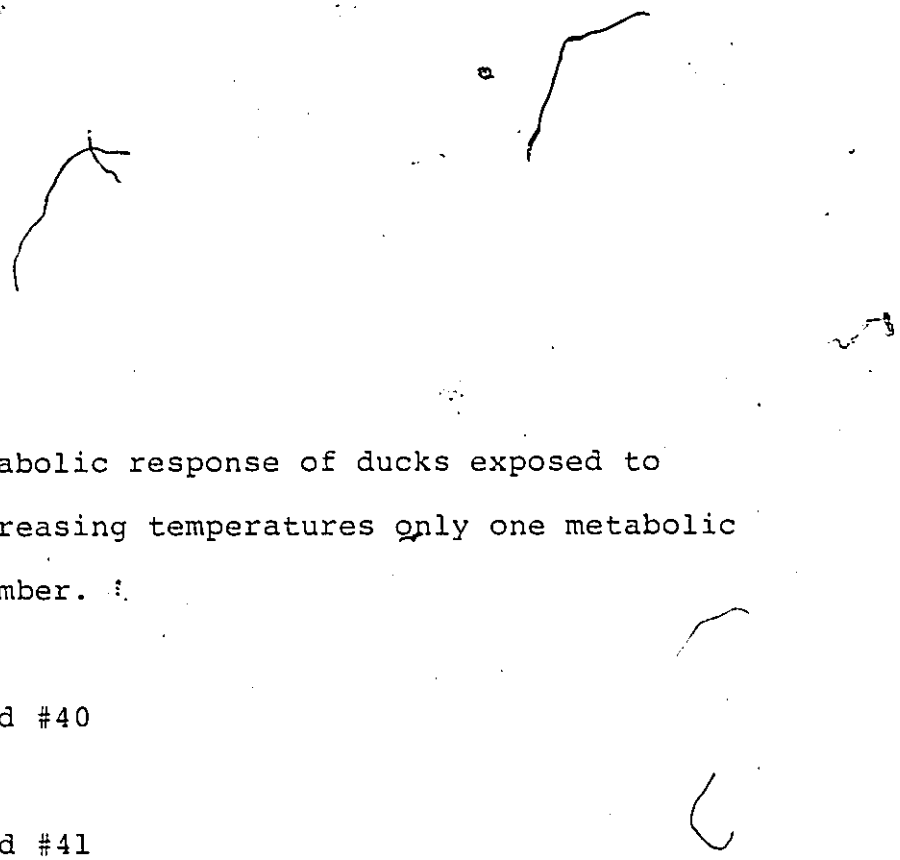


FIGURE 23: Metabolic response of ducks exposed to decreasing temperatures only one metabolic chamber.

A: Bird #40

B: Bird #41

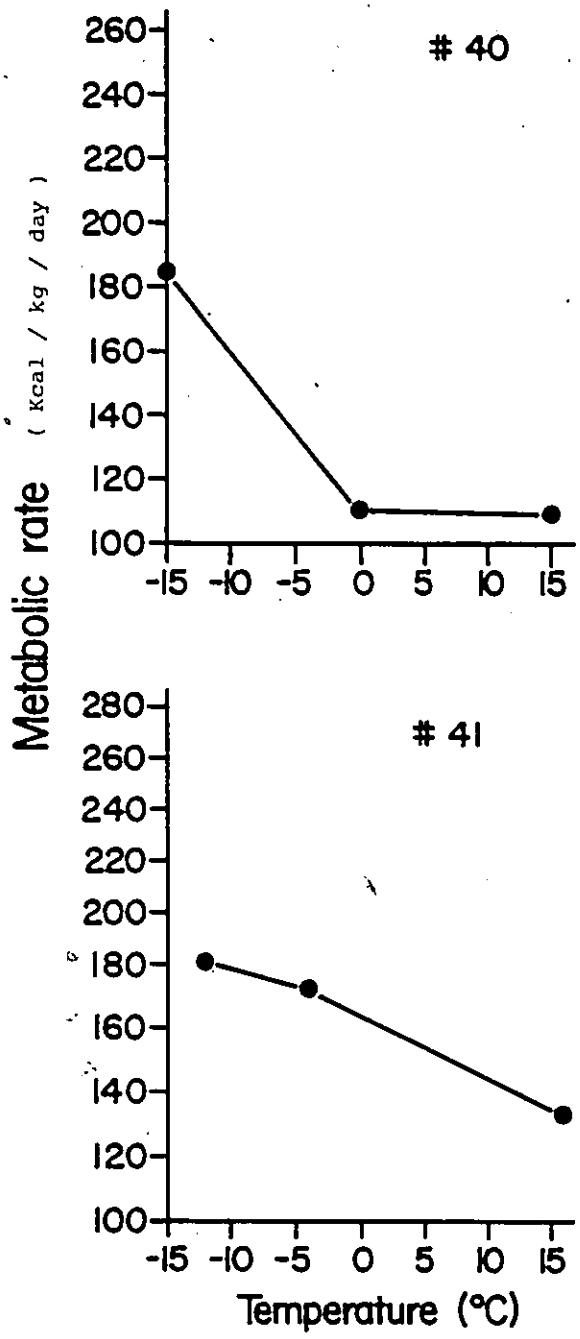

Metabolic rate (Kcal / kg / day)
40
260
240
220
200
180
160
140
120
100
-15 -10 -5 0 5 10 15
41
280
260
240
220
200
180
160
140
120
100
-15 -10 -5 0 5 10 15
Temperature (°C)

FIGURE 24: Metabolic response of ducks exposed to decreasing temperatures using only one metabolic chamber.

A: Bird #42

B: Bird #43

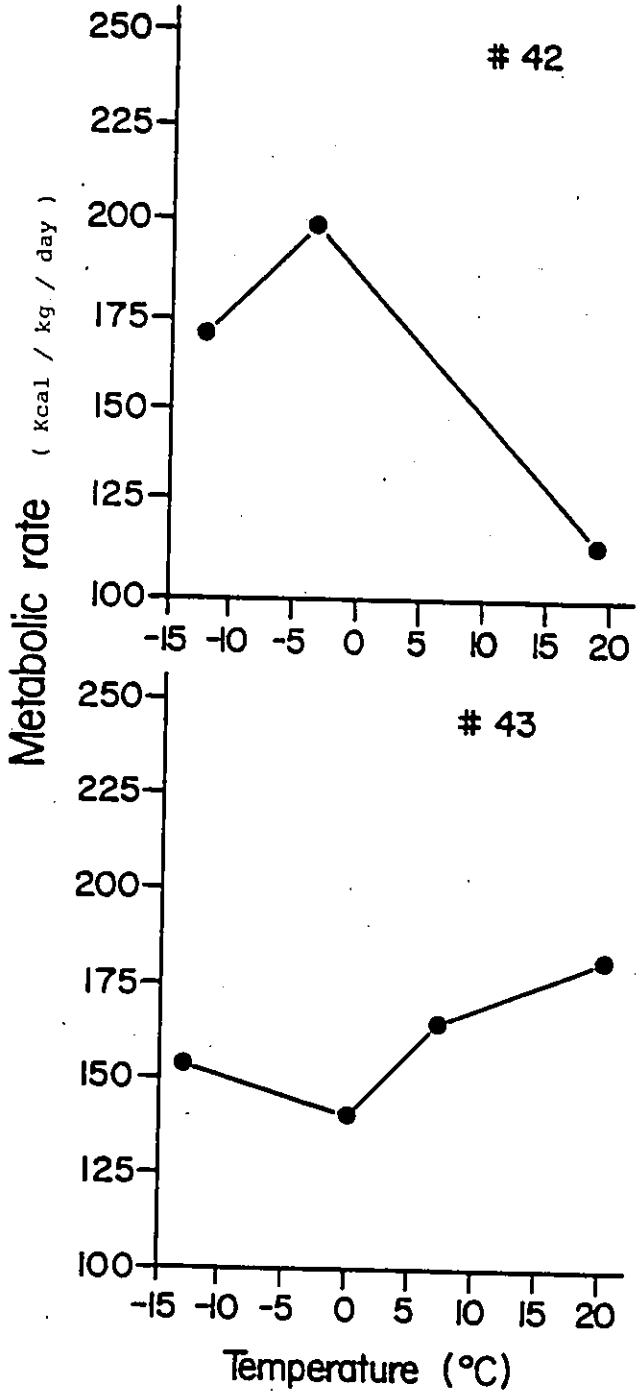

Metabolic rate (kcal / kg / day)
250
225
200
175
150
125
100
42
-15 -10 -5 0 5 10 15 20
43
Temperature (°C)

FIGURE 25: Metabolic response of ducks exposed to decreasing temperatures using only one metabolic chamber. Each bird was exposed more than once to check the consistency of the results.

A: Bird #44

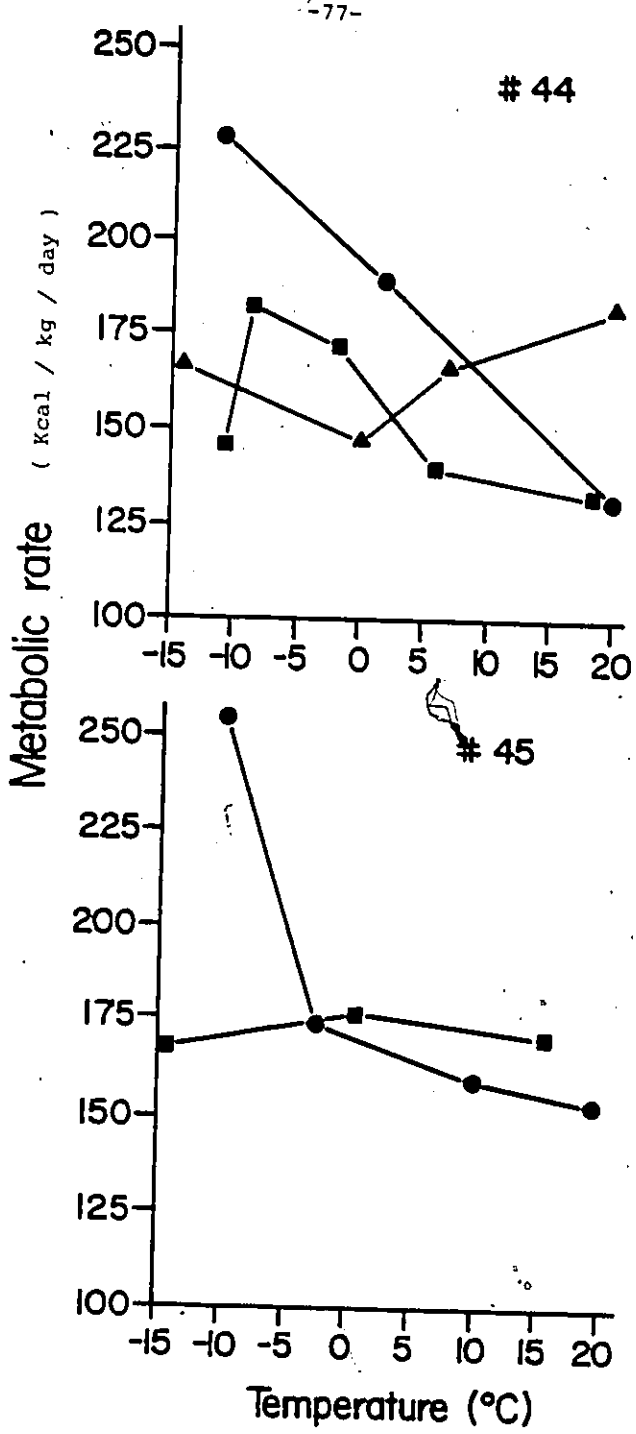
B: Bird #45

LEGEND

● 1st exposure

■ 2nd exposure

▲ 3rd exposure


#44
#45
Metabolic rate (Kcal / kg / day)
250
225
200
175
150
125
100
-15 -10 -5 0 5 10 15 20
Temperature (°C)

3.1.2.4 Study four

After redesigning the metabolic box to ensure better perature control, as shown on page 62, two birds (#46 & #47) were used, each of them twice under identical experimental conditions, to verify if this brought about an improvement in the consistency of results. With one of them (bird #46), results on a first run were fairly good until an ice plug formed in the tube between metabolic chamber and analyzers (Fig. 26A). This interfered with the experiment in progress by changing the volume and pressure of airflow to the analyzers. On a second run with the same bird, the first and last drops in temperature resulted in unexpected decreases in metabolic rate while the second drop produced the expected increase.

In a first run, another bird (bird #47) responded as expected when comparing with similar experiments reported in the literature (Hartung, 1967; McEwan & Koelink, 1973), although the increase in metabolic rate at each temperature was smaller than expected (Fig. 26B). In a second run, the second drop in temperature resulted in a decrease in metabolic rate, which, as seen earlier, is either due to formation of an unnoticed ice plug, a rapid change in pressure inside the analyzer or some unexpected and unidentified factor.

These results were very disconcerting and showed that it was useless to go any further with the method and equipment as designed.

FIGURE 26: Metabolic response of ducks using redesigned metabolic box.

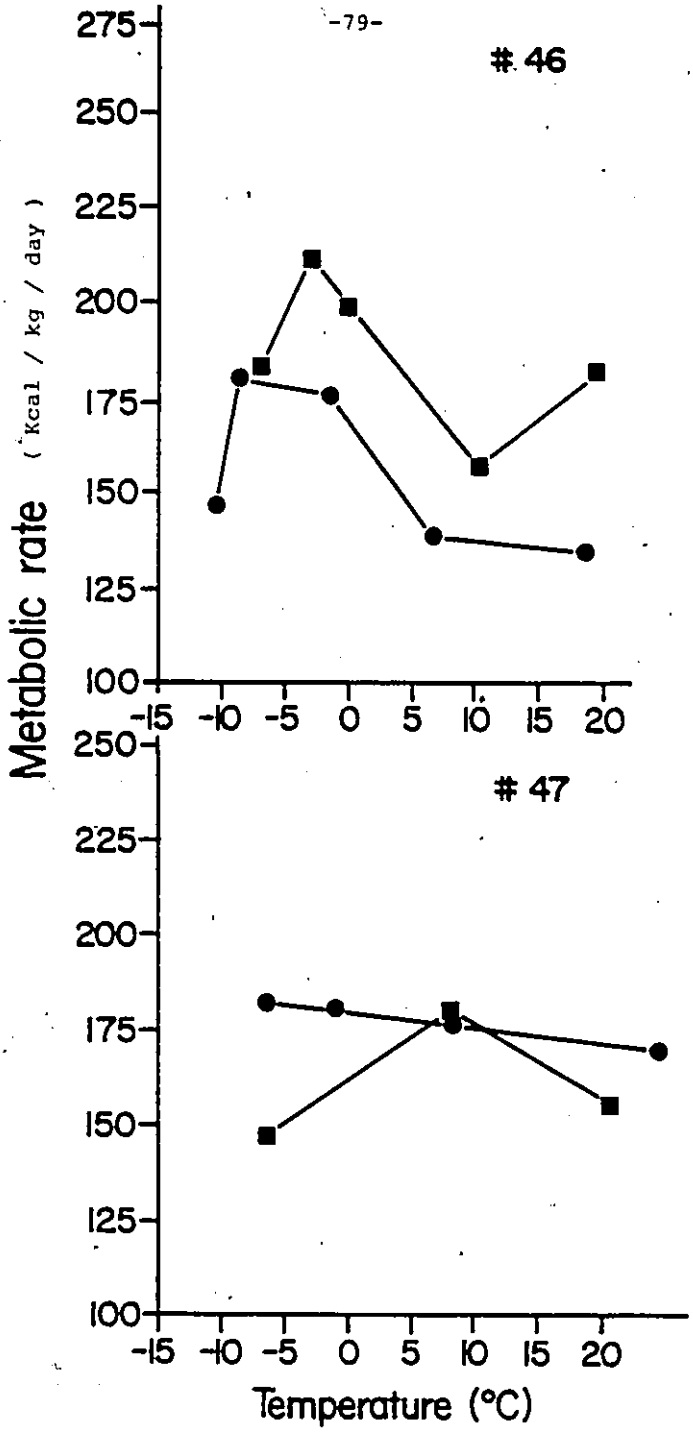
A: Bird #46

B: Bird #47

LEGEND

● 1st exposure

■ 2nd exposure


#46
#47
Metabolic rate (Kcal / kg / day)
275
250
225
200
175
150
125
100
-15 -10 -5 0 5 10 15 20
Temperature (°C)

3.1.2.5 Study five

Results from these tests described on page 61 showed that there is a period of fluctuation at the beginning of birds' adaptation to confinement or confinement + cold stress, after which the metabolic rate is fairly stable. However, the duration of this acclimation period varied from one bird to the next.

Individual birds react differently to handling and restricted confinement in the metabolic chamber. Some birds, such as bird #56, reacted violently and struggled vigorously, pecking at the walls of the metabolic box, at the thermal probe or the metal grid on which they stood, shaking water off their wings, or moving up and down trying to find an escape out of the box. Their metabolism consequently started off very high and required some time to settle down to a resting level (Fig. 27). Other birds, such as bird #57, started with a very low metabolic rate as if "holding their breath" which mallards can do without great stress, being used to diving and feeding under water (Jones, U.B.C.; personnal communication). After a while, they resumed their normal behaviour and their metabolism also settled to a fairly stable level (Fig. 28).

3.1.2.6 Study six

The results summarized in Fig. 29, 30, 31, 32 showed that, with only one very clear exception (bird #52 - ■) where there might have been some technical problems or maybe

FIGURE 27:

Metabolic response of a duck to confinement in a metabolic chamber at 18°C over a period of six hours.

Bird #56

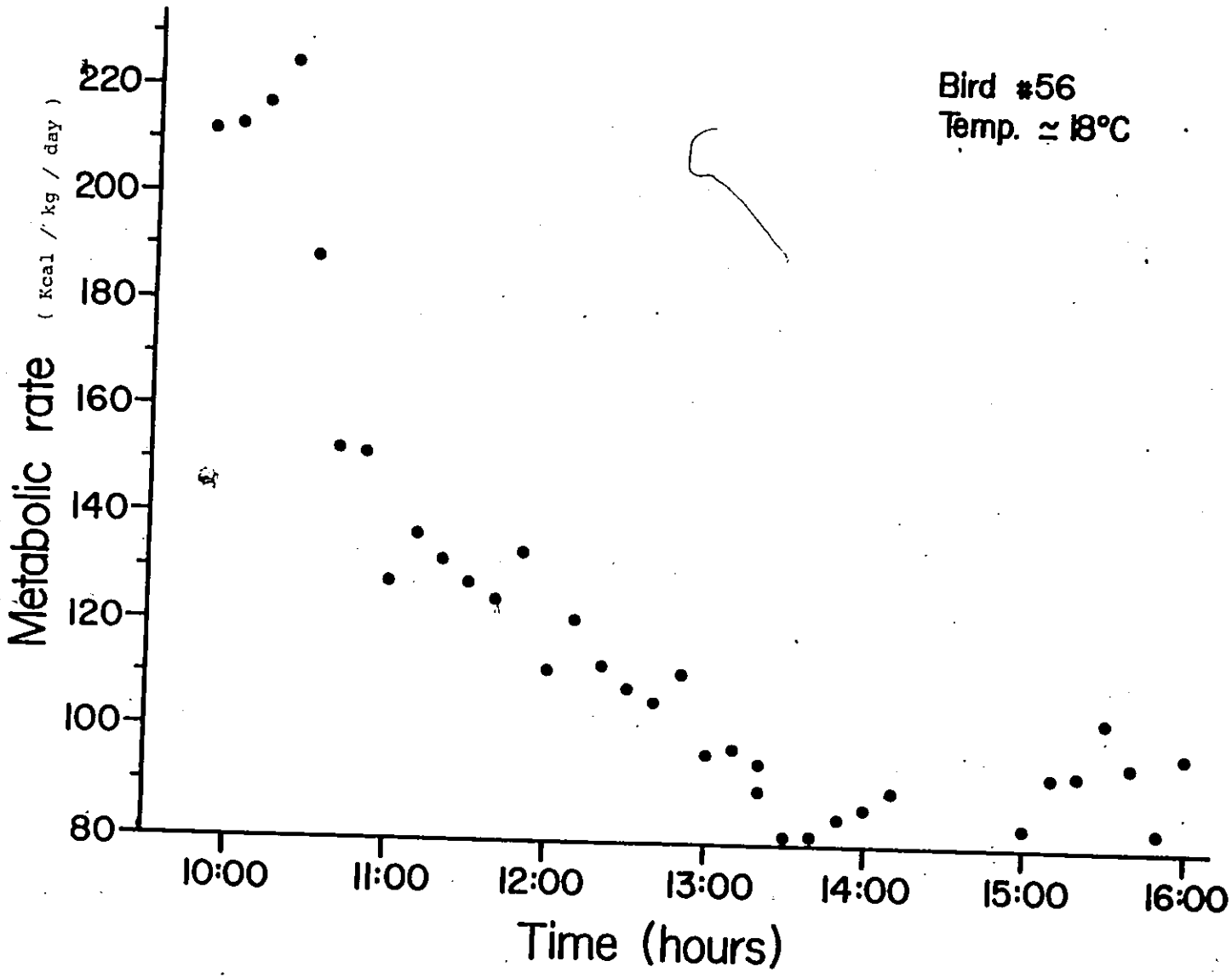

Bird #56
Temp. ≃ 18°C
Metabolic rate (Kcal / kg / day)
220
200
180
160
140
120
100
80
10:00
11:00
12:00
13:00
14:00
15:00
16:00
Time (hours)

FIGURE 28:

Metabolic response of a duck to confinement in a metabolic chamber at $0^{o}C$ over a period of six hours.

Bird #57

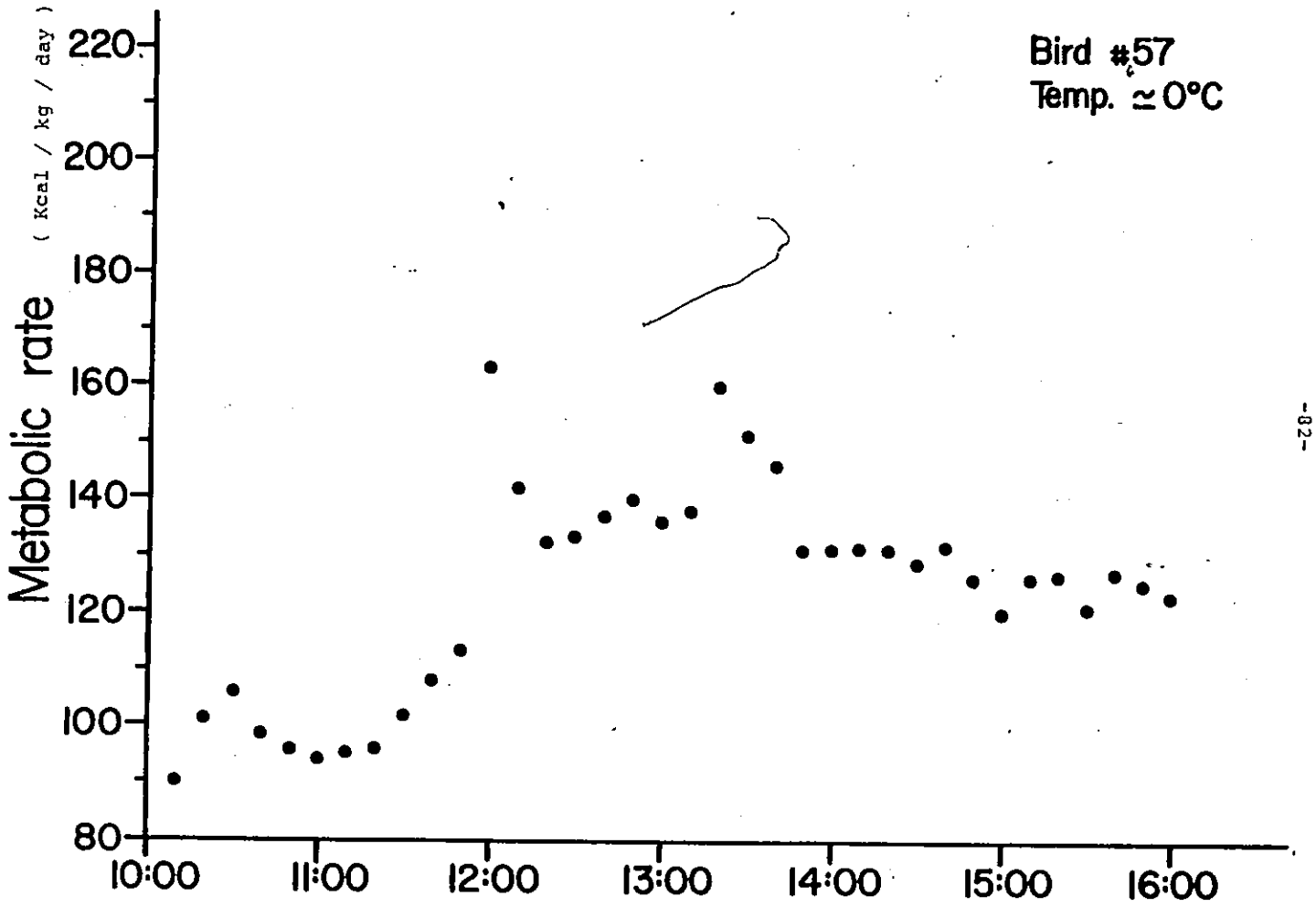

Bird #57
Temp. ≃ 0°C
Metabolic rate (Kcal / kg / day)
220
200
180
160
140
120
100
80
10:00
11:00
12:00
13:00
14:00
15:00
16:00

FIGURE 29: Comparison between the metabolic response of ducks to cold temperatures during dry runs, wet runs and after exposure to dispersant.

A: Bird #48

B: Bird #49

LEGEND

▲ dispersant

● 1st dry run

■ 2nd dry run

○ 1st wet run

△ 2nd wet run

□ 3rd wet run

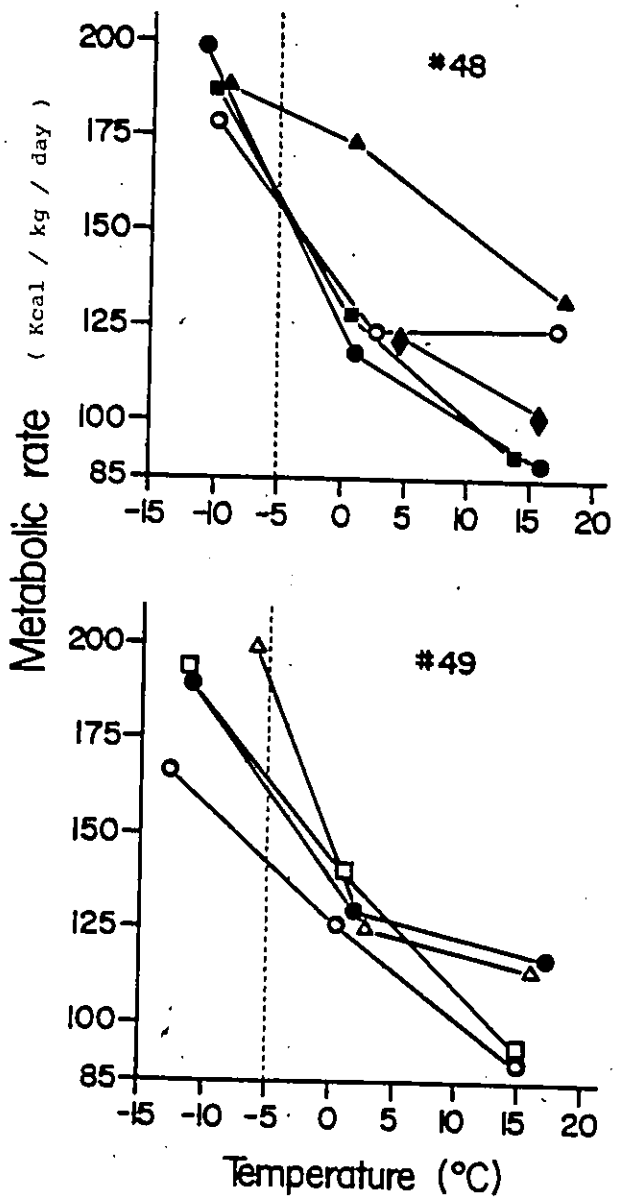

#48
#49
Metabolic rate (kcal / kg / day)
200
175
150
125
100
85
-15
-10
-5
0
5
10
15
20
Temperature (°C)

FIGURE 30: Comparison between the metabolic response of ducks to cold temperatures during dry runs, wet runs and after exposure to dispersant.

A: Bird #50

B: Bird #51

LEGEND

▲ dispersant

● 1st dry run

■ 2nd dry run

O wet run

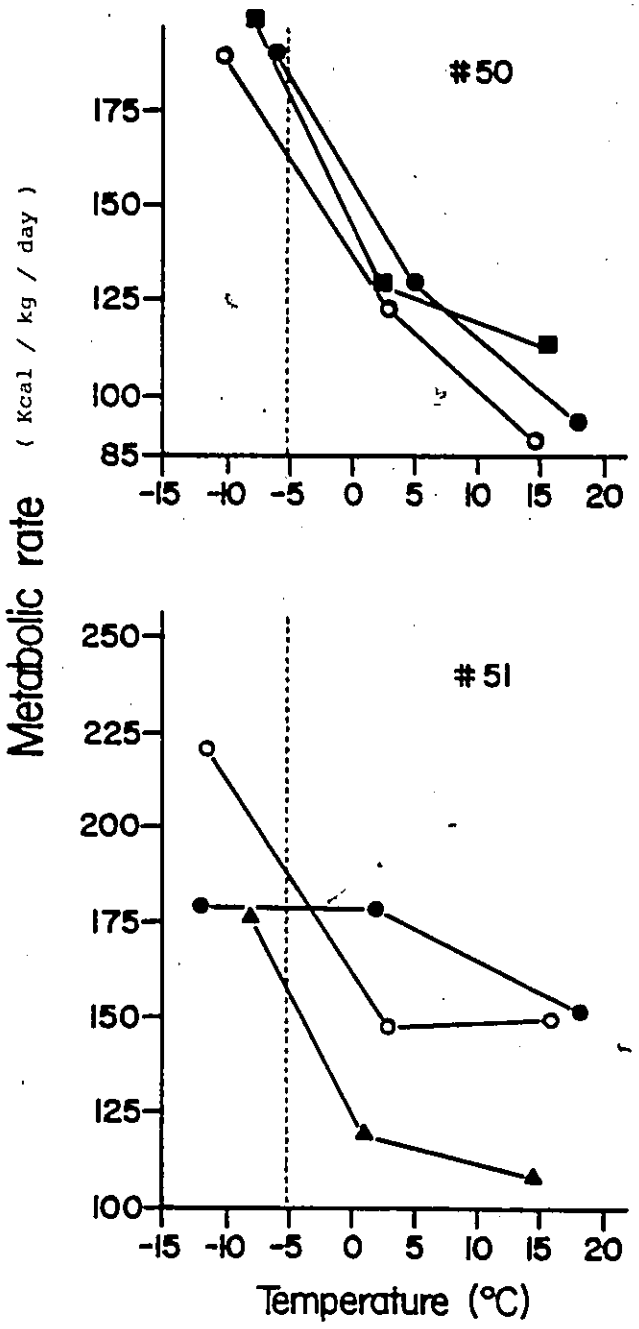
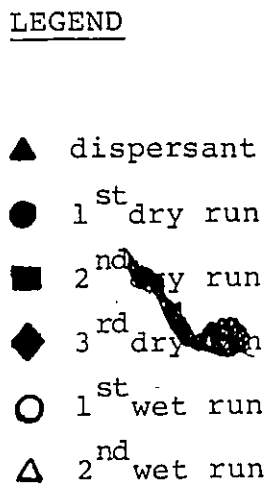

#50
175
150
125
100
85
-15 -10 -5 0 5 10 15 20
Metabolic rate (Kcal / kg / day)
#51
250
225
200
175
150
125
100
-15 -10 -5 0 5 10 15 20
Temperature (°C)

FIGURE 31: Comparison between the metabolic response of ducks to cold temperatures during dry runs, wet runs and after exposure to dispersant.

A: Bird #52

B: Bird #53



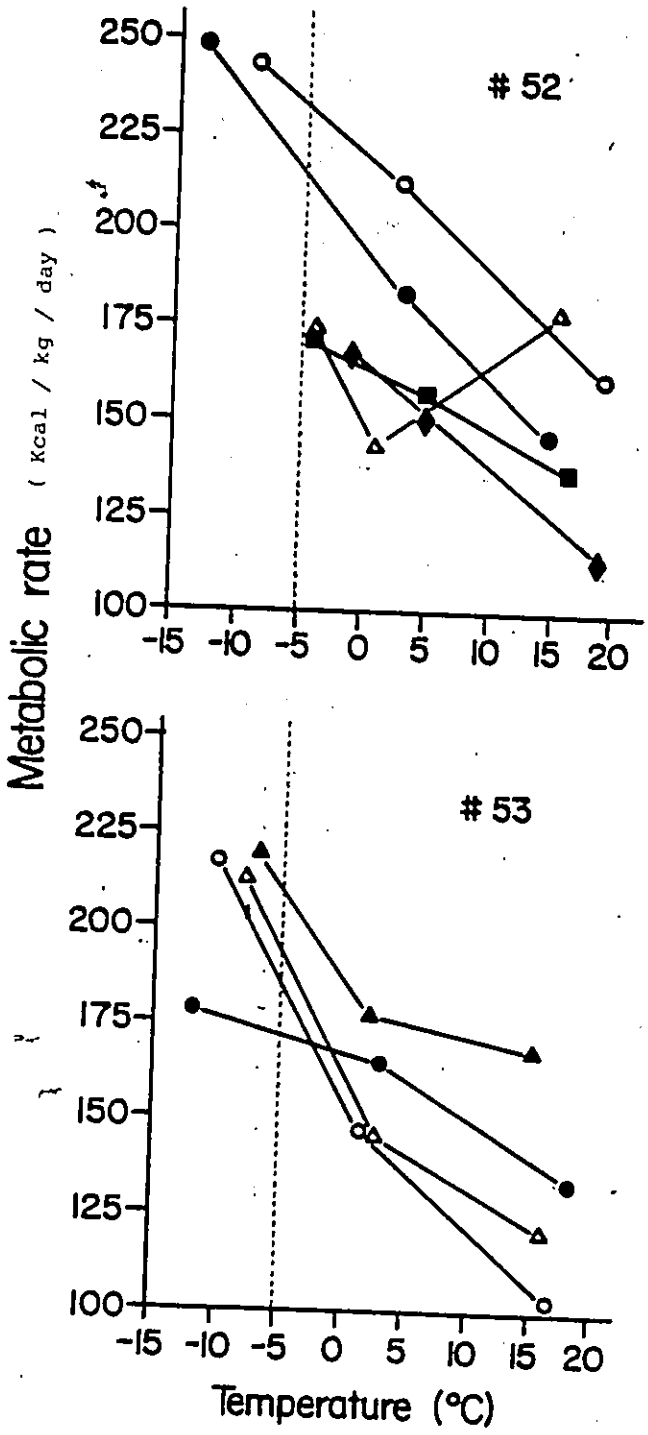

Metabolic rate (Kcal / kg / day)
250
225
200
175
150
125
100
52
53
-15 -10 -5 0 5 10 15 20
Temperature (°C)

FIGURE 32: Comparison between the metabolic response of ducks to cold temperatures during dry runs, wet runs and after exposure to dispersant.

A: Bird #54

B: Bird #55

LEGEND

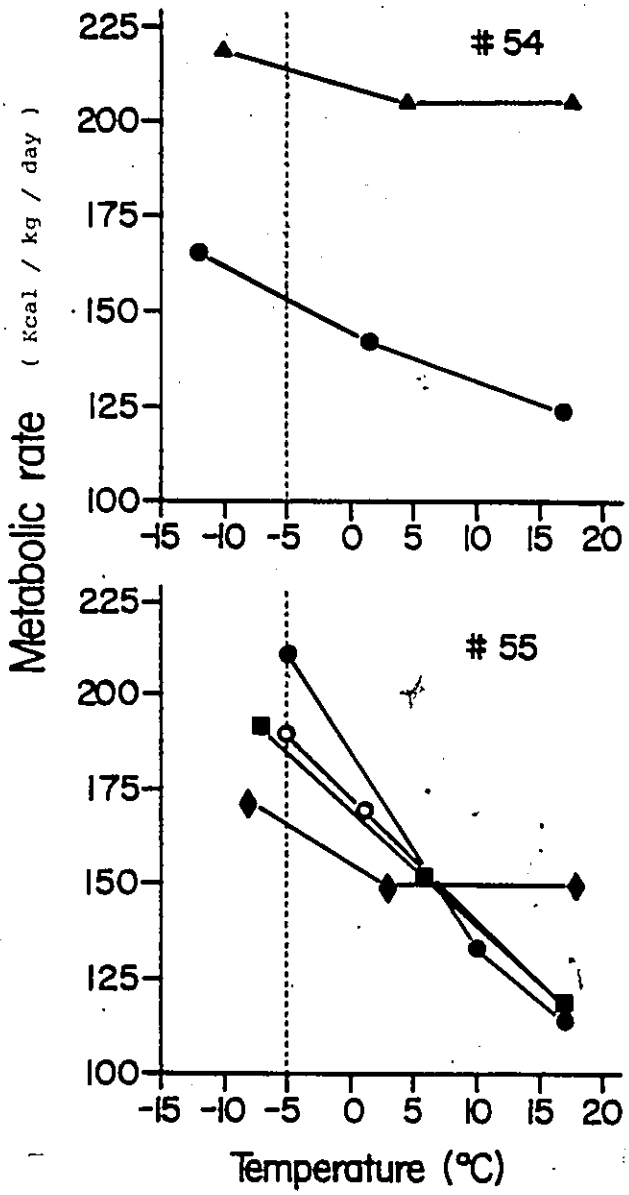
▲ dispersant

● 1st dry run

■ 2nd dry run

◆ 3rd dry run

○ wet run


#54
#55
Metabolic rate (Kcal / kg / day)
225
200
175
150
125
100
-15 -10 -5 0 5 10 15 20
Temperature (°C)

some misreading of the first set of data (Fig.31A), results from experiments following the method described on page 63 were definitely in agreement with expectations based on previous studies, in as much as a decrease in temperature consistently resulted in an increase in metabolism. The ranges of metabolic rates obtained here was also very similar to those found in the literature. This seemed to indicate that the method and equipment used were finally adequate for the purpose of this study which was to compare the metabolism of ducks treated with different pollutants.

From the same set of results, a comparison between "dry" and "wet" runs yielded inconclusive results: 4 out of 7 times, exposure of the ducks to clean seawater (or "wet" runs) resulted in metabolic rates slightly lower than "dry" runs where birds were taken from their pen directly to the metabolic chamber. Two of the remainers (birds #48 and #52) showed a very small increase in metabolism during the wet run and the last one (bird #55) showed no difference at all between wet and dry runs. Of the 4 ducks exposed to dispersant, 3 responded by a sensible increase in metabolism (birds #48, #53, #54) while another showed a decrease (bird #51).

Although the metabolic rates did increase consistently with a decrease in temperature, the calculated slopes showed a wide range of variation. The averages and standard deviations calculated for each group therefore greatly overlapped (Table 4) and were therefore inadequate for comparisons between groups.

Table 4. Variations in metabolism as a function of temperature

(This table presents the salient features of figures 29 to 32)

Group	n	Average slope	Standard deviation	Significance
Dry	16	- 2.76	1.19	*
Wet	10	- 3.08	0.69	*
Dispersant	4	- 1.69	1.04	*

* Student's T-tests on all of them showed that the averages were not significant.

3.1.3.0 Discussion

3.1.3.1 Study one

The response of ducks to temperature changes, as seen on page 65, did not follow the usual pattern of an increase in metabolic rate proportional to the decrease in temperature. In fact, one bird reacted in exactly the opposite way (bird #16, Fig. 17), decreasing its metabolism with a decrease in temperature. Another bird (bird # 17) did not react at all to a change in temperature, maintaining its metabolism stable throughout the experiment. In some cases (birds #18, #20, #21), the same bird reacted differently to two consecutive drops in temperature.

These results are difficult to analyze and explain individually since they were not only inconsistent with our expectations, but also inconsistent from one bird to another. This seems to indicate that an unidentified factor in the material involved or method used was inadequate and interfered with the consistency of results. Results which we derived from experiments, as presently designed, were not a valid indication of birds' responses to changes in temperature. They were merely an artifact hiding a variety of parameters which affected, to the point of completely overshadowing, the actual response of birds to cold temperature. Their impact was underestimated and the experimental design therefore had to be modified.

Maintaining a low temperature in the two incubators turned out to be a major problem since the incubators warmed up rapidly everytime the door was opened to transfer a duck or to make an adjustment to the metabolic chamber. Temperature variations were very wide, which somewhat interfered with derivation of a slope from the metabolic rate of ducks at three clearly distinct temperatures.

Another factor that very likely affected the results in previous experiments was the rapid changes in pressure resulting from alternate analysis of air from each of the chambers. When all three chambers were functionning simultaneously, only one a time could be connected to the analyzers, while the other two were vented into the room. Consequently, pressure build-up in metabolic chambers was minimal during venting since there was no resistance opposing to outflow of air from the chamber. During analysis, however, air was pushed through a system of tubes, valves, drierite column, filters and analyzers. This resulted in a very high pressure build-up in the metabolic chamber, required to force air along this highly resistant path. This big difference in pressure to which a bird was submitted was very likely one of the major reasons for the inconsistency of results.

The sizable drop in metabolic rate at the lowest temperature in birds #18, #20, #21 (Fig. 17) might also be due to icing up in outgoing tubing of the coldest metabolic chamber (below $0^{o}C$). This ice "plug" further restricted an already

highly resistant air path, causing an even greater pressure build-up than normally. This not only affected the birds' metabolism directly but also modified the pressure at which air was delivered to the analyzers, thus causing unexpected variations in the results.

Modifications required here to improve the system are therefore: a better control of temperature and pressure; prevention of ice formation in tubes between metabolic chamber and analyzers.

3.1.3.2 Study two

Results shown on pages 66 to 69 were certainly a great improvement over the previous ones. But in one case out of four, results still deviated, at times slightly, at times strongly, from logically expected results. This proportion of unusual results was much too high to be overlooked or ignored.

Of 15 decreases in metabolic rates following a decrease in temperature, 4 corresponded to the first drop (from approx. $+20^{o}$ to approx. $0^{o}C$), while the 11 others corresponded to the second drop (from approx. 0^{o} to approx. $-15^{o}C$). This difference was strong evidence that there still was something happening, especially at very cold temperatures, which interfered with the proper response of birds to a further temperatures decrease. Another strong possibility was that the "unknown factor" did not actually affect the birds' response but in fact, prevented an adequate monitoring of a proper response.

A possible cause for inconsistencies of the results seen here is the difference in birds' metabolism during a "dry" run, where birds are not exposed to water, and a "wet" run, where a bird is exposed to seawater with or without a pollutant. In these experiments, pre- and post-exposure runs were "dry". Birds were taken directly from their pen to the metabolic chambers. In contrast, on the day of exposure, ducks were exposed to seawater, either alone or with oil and/or dispersant. It is very likely that insulating properties of the plumage at very cold temperature differed according to its state of wetness or dryness.

It is also known that birds, and consequently their metabolism, are very sensitive to any kind of handling. The method as designed required handling each duck from its cage to a swimming tank, then to a first metabolic chamber, again to a second and later to a third chamber. All this handling, no doubt, stressed the ducks and very likely affected their behaviour, their endocrine state and consequently their metabolism. The easiest way to avoid this was to redesign experiments so that only one metabolic chamber was used for all three temperatures. This not only eliminated the problem of handling and its possible consequence on ducks' metabolism but also allowed for uninterrupted analysis of air coming out of the chamber. This, at once, eliminated all possible pressure variations due to switching on and off of valves directing air to the analyzers. These, although greatly reduced by

the removal of millipore filters, might still contribute to variability in results.

Another parameter possibly compromising the accuracy of readings is the variability in barometric pressure during experiments. In the CO_2 analyzer, these variations in atmospheric pressure did not introduce any error in instrument readings, as long as the same flow rate was used for calibration and for sample analysis. However, in the oxygen analyzer, barometric pressure variations affected the flow of air through the analyzer and consequently, the accuracy of readings. A 1% barometric pressure change after calibration could have resulted in as much as 20% readout error.

The analyzers were calibrated only once a day, early in the morning. A back-pressure regulator was therefore necessary to monitor and correct for any change in barometric pressure during the day. Although tubes linking metabolic chambers to analyzers were replaced by much bigger ones, this was not sufficient to prevent icing up within the tubes and a resulting escalation of pressure inside the box. A much closer and more frequent verification of the tubes throughout the experiments was therefore mandatory.

3.1.3.3 Study three

By creating themselves a warm environment within the box, ducks were never really exposed to cold as was speculated.

Impact of cold temperature depended therefore exclusively on the effectiveness of a birds' position within the box. This explains to a great extent why some birds reacted as though "untouched" by cold, as can be seen on pages 74 to 77. Indeed, they hardly felt it at all. Results obtained so far were thus related to the temperature measured in the upper corner of the metabolic chamber, which was very different from the actual stress imposed on the bird. This strongly stressed the need to redesign the metabolic box, to ensure a better air circulation throughout the box. A better monitoring of temperature which would help detect any discrepancies between different points in the metabolic chamber was also essential.

3.1.3.4 Study four

Redesigning of the metabolic box did not bring about the expected improvement. Apparent decreases in metabolic rate in both runs of bird #46 and in the second run for bird #47 (Fig 26, page 79) were very likely an artifact resulting from restriction of the air flow going through the analyzer, by formation of an ice plug in the tubing. This therefore did not necessarily represent an actual phenomenon at the level of birds' metabolism but simply an inability to properly measure metabolism with the present equipment, applying the current methods of monitoring and calculating.

The new design of the box and close monitoring of temperature with telethermometer probes ensured that the temperature

read on the telethermometer was, in fact, the temperature to which birds were exposed. Similarly, pressure monitored and controlled through a manometer and back-pressure regulator should not have varied sufficiently to affect results of the present experiment.

One factor possibly affecting these experiments and not taken into account so far was the time required by ducks to adjust to a certain temperature and respond to it. The 45 minute acclimation period allowed so far might simply not have been sufficient for birds' metabolism to reflect accurately a direct effect of cold stress.

3.1.3.5 Study five

Results on Fig. 27 & 28 (pages 81 & 82) have shown the variability in time required for a bird's metabolism to reach stability. This implies that a great flexibility is required in timing of experiments. Not only do individual birds react differently from one another, but they also differ in rapidity of their response to two consecutive changes in temperature. This implies also that metabolism of a duck has to be monitored constantly from the time of its transfer to a metabolic chamber to the time at which a certain stability is reached. This point of relative stability can be fairly easily detected and readings therefore, if monitored over a long enough period of time, are quite consistent. From that point on, a minimum of

20 readings constitute a fair sample from which actual metabolic measurements can be calculated and used as an indication of this bird's response to specific experimental conditions. If the acclimation period before data gathering is too short, one may end up comparing the metabolic rate of a bird at steady state at one temperature, with its metabolism in a transient state, during fluctuations due to a longer adaptation period at another temperature. This obviously introduces a considerable error reflected by a variety of aberrant results, such as seen so far. Consequently, experimental procedures had to be modified at three different levels to adequately measure the actual response of a bird to cold stress. First, it was necessary to fast birds longer to make sure that measurements obtained really reflected the duck's post-absorptive state. Then, it was also essential to wait longer after reaching a desired temperature, to ensure that the metabolism measured indeed reflected the duck's actual response to cold stress and not merely a segment of fluctuations of metabolism while the bird was adapting to a new temperature. Finally, it was also necessary to read over a longer period, once a point of stability had been reached, so that results would correspond to the bird's metabolism after adaptation to cold and not a random sample of its metabolism during acclimation.

3.1.3.6 Study six

The second "wet" run of bird #52 was the only one showing a decrease in metabolism corresponding to a decrease in temperature, out of the 30 experiments seen on pages 83 to 86. This clearly indicates that something went wrong, either technically, behavourly or in data recording. A technical problem is ruled out since all known parameters were closely monitored and controlled. Behaviour of the bird, such as a high level of activity as a reaction to confinement in a metabolic box, might have affected the results. This is however less likely to occur during a bird's fifth exposure to a metabolic chamber over a period of 4 months, than it would have been on the first run. Although no clear explanation can be given for this aberrent result, it seems justified to exclude it from the overall analysis of data (Table 4).

Although there did not seem to be any clear difference between wet and dry runs, it is certainly safer to expose all birds to identical experimental procedures on each run. Therefore, from this point on, all ducks were exposed to clean seawater before any metabolic measurements.

Results as plotted on Fig. 29 to 32 are very consistent but when comparing the slopes, only a very poor correlation can be drawn between groups. This therefore means that slopes used in this way are a very poor criterion for comparison between treatments since this variability is greater than

the difference between groups (Table 4).

The only useful comparison that can be made, using the data available so far is between metabolic rates of a duck at the same low temperature and under different experimental conditions (as indicated by the dotted line on Fig. 29 to 32). This therefore means that the whole experimental design can be simplified to expose ducks to only one low temperature and compare results at that temperature, since this is the only useful point on the present plots. It is then easier to reach and maintain a lower temperature, since temperature settings on an incubator don't have to be changed between metabolic readings. It also allows for a much shorter experiment, thus reducing stress of confinement to birds. Results of such experiments are then numerical values, representing the metabolic rate, instead of a slope and thus allow for much easier statistical tests and comparisons between treatment groups.

3.2.0 Hypothesis testing

Having developed an adequate method and improved the equipment to suit the requirements of the present experimental procedures, it is now possible to investigate the actual effects of contamination with oil and/or dispersant on mallard ducks.

From the modifications of chemical composition and behaviour of oil seen when a dispersant is used, it is also expected that the effects of oil alone on birds will be modified when oil is combined with a dispersant.

3.2.1 Materials and Methods

To test this, ducks were exposed to only one low temperature (approximately 12° C) to simulate the kind of cold stress a bird could be exposed to when returning to shore, in a cold environment. The complete treatment of each bird included two exposures to seawater alone (pre-exposure runs), a few days apart to establish the basic metabolic rate of each individual bird, then a one-time exposure to either PBCO, Corexit 9527, or a combination of the two (exposure run). After exposure to a pollutant, each bird was exposed two more times to seawater alone over the following two-week period to follow its recovery from contamination with the particular pollutant.

Metabolic rate at this low temperature was calculated from 20 to 30 readings. Using each bird as its own control, it was possible to compare the individual response of each bird to exposure to seawater, then to a pollutant and finally to seawater again. The response of each bird was also compared with other birds within its own treatment group and the average from each group used to compare different treatment groups. Percent change over basic metabolic rate (indicated by the control value) was calculated for each bird and averages for each exposure and treatment also calculated.

Normality of distribution of each group of data was verified using Kolmogorov-Smirnov statistical test. ANOVA (analysis of variance) tests and Duncan's multiple range tests were used to estimate the significance of differences among treatment groups and also of different runs (control, exposure, 1^{st} post- and 2^{nd} post-exposure) within each treatment group.

3.2.2 Results

Matting of feathers into clumps, as a results of contamination with oil, allows water to infiltrate the duck's plumage. This reduces insulating properties of the plumage by displacing the layer of warm air normally trapped between feathers that usually protects a bird against cold. During and after exposure to oil, birds were often observed shivering, either while floating on water or sitting in a metabolic chamber.

After having exposed each bird twice to seawater, the average between the two metabolic rates obtained was calculated in order to establish the basic metabolic rate (BMR) of each individual bird. In 29 out of 37 cases, the first experiment yielded a higher metabolic rate than the second time around and, at times, to a very large extent. It was therefore decided to use the second reading as BMR and to consider the first run as a "breaking-in" to accustom the bird to experimental procedures which included one-hour forced swimming, transfer to a metabolic chamber and a 2 1/2 to 3 hours confinement inside a cold metabolic chamber.

Readings taken every 5 minutes showed that all birds required from 45 minutes to 3 hours for their metabolism to reach a point of stability. From that point on, a minimum of 20 readings were taken for actual measurements of their metabolic rate.

Table 5 summarizes the average values of metabolic rates for each exposure and treatment, which are given in more details in appendix I. The three groups showed an almost identical basic metabolic rate, with an overall average of 200.1 kcal/kg/day $\pm$ 3.0 (or $\pm$ 1.9%), (n = 37) and an Anova test confirms that these control values are indeed not statistically different and therefore belong to the same "population" of data (Appendix II a.). On this basis, the results from further tests using these arbitrarily distributed ducks can be used for comparing the effect of specific treatments.

Table 5. Metabolic rates of mallards exposed to oil and/or dispersant.

Figures are arithmetic means ($\bar{X}$) in kcal / kg day, standard error (SE) and sample size (n).

	OIL				OIL + DISPERSANT				DISPERSANT			
	$\bar{X}$	SE	n		$\bar{X}$	SE	n		$\bar{X}$	SE	n	
CONTROL	206.8	6.5	(15)		195.6	4.0	(12)		195.5	9.5	(10)	
EXPOSURE	222.7	9.5	(15)	*	241.5	10.3	(11)	**	204.7	6.0	(10)	Δ
1° POST-EXPOSURE	232.6	6.8	(15)	*	231.9	12.0	(12)	**	193.0	10.8	(10)	Δ
2° POST-EXPOSURE	232.0	10.2	(15)	*	237.7	9.1	(12)	**	201.2	11.5	(9)	Δ

*, ** significantly different from the CONTROL value but not significantly different from one another.

Δ not significantly different from the CONTROL value or from one another

When birds were exposed to oil, dispersant or oil + dispersant, changes in metabolic rates over a two-week period depended on the individual treatment. Anova tests show that the results for the three treatment three treatment groups at the time of exposure are statistically different from one another ($p = 0.04$, Appendix II b). This difference is maintained through the 1st post-exposure run ($p = 0.01$, Appendix II c) but decreases slightly by the 2nd post-exposure measurement ($p = 0.06$, Appendix II d).

Another set of Anova tests within each treatment group has shown that the measurements of metabolic rate at the time of exposure and on both post-exposure runs in the oil and oil + dispersant groups differed significantly from the control values ($p = 0.045$ and $p = 0.004$ respectively, App. III a & b), while the ones from birds exposed to dispersant alone showed no difference at all ($p = 0.864$, App. III c). This therefore indicates that exposure to dispersant alone had no effect on the metabolic rate of ducks, either at the time of exposure or during the two-week post-exposure period.

Table 6 shows the average percent change of each treatment using each bird as its own control. The percent increase or decrease in metabolic rate over the BMR was calculated for each experiment (exposure, 1st post- and 2nd post-exposure) and for each individual birds. The average percent seen on Table 6 are therefore not a mere calculation drawn from the average metabolic rates on Table 5, but an actual averaging of the individual percent change calculated for each bird.

Table 6. Percent change of metabolic rate of mallards exposed to oil and/or dispersant. Figures are arithmetic means ($\bar{X}$) in percent change over the basic metabolic rate, standard error (SE) and sample size (n).

	OIL			OIL + DISPERSANT			DISPERSANT		
	$\bar{X}$	SE	n	$\bar{X}$	SE	n	$\bar{X}$	SE	n
CONTROL	-			-			-		
EXPOSURE	+ 10.6	5.4	(14)	+ 22.3	4.6	(11)	+ 6.2	4.2	(10)
1^{o} POST-EXPOSURE	+ 16.3	5.3	(14)	+ 18.2	4.9	(12)	+ 0.2	5.6	(10)
2^{o} POST-EXPOSURE	+ 16.6	7.5	(14)	+ 21.8	4.6	(12)	+ 5.2	6.4	(9

Percent changes in metabolic rates were calculated using each bird as its own control

This method allowed for an evaluation of the increase or decrease in metabolic rate, independently from the BMR , thus eliminating differences between individual birds with either very high or very low BMRs. Here again, Anova tests were done on each set of results to verify the significance of differences observed in averaged. These tests confirmed the results obtained from actual metabolic rates of Table 5.

Exposure of birds to PBCO caused an increase in metabolic rates from an average of 206.8 kcal/kg/day for control to 222.7 kcal/kg/day for the same birds on day of exposure to oil. This represents an increase of 10.6% over control value (Table 6). Metabolic rates of these same birds, when measured one and two weeks after exposure showed a further and sustained increase to 232.6 and 232.0 kcal/kg/day respectively. This corresponds to increases of 16.3 and 16.6% over control values (Table 6). According to the statistical tests, metabolic rates for exposure, 1^{st} and 2^{nd} post-exposure were all different from the control but not from one another ($p = 0.75$, Appendix IV a).

Exposure of birds to dispersant Corexit 9527 hardly caused any effect on metabolism. The average metabolic rate ($n = 10$) went from 195.5 kcal/kg/day for control to 204.7 kcal/kg/day when birds were exposed to dispersant. This 6.2% average increase in metabolic rate results from averaging values of a very wide range (from - 9.9 to - 30.4%) as shown by the very high standard error ($\pm$ 4.2 or 68%). This lack of effect of dispersant observed on the day of exposure continues over the post-exposure period, with 193.0 and 201.2 kcal/kg/day for first and second post-exposure runs respectively. These

values correspond to an average of + 0.2% ± 5.6 (n = 10) for first and + 5.2% ± 6.4% (n = 9) for second post-exposure runs (Table 6). Here again, very high standard errors indicate that these averages result from a wide range of values (- 21.0% to + 22.7% and - 19.2% to + 42.3%, respectively). An Anova test has shown no significant difference between the control, exposure, 1st and 2nd post-exposure runs (p = 0.67, App. IV b), indicating a lack of response of the ducks' metabolism to the presence of dispersant.

Exposure of birds to PBCO + Corexit 9527 resulted in a clear increase in their metabolic rate from an average of 195.6 kcal/kg/day for control to 241.5 kcal/kg/day for exposure runs (n = 11). This represents a 22.3% average increase (Table 6). Although oil + dispersant would go through a much greater dilution at sea than it did in our swimming tanks, it still is interesting to see that oil which had "disappeared" from the surface with the addition of dispersant, seems to resurface from the clean-looking water to adhere to ducks' plumage. After a few minutes, oil contamination on the plumage of a bird exposed to oil + dispersant was indistinguishable from that of a bird exposed to oil alone. By the end of a one-hour period, a bird exposed to oil + dispersant was just as soaked an waterlogged as birds exposed to dispersant alone, yet its plumage was as stained and matted as those exposed to oil alone (Fig. 33 A & B). The increase in metabolic rate seen on day of exposure to oil + dispersant extends to both first and second post-exposure measurements. The average metabolic rate for the first post-exposure period is 321.9 kcal/kg/day corresponding to an 18.2% increase and the second, 237.7 kcal/kg/day, to a 21.8% increase over control values.

Here again, as in the case of birds exposed to oil, the Anova test showed that the results from exposure, 1st and 2nd post-exposure runs were different from those of control runs but not from one another (p = 0.80. App. IV c), indicating that the effect of oil + dispersant on the birds' metabolism on the day of exposure is significant through the two-week post-exposure period.

There is therefore no difference between runs within each treatment group but there are differences when comparing for the same run among treatments (p = 0.09, 0.05 & 0.25 respectively, App.V). A Duncan's multiple range test was used to identify the groups and treatments yielding values significantly different from one another. Such grouping of results thus allows for a classification of results into three classes (App. VI): the first post-exposure run with birds exposed to dispersant alone showed no response at all to the treatment; all others showed "some" increase in metabolic rate; but only the exposure, 1st and 2nd post-exposure runs of the birds exposed to oil + dispersant showed a definite and marked increase in metabolic rate. This ranking of response possible with Duncan's multiple range test confirms that although dispersant has no effect on the birds' metabolism when used alone, when combined with oil, it increases the deleterious effects that oil has on birds and therefore increases the threat oil represents for birds in their natural environment.

FIGURE 33:

A & B: Plumage of birds exposed to oil + dispersant showing the staining due to oil and the physical disruption of the plumage caused by the dispersant.

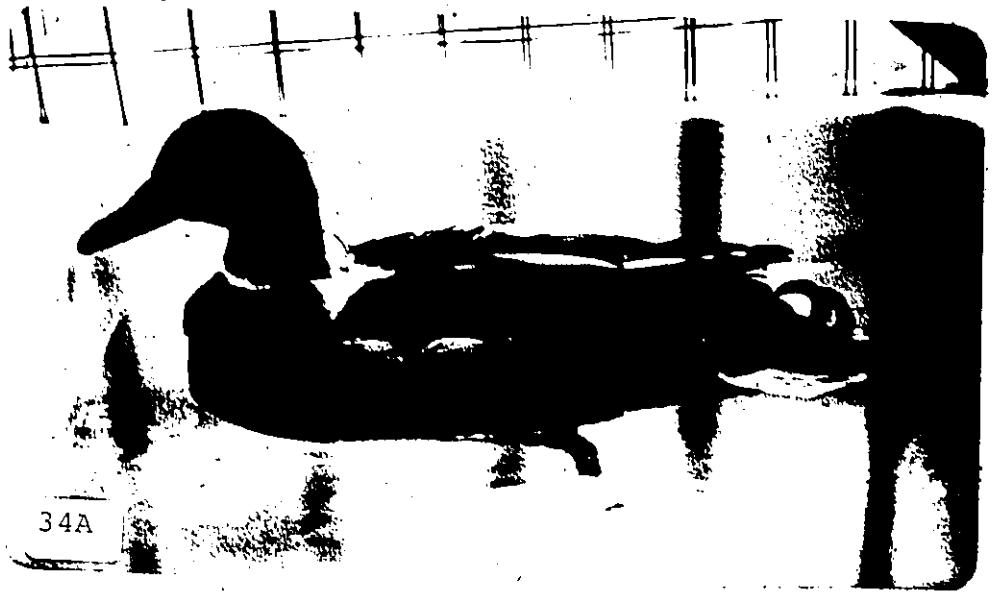
33A

33B

FIGURE 34:

A: Ducks swimming in clean seawater and floating very high on the surface of the water.

B & C: Ducks swimming in seawater to which 1.2 ml of dispersant had been added. The bird sank to a much lower level than under normal conditions.



34A

34B

34C

FIGURE 35:

Feathers after exposure to oil.
After two weeks of preening, it still shows the matting of some of the barbules.

Figure 35

FIGURE 36:

Feathers after exposure to oil + dispersant. After two weeks of preening, it still shows the matting of some of the barbules and also the disruption of the physical integrity of the feather.

Figure 36

3.2.3 Discussion

Contamination of ducks with oil results in a loss of metabolic heat that needs to be compensated by a proportionally increased metabolism (Hartung, 1967; McEwan and Koeling, 1973). Results of experiments reported in Tables 5 & 6, pages 102 & 104, confirm this, showing a 10.6% increase over basic metabolic rate at time of exposure to oil. Further increases (to 16% over BMR) measured one and two weeks after exposure may be partially caused by direct effects of oil on plumage. Although part of its integrity is restored through preening, the plumage may require up to a few months to completely rid itself of oil stains. A bird might go through a molt during that period, thus accelerating the cleaning process. It is expected that, as long as the plumage shows signs of contamination, it will not completely regain its full insulating effectiveness. It is also known from previous studies that oiled birds do ingest a significant amount of oil (Hartung, 1963) which is known to cause systemic disturbances (Croxall, 1977; Hartung & Hunt, 1966; Holmes & Cronshaw, 1977). Ingestion of oil might have caused modifications in metabolism or adaptive pathways, therefore contributing in part to the increase in metabolic rate measured here.

Exposure of ducks to Corexit 9527 resulted in a noticeable sinking of ducks which is assumed to be caused by the surface tension reducing property of dispersant. Surfactant

in dispersant renders feathers hydrophilic and therefore breaks the water-repellent barrier that normally allows birds to swim on water while remaining completely dry. Feathers then get saturated with water which disrupts the precise and regular physical arrangement (Croxall, 1977) that normally gives them their firmness and strenght on which their impermeability depends (Fig. 35 and 36). Water can thus penetrate the plumage and the bird soon becomes waterlogged, increasing its weight proportionally and accelerating the sinking process. Many birds reacted to this loss of buoyancy by trying to escape from swimming tanks. They were often seen flapping their wings vigorously in an attempt to take off from contaminated water. The only ledge they had in a swimming tank was about 10 cm wide but birds often preferred that very uncomfortable position rather than go to contaminated water. This may have resulted in a stress reaction. It was also noticed that birds could not shake or preen the water off their plumage as usual. In spite of their efforts to dry themselves, they remained dripping wet for much longer than birds exposed to only water or even to oil.

Although birds swimming in seawater + Corexit 9527 showed signs of loss of buoyancy, there was no increase in metabolic rate as could have been expected. This lack of response may be due to the fact that the air entrapped between feathers was still intact and only the surface feathers were soaked, therefore not causing as great a stress as oil which actually

matts and opens the plumage to infiltration of water and to escape of metabolic heat. In these experiments, metabolism of ducks was measured after a one-hour exposure period. Its effect would have probably been much greater after a few hours if one considers the progressive wetting, waterlogging and resulting sinking described earlier. This would then indicate that a bird's plumage can withstand exposure to a dispersant for one hour and still retain its insulating properties in spite of its partial loss of impermeability. It is possible also that when the plumage is wet, as long as it retains its flexibility and suppleness, it may still protect the bird againts cold air. This insulating protection would then be analogous to a scuba-diver's "wet-suit" used in ice-cold waters which allows water to penetrate down to his skin but, when water is trapped in the suit's foam-like material, it soon warm up from metabolic heat and then serves as a "wet" insulating layer.

Behaviour of birds trying to escape contaminated water suggests that if their metabolism was measured during the struggle, a rapid and sustained increase would take place for the whole duration of exposure period. It can also be speculated that, when a bird is taken out of such a stressful situation, its metabolism might fall back to a lower level as a result of exhaustion. This could be verified in an experiment using radiotelemetry to monitor the bird's heart rate, as an indication of its overall metabolism (Flynn & Gessaman, 1979).

The lack of response seen in both post-exposure periods also seems to indicate that possible ingestion of Corexit 9527 by ducks while preening does not significantly raise their metabolism.

Ducks exposed to oil + dispersant show a 22.3% average increase in their metabolic rate on day of exposure, which is greater than the increase due to dispersant alone, or to oil alone. In fact, it is even greater than the summation of the separate increases due to oil and due to dispersant. This seems to indicate a synergistic action between oil and dispersant on the day of exposure, which can be understood by examining the effect that oil + dispersant mixture had on a duck's plumage (Fig. 33A, B and 36). Dispersant, as seen earlier, allows penetration of water into the plumage until saturation and disrupts physical integrity of feathers (Fig. 35). This results in a loss of buoyancy, while oil causes matting of feathers, opening channels where heat loss occurs and exposing the bird to chilling. When combined, oil and dispersant cause a greater loss of metabolic heat and consequently, a higher increase in metabolic rate.

There is hardly any difference between the metabolic rate of ducks exposed to oil + dispersant on day of exposure and during both post-exposure periods. It was not possible in the current study to determine whether this is due to sustained physical damage caused by contact with oil + dispersant or, at least partially, to ingestion of oil through preening.

These two post-exposure measurements are very similar to those seen in birds exposed to oil alone, showing that the use of a dispersant, at 30:1 (oil:dispersant) ratio, does not appreciably increase the effects of crude oil alone on birds, beyond the day of exposure.

3.3.0 Summary and conclusions

The present work has shown that metabolism of birds can be readily monitored but, for an accurate data gathering method, the impact of a great number of factors demand a thorough evaluation. Parameters that were at first overlooked or ignored, such as atmospheric pressure variations, temperature distribution within the metabolic box or even formation of ice plugs in tubing, turned out to be major interferences. Progress of experiments was seriously affected until required modifications to either equipment or method were implemented to monitor, control or compensate adequately.

This study has also shown that the use of slopes as a basis for comparison was definitely inadequate. Being derived from a set of data which themselves involve an error factor, slopes exhibit too great a sensitivity and variability to be used as a significant representation of bird's response to a series of changing experimental conditions.

The great importance of the duck's behaviour and reactions during experiments also stresses that, when working with biotic systems, a great flexibility must be exercised to allow each individual organism to behave, react or adapt according to its own "personnality". One can not expect an automatic, immediate and consistent response, as can be expected of mechanical or electronic equipment. Much better results were

obtained after reducing unnecessary stresses imposed on birds, such as handling and very long confinement in a metabolic box. This required a completely new experimental design which turned out not only to be more adequate and precise but also greatly simplified experimental procedures and consequent calculations.

From the results of the last study (section 3.2.), one can see that contamination of birds with oil imposes a significant metabolic demand on them, while exposure to dispersant alone did not cause any significant effect, under the present experimental conditions. However, on the day of exposure, a combination of oil and dispersant caused an increase in metabolism greater than the sum of increases due to each of the pollutants separately. This, though, must be kept in perspective and the obvious question that needs to be asked is: Will seabirds actually encounter concentrations of oil + dispersant such as the ones used in these experiment, when a dispersant is used in the natural environment to disperse and dilute oil rapidly after a spill?

Effects of contamination with oil + dispersant were sustained for at least 2 to 2½ weeks but the difference between oil and oil + dispersant fades away, so that dispersant used in a 30:1 ratio does not appreciably increase the effects caused by crude oil beyond the day of exposure.

When considering the possibility of using a dispersant after an oil spill, one should keep these results in mind and evaluate the potential of dilution that oil and dispersant would have after application. If an oil spill occurres near shore and dispersant is to be applied by aerial spraying, the possibility of some dispersant drifting on shore, in small pool or in shallow estuarine areas should be considered. As seen earlier, the presence of dispersant in water could cause sinking and drowning of some birds. When mixed with oil in shallow water, dispersant would not dilute and disperse oil as rapidly as it would at open sea, and would consequently be more of a threat to birds than it would further from shore.

When faced with the decision to either use dispersant or not to help clean-up an oil spill, one should therefore not only consider the short-term and rapid aesthetic improvement brought about by dispersant but also the longer-term and certainly more subtle effects of the dispersant on birds' survival. Even a very small quantity of dispersant finding its way into a pool of water or a marsh area could represent a lasting threat to birds which might try to swim or feed on that water. The presence of dispersant in a bassin with limited potential for dilution could therefore prevent birds from feeding normally or cause them to get waterlogged and drown.

APPENDIX I Metabolic rate of ducks in kcal/kg/day

Group	Control	Exposure	1st post-exp	2nd post-exp.
Oil	190.39	198.71	227.54	216.76
	186.91	158.01	194.53	200.77
	237.17	216.19	209.43	193.09
	251.58	295.40	212.75	217.05
	170.33	245.28	280.19	331.71
	194.14	174.50	224.61	194.80
	214.41	219.57	253.41	290.74
	208.73	227.80	245.87	254.30
	183.16	271.29	218.11	231.50
	237.20	205.84	254.90	239.94
	184.43	241.29	256.54	264.13
	218.98	258.55	265.22	238.04
	196.00	238.62	239.68	202.95
	188.61	189.47	210.78	205.65
Oil + Disp	190.01	294.46	182.33	266.72
	176.34	211.23	191.25	232.07
	212.88	252.13	215.72	247.12
	201.04	235.94	262.49	269.25
	200.89	247.94	323.19	289.27
	174.19	-	187.60	192.59

APPENDIX I (cont'd)

Oil + Disp	218.59	311.42	281.71	255.38
(cont'd)	185.04	210.47	208.74	208.27
	207.06	225.80	244.55	209.84
	198.71	206.13	228.10	206.94
	183.55	239.64	224.98	263.55
	198.97	221.00	231.71	211.58
Dispersant	207.87	207.28	165.74	-
	187.03	227.11	229.62	204.83
	213.68	208.29	243.76	201.23
	235.52	212.29	208.25	211.87
	196.51	227.19	237.54	279.66
	141.57	184.60	170.09	178.39
	209.83	190.73	173.63	204.57
	158.81	176.12	147.74	152.51
	175.31	188.21	166.60	193.90
	227.10	224.97	187.03	183.56

APPENDIX II

a/ all control data from - oil
- oil + dispersant
- dispersant

Anova (Analysis of variance)

Source	df	SS	F
Total	36	20215.0	
Treatments	2	1130.7	1.007
Error	34	19084.3	

Probability of F > 1.007: 0.38 → No difference

b/ all exposure data from - oil
- oil + dispersant
- dispersant

Anova (Analysis of variance)

Source	df	SS	F
Total	35	40958.3	
Treatments	2	7103.6	3.462
Error	33	33854.8	

Probability of F > 3.462. 0.04 → different

APPENDIX II (cont'd)

c/ All 1st post-exposure data from - oil
- oil + dispersant
- dispersant

Anova (Analysis of variance)

Source	df	SS	F
Total	36	50257.2	
Treatments	2	11256.4	4.907
Error	34	39000.9	

Probability of F > 4.907: 0.01 ⟶ different

d/ All 2nd post-exposure data from - oil
- oil + dispersant
- dispersant

Anova (Analysis of variance)

Source	df	SS	F
Total	35	50176.9	
Treatments	2	7738.4	3.009
Error	33	42438.5	

Probability of F > 3.009: 0.06 ⟶ different

APPENDIX III

a/ all <u>OIL data</u> from - control
- exposure
- 1st post-exposure
- 2nd post-exposure

<u>Anova</u>

Source	df	SS	F
Total	58	65649.4	
Treatments	3	8849.7	2.856
Error	55	56799.7	
Probability of F > 2.856:			0.045 ⟶ different

b/ all <u>OIL + DISPERSANT data</u> from - control
- exposure
- 1st post-exposure
- 2nd post-exposure

<u>Anova</u>

Source	df	SS	F
Total	46	59351.5	
Treatments	3	15771.1	5.187
Error	43	43580.4	
Probability of F > 5.187:			0.004 ⟶ different

APPENDIX III (Cont'd)

c/ all DISPERSANT data from - control
- exposure
- 1st post-exposure
- 2nd post-exposure

Anova

Source	df	SS	F
Total	38	65675.0	
Treatmants	3	1350.9	0.245
Error	35	64324.1	

Probability of F > 0.245: 0.864 → No difference

APPENDIX IV

a/ all % change from birds exposed to OIL from - exposure
- 1st post-exp.
- 2nd post-exp.

Anova

Source	df	SS	F
Total	41	21103.1	
Treatments	2	315.1	0.296
Error	39	20788.0	

Probability of F > 0.296: 0.75 ⟶ No difference

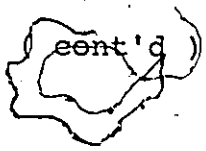
b/ all % change from birds exposed to DISPERSANT from - exposure
- 1st post-exp.
- 2nd post-exp.

Anova

Source	df	SS	F
Total	28	7600.7	
Treatments	2	232.5	0.41
Error	26	7368.1	

Probability of F > 0.41: 0.67 ⟶ No difference

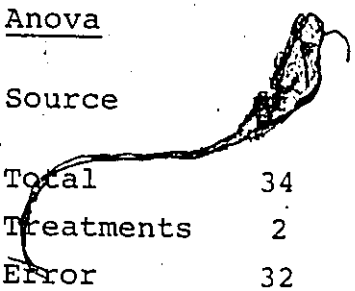
APPENDIX IV ~~cont'd~~



c/ all % change from birds exposed to OIL + DISPERSANT

from - exposure
- 1st post-exp.
- 2nd post-exp.

Anova



Source		SS	F
Total	34	8351.4	
Treatments	2	115.2	0.224
Error	32	8236.3	
Probability of F > 0.224			0.80 → No difference

APPENDIX V

a/ all % change during EXPOSURE to - oil
- oil + dispersant
- dispersant

Anova

Source	df	SS	F	
Total	34	10066.0		
Treatments	2	1397.7	2.58	
Error	32	8668.3		
Probability of F > 2.58:			0.09	→ slightly different

b/ all % change during 1st POST-EXPOSURE to - oil
- oil + dispersant
- dispersant

Anova

Source	df	SS	F	
Total	35	13183.1		
Treatment	2	2200.0	3.31	
Error	33	10983.2		
Probability of F > 3.31:			0.05	→ different

APPENDIX V (Cont'd)

c/ all % change during 2nd POST-EXPOSURE to - oil
- oil + dispersant
- dispersant

Anova

Source	df	SS	F
Total	34	17541.5	
Treatments	2	1442.8	1.43
Error	32	16098.7	

Probability of F > 1.43: 0.25 ⟶ different only at 75%

APPENDIX VI

Coding #

run \ treatment	oil	oil + disp	dispersant
Control	-	-	
Exposure	1	4	7
1st post-exposure	2	5	8
2nd post-exposure	3	6	9

Duncan's multiple range test

Variable: Metabolic rate

Alpha level: 0.05

df: 97

Grouping:

Definite and marked response	# 4	A	
	# 6	A	
	# 5	A	
" Some " response	# 2	A	B
	# 3	A	B
	# 1	A	B
	# 7	A	B
	# 9	A	B
No response	# 8		B

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