A BIOINDICATOR APPROACH TO MEASUREMENT OF CHRONIC STRESS IN TERRITORIAL POPULATIONS OF CUNNER, Tautogolabrus adspersus (WALBUM, 1792), ADJACENT TO A NON-CHLORINATED PULP AND PAPER MILL IN THE HUMBER ARM ESTUARY, NEWFOUNDLAND, CANADA

SONYA MARIE BILLIARD

A bioindicator approach to measurement of chronic stress in territorial populations of cunner, *Tautogolabrus adspersus* (Walbum, 1792), adjacent to a non-chlorinated pulp and paper mill in the Humber Arm Estuary, Newfoundland, Canada.

by

Sonya Marie Billiard (B.Sc.)

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Department of Biology
Memorial University of Newfoundland
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Abstract

Signs of pathogenicity were quantified in cunner, *Tautogolabrus adspersus*, living adjacent (Birchy Cove and Summerside) to a non-chlorinated pulp and paper mill in the Humber River Estuary, western Newfoundland, compared with a reference site (Gillams) approximately 12 kilometers downstream of effluent outfall(s). Several bioindicators of pulp toxicity were utilized, including condition factor, organ somatic indices, macroscopic and microscopic irregularities, parasitofauna, age structure and induction of hepatic mixed function oxygenases. Condition factors were non-significant among sites in contrast to exaggerated growth at impact sites compared to Gillams' fish. Male and female gonadosomatic indices (GSIs) and histological analyses suggested a delay in spawning and/or gametogenesis of impacted fish versus mature, reference cunner. However, consideration of the short reproductive cycle of this species it is possible that polluted fish may have been sampled at the beginning of the maturational phase in comparison to Gillams' counterparts. Hepatosomatic indices (HSIs) were exaggerated at polluted sites and may reflect proliferation of hepatic smooth endoplasmic reticulum (SER). This was consistent with significant induction of 7 ethoxyresorufin deethylase at the Birchy Cove site, since SER is the site of both Phase-I and Phase-II enzymes. However, increased liver size may be attributed to vitellogenesis of female fish. No consistent differences were observed in visceralsomatic indices (VSIs), but splenosomatic indices (SSIs) were, in general, larger at Birchy Cove and Summerside than at Gillams. Percent splenic deposits did not demonstrate site significance. Histological surveys indicated more pronounced tissue pathology at Birchy Cove and Summerside. Quantification of *Cryptocotyle lingua* did not establish any site-specific trends, however, enteric parasitization was considerably more intense at the reference site compared to Birchy Cove and Summerside, possibly due to voiding or reduced

proliferation in effluent-exposed cunner. Otolith analysis (1994) showed the mean age of the Gillams' sample to be significantly greater than at polluted sites. Chronic stress observed in cunners suggests that evidence of pulp-induced toxicity in this population is not necessarily due to chlorine constituents.

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List of Abbreviations and Symbols

± : plus or minus
(♂) : male
(♀) : female
% : percent
2^0 : secondary
ANOVA : analysis of variance
Ah : aryl hydrocarbon receptor
BC: Birchy Cove
BKME : bleached kraft mill effluent
BOD : biochemical oxygen demand
BSA : bovine serum albumen
^{0}C : degrees celsius
CBPP : Corner Brook Pulp and Paper
cc : cubic centimeters
cm : centimeter
$CuSO_4$: copper sulphate
ER : ethoxyresorufin
EROD : 7 ethoxyresorufin
F : female
G : gauge
g : grams
GM : Gillams
GSH : glutathione
GSI : gonadosomatic index
HPLC : High Performance Lipid Chromatography
HSI : hepatosomatic index
K-Factor : condition factor
K-S : Kolmogorov-Sminrnov
L : liter
ln : natural logarithm
log_{10} : base 10 logarithm
M : male
m^3 : cubic meters
MFO : mixed function oxygenase/oxidase
mg : milligrams
min : minute
MMC : melanomacrophage center
ml : millimeters
mM : millimoles
mRNA : messenger RNA
N : normal

NADPH : nicotinamide adenine dinucleotide phosphate (reduced form)
Na_2CO_3 : sodium carbonate
NBF : neutral buffered formalin
nm : nanomoles
npar : non-parametric statistic
PCB : polychlorinated biphenyl
RNA : ribonucleic acid
s.e. : standard error
SER : smooth endoplasmic reticulum
SI : somatic index/indices
SS : Summerside
SSI : splenosomatic index
sqrt : square root
TMP : thermomechanical processing
μl : microliters
μm : micrometers
VSI : visceralsomatic index
w : v : weight to volume
X g : specific gravity

Introduction

Concomitant with increasing public awareness of the environmental influx of xenobiotics, there has been recent emphasis on the study of anthropogenic consequences to the aquatic habitat and its residential biota. Several field and experimental studies have reported deleterious downstream effects of pulp and paper discharges on localized fish populations. These include weight loss or anorexia, altered hepatic activities, cellular hyperplasia, skeletal deformities, impaired immunocompetence, tumours of various origins, (i.e. liver and epidermal neoplasms), cutaneous and/or subcutaneous lesions and modification of typical parasitic profiles (Lehtinen *et al.*, 1984; Andersson *et al.*, 1988; Couillard *et al.*, 1988; Neuman and Karås, 1988; Sandström and Thoresson, 1988; Lehtinen, 1990; Lindesjöö and Thulin, 1990; Lindström - Seppä and Oikari, 1990; Khan *et al.*, 1992). In addition, exposure to pulp mill effluents has been shown to induce stress-provoked mutations, such as, reproductive and steroidogenic dysfunction (Adams *et al.*, 1992; Barker *et al.*, 1994; Munkittrick *et al.*, 1994). Activities of cytochrome P-450 dependentenzymes, particularly monooxygenases, appear to be sensitive biochemical indicators of pulp mill wastewaters (Otto *et al.*, 1994). Mechanisms of various monooxygenases (such as benzo (a) pyrene hydroxylase and aryl hydrocarbon hydroxylase) have been empirically resolved and all reflect strong induction in response to a plethora of xenobiotics; however, 7 ethoxyresorufin O-deethylase (**EROD**) activity appears to be extremely sensitive to pulp and paper effluent exposure (Lindström-Seppä and Oikari, 1990). Induction is the mechanism by which aromatic compounds bind with a protein, **Arylcarbon (Ah)**, receptor. The receptor-inducer complex merges with gene-coding for the enzyme and **messenger RNA (mRNA)** and enzyme protein are synthesized, effecting appreciation of **mixed function oxidase activity (MFO)**. Mixed function oxidase animation, in contrast to other biochemical

modifications, is a primary detoxification response. Thus, enzyme stimulation can serve as an early warning signal of potentially more debilitating pathologies, such as, liver lesions, in fish species, particularly if hepatic induction continues to be both intense and persistent.

The pulp and paper industry is an integral component of both the socioeconomic and environmental fabric of many industrialized countries, including Canada. There exist over 100 pulp mills in this country alone and in several communities they comprise the major industrial and employment source (Robinson *et al.*, 1994), as in the system under current investigation. Pulp industries produce more contaminated wastewater than any other occupational yield in the Atlantic region, ranking second only to sewage outfall and environmental decay originating from metropolitan and/or agricultural areas (Waldichuk, 1988). A typical large-scale pulp and paper operation can release anywhere from 50,000 on upwards to 150,000 m^3 of effluent daily (Robinson *et al.*, 1994).

The bulk of the literature has focused on bleached kraft mill effluent (**BKME**), which produces a plethora of chlorinated compounds derived from the bleaching sequence; subsequently, organochlorides (particularly, polychlorinated dibenzo-p-dioxins and dibenzofurans) have been identified as the predominant toxic culprits. These compounds (in addition to polychlorinated biphenyls (**PCBs**)), are resistant to degradation and bind strongly to the **Ah** receptor, therefore, induction tends to be characteristically persistent. However, recent research has illustrated that non-chlorinated pulping processes elicit measurable and, often, more pronounced negative effects (Bengtsson *et al.*, 1988; Lehtinen, 1990; Axelsson and Norrgren, 1991; Pesonen and Andersson, 1992; Lindström - Seppä *et al.*, 1992; Munkittrick *et al.*, 1994; Otto *et al.*, 1994; Robinson *et al.*, 1994). Therefore, effluent toxicity in the latter, at least, may be associated with specific types of

wood and/or particular pulping procedures.

Exposure to unbleached effluents, for example, has been correlated with elevated frequencies of biochemical and mechanical spinal deformities; this wastewater effect has proven to be significantly more potent in adjacent marine fish species than its bleached counterpart (Bengtsson *et al.*, 1988).

Both chlorinated and non-chlorinated effluents can induce significant cytochrome P450-dependent **EROD** activity; however, chlorine-alternative pulping processes indicate considerable inhibition of hepatic enzyme induction at higher effluent concentrations (Lindström-Seppä*et al.*, 1992; Pesonen and Andersson, 1992). This also alludes to problems of government legislation based on concentration, such as acute lethality testing, which can be counter-productive or misleading. Operations which discharge large volumes of weak effluent may meet requirements simply because of its low concentration compared to a mill that produced less wastewater in total, but was more concentrated (Doering *et al.*, 1992).

Pesonen and Andersson (1992) reported that extractable constituents from thermomechanical and/or sulphate/sulphite procedures exerted stronger consequences on the integrity of cultured hepatocyte plasma membranes and cellular glutathione (**GSH**) content than bleached effluent. Glutathione is an indicator of oxidative stress and the formation of reactive metabolites. Since toxic metabolites manufactured by the detoxification system (**MFO**), i.e. free radicals, may bind irreversibly with lipid substrates in the liver, these enzymatic studies may indicate significant membrane injury or hepatic necrosis at unbleached sites (Lehtinen, 1990). Axelsson and Norrgren (1991) reported an increase in gill parasite frequencies and liver anomalies in populations of three-spined stickle back (***Gasterosteus aculeatus***) adjacent to non-chlorinated (softwood pulp) discharges;

unbleached acute toxicity to rainbow trout (***Oncorhynchus mykiss***) was also more significant when compared to chlorinated exposure.

These studies suggest that alternative substances, such as, resin acids, for example, may be responsible for the toxicity that has been attributed to pulp mill effluents. Since the first published North American report addressing the sublethal effects of non-chlorinated effluent on wild fish populations was a detailed study by Munkittrick *et al.* (1994), research concerning the deleterious effects and mechanisms of unbleached waste-water toxicity is warranted. Selected biological indicators of stress were used in the current study to evaluate the relationship between exposure to non-chlorinated, untreated pulp discharge and pathological manifestation in territorial populations of cunner, ***Tautogolabrus adspersus***.

I. Corner Brook Pulp and Paper Mill

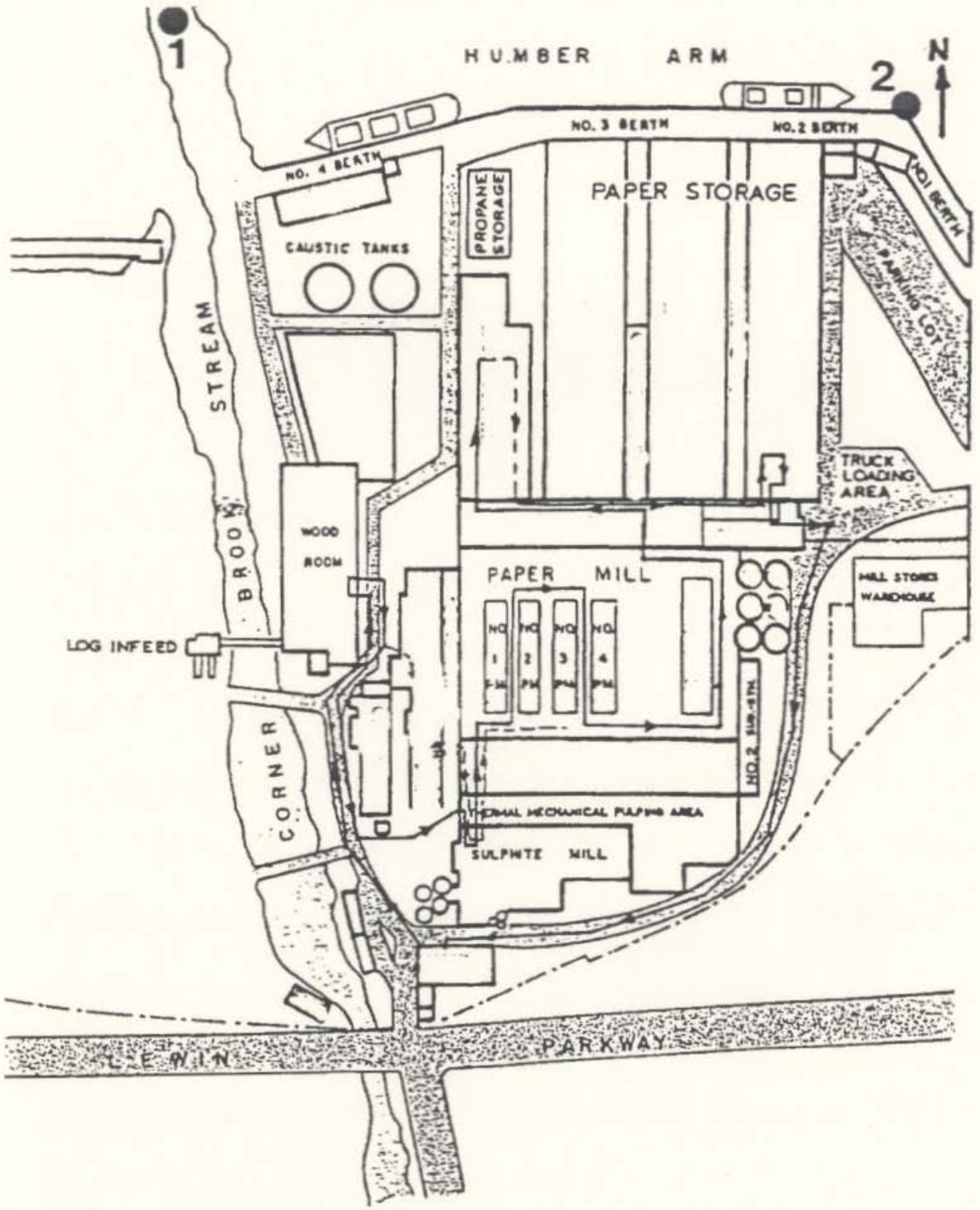
Since the early 1920s, a pulp and paper mill has been operated near the head of the Humber Arm Estuary. The Corner Brook Pulp and Paper Mill (**CBPP**) has been owned and operated by Kruger Incorporated since 1984, when ownership was acquired from Bowaters International. Before 1992, the mill produced newsprint from a combination of thermomechanical (**TMP**) and sulphite-pulping processes. The latter has since been discontinued and the incorporation of recycled paper (approximately 6 to 8 % of total yield) is now routine. In 1992, the Pulp and Paper Effluent Regulations under the Fisheries Act were outlined to decrease discharges of suspended solids, biochemical oxygen demand (**BOD**) and acutely lethal effluents (SeaConsult Limited, 1993). This paper mill currently meets standard regulations for suspended solids only-a secondary treatment facility for compliance with BOD and acute toxicity legislation is presently under construction with a projected start-up date of June 1996 (R. Billiard, C. House, personal communication). A bark

boiler to has been in operation since late 1995 to improve quality of aerial emissions and also employs bark remnants as fuel, eliminating disposal problems at the mill site and minimizing transportation costs inherent in trucking refuse to the municipal disposal site, approximately fifteen kilometres across the bay (C. House, personal communication). Previous to construction, bark refuse had been deposited near the mouth of the Corner Brook Stream. Consequently, wood fibre has precipitated on the bottom of the Humber Arm for up to two kilometres north and northeast of the two wastewater outflows (Eaton, 1994). This bark isthmus not only degrades the benthic habitat by increasing factors such as suspended solids and turbidity, but methane and hydrogen sulphide gas, nutrients, acids and organic toxins may also be leached into the water column (Colodey *et al.*, 1990).

For more than 50 years coniferous logs (both spruce and fir) were floated to the Bowater site through Deer Lake and the Humber River and were temporarily housed at the booming grounds adjacent to the pulp mill (SeaConsult Limited, 1993). Between 1987 and 1991 this holding pattern was discontinued in favour of trucking logs to a dry land sorting yard. The legacy of former river transport and wet debarking, however, has resulted in considerable loss of wood and bark residue that have been deposited on the estuary's benthic substrate and, presumably, throughout the head of the inlet (SeaConsult Limited, 1993).

Wastewater is discharged directly into the Humber Arm from two mill outfalls (**Figure 1**.). The untreated effluent which is comprised of suspendedsolids (79-93 mg L^{-1}), tannin and resin acids (approximately 27 mg L^{-1}), particularly dehydroabietic (600-800 μg L^{-1}) and abietic acids (600-1200 μg L^{-1}), disperses after discharge and is quite visible at the freshwater surface layer for up to 2 kilometres from the mill's outflow (Environment Canada, unpublished data). An estimated 70 percent of the total mill effluent is unloaded via the west sewer, a concrete extension of an open drain

Figure 1. Property layout of the Corner Brook Pulp and Paper Mill illustrating effluent discharge points via the west (1) and east (2) sewers (from SeaConsult Limited, 1993).


1
HUMBER ARM
2
N
NO. 3 BERTH
NO. 2 BERTH
NO. 4 BERTH
NO1 BERTH
PROPANE STORAGE
PAPER STORAGE
CAUSTIC TANKS
PARKING LOT
STREAM
BROOK
TRUCK LOADING AREA
WOOD ROOM
PAPER MILL
MILL STORES WAREHOUSE
LOG INFEED
NO. 1 PM
NO. 2 PM
NO. 3 PM
NO. 4 PM
CORNER
THERMAL MECHANICAL PULPING AREA
SULPHITE MILL
PARKWAY

system that services the mill's thermomechanical plant and wood rooms (SeaConsult Limited, 1993). The remaining 30 percent (originating from the paper mill) is released through the east sewer which is located at the junction of number 1 and number 2 loading berths (**Figure 1**.). Prior to 1992, sulphite refuse was unloaded directly into the Corner Brook Stream (SeaConsult Limited, 1993). Effluent discharge at the Corner Brook Pulp and Paper Mill has been calculated at 89, 639 m^3/daily (Eaton, 1994).

II. Sanitary Sewage

Untreated sewage is discharged directly into the surface waters of this inlet. Twelve drainage pipes service Corner Brook and the amalgamated community of Massey Drive, with an estimated cumulative flow per day of 10,347,000 litres (Newfoundland Design Associates Limited, 1974; SeaConsult Limited, 1993). Summerside and Gillams discharge 161,000 and 60,000 litres, respectively, of raw waste daily into the Humber Arm, which receives a total of 13,880,000 litres*day^{-1} of untreated sanitation (**Table 1**.).

III. Other Sources of Non - Pulp Industrial Wastes In The Humber Arm

In conjunction with the Corner Brook Pulp and Paper Mill, several regional industries are a contributing source to aquatic pollution characteristic of the Humber Arm Estuary. These alternate pollutant derivations are outlined in **Table 2**.

The necessity for level industrial tracts contiguous to the waterfront at the estuary's head has precipitated a significant in-fill of the Corner Brook Harbour shoreline (SeaConsult Limited, 1993). This practise, spanning several decades, has two fundamental adverse effects on this system : coverage of the benthic habitat and its residential biota at these sites and the introduction of fine silt or dissolved material to the estuary (SeaConsult Limited, 1993). The principal biological effect at

Table 1. Community summary of monitored outfalls discharging untreated sewage into the Humber Arm Estuary (adapted from SeaConsult Limited, 1993).

Community	Outfall(s)	Population	Number of Services	Population Serviced (%)	Cumulative Flow/Day (000's litres)
Corner Brook/ Massey Drive	12	23226	9271	99	10347
Mount Moriah	3	692	240	94	293
Benoit's Cove	3	2200	220	35	347
Irishtown	1	804	131	49	177
Summerside	2	796	120	45	161
Meadows	3	671	261	90	272
Gillams	1	512	44	26	60
McIvers	1	738	26	10	33
Cox's Cove	1	1000	200	54	243
Deer Lake	1	4327	1537	100	1947

Note : Cumulative daily flow rates are literature estimates.

Table 2. Non - pulp mill sources of industrial refuse into the Humber Arm Estuary (adapted from SeaConsult Limited, 1993).

Industry	Potential Waste Contribution
Genesis Organics	Organic material and bark as fertilizer ingredients
North Star	Dry cement spills during unloading at Brake Point
Atlantic Gypsum	Fine $CaSO_4$ particulates from processing and/or storage
Atlantic Ready - Mix	Discharge of fine complexes or cement
Terra Transport (Seal Head)	Steam cleaning of equipment
Imperial/Irving Oil (Curling)	Potential petroleum spills
Ultramar (Church Cove)	Potential spills
Barry Fisheries (Curling)	Fish offal discharge

these in-fill sites has been the sacrifice of the intertidal zone and its associated fauna (SeaConsult Limited, 1993). A list of in-fill project locations and industrial nature are referenced in **Table 3**.

IV. The Indicator Species - *Tautogolabrus adspersus*

The eastern coast of North America supports an abundant population and widespread distribution of the cunner, *Tautogolabrus adspersus*, Walbum 1792 (Scott and Scott, 1988). This species inhabits shallow, inshore waters. It is epibenthic in habit, typically occupying restricted home ranges within a few kilometres of shore (Scott and Scott, 1988). Tagging studies have illustrated that cunners are non-migratory - individuals have been observed to return to home sites following displacements of at least four kilometres and that this homing faculty was not compromised in specimens maintained under laboratory conditions for a period of nine months (Green, 1975). Cunners congregate in masses or schools about wharves, man-made reefs and submerged seaweed beds-these shelters present sufficient food resources to attract fish and have the potential for nutritive accretion (Olla *et al.*, 1975). In Newfoundland, spawning commences in mid to late July and lasts approximately three to four weeks to the beginning of August (Pottle and Green, 1979b; Martel and Green, 1987). Time of spawning has been based on field observations from both the east (Conception Bay) and west (Norris Point, approximately 80 km north of the Humber Arm system) coasts of Newfoundland.

In contrast to other Newfoundland coastal species which migrate to deeper, warmer waters with the onset of colder temperatures, cunner enter and remain submergedwithin the substratum (i.e., under rocks) in late fall or early winter in a torpid state until the following spring (usually when water temperatures rise above approximately 5 ^{0}C). As a result, populations indigenous to Newfoundland coastal regions are metabolically inactive for five to six months, during which time

Table 3. List of industrial in-fill projects near the head of the Humber Arm and Corner Brook harbour shoreline (adapted from SeaConsult, 1993).

Location	Project Type
Atlantic Ready-Mix quarry	Aggregate storage proximate to the mouth of the Humber River
Pre-stresses Concrete Fabrication Yard	Industrial plant at mouth of the Humber River
Atlantic Gypsum	Industrial plant at Brake's Point
Terra Transport	Loading and parking area
Corner Brook Pulp and Paper Mill	Mill east of the mouth of the Corner Brook Stream
Corner Brook Pulp and Paper Mill	Bark pile at the mouth of the Corner Brook Stream
Corner Brook Pulp and Paper Mill	Dry land storage area west of the mouth of the Corner Brook Stream
Curling Waterfront Arterial Road	Roadway built between mill and Curch Cove oil terminal (Ultramar)
Church Cove In-fill projeccts	City of Corner Brook in-fill areas
Curling fish plant development	Loading and cold storage area
Stan Dawe Limited	Lumber Yard at the mouth of the Humber River
Pleasant Cove Yacht Club	Dredging in bay proximate to berths

they cease feeding behaviour (Green and Farwell, 1971). Metabolic depression induced by low temperatures is perhaps the deciding factor in its year-round inshore quarantine. However, this does not preclude, perhaps, some form of physiological compensation during the torpor period. Graham and Fletcher (1986) concluded that large concentrations of functional haemoglobin in non-migratory, territorial cunner could have a useful oxygen-storage function during the winter when this species is more metabolically quiescent and ventilation is practically undetectable. Chiasson (1995) concluded that some form of somatic recompensation may allow Newfoundland cunner populations to grow as fast as their southern counterparts. Because the cunner is an abundant inshore resident and, typically, the most abundant species in harbours and about wharves, it has considerable potential as a bioindicator species of acute and/or chronic environmental perturbations, without the compounding effects of migratory behaviour. Payne (1976) first suggested its use as a monitoring species because of capture and maintenance facility, as well as, induction sensitivity of benzopyrene hydroxylase with petroleum exposure regimes. Its potential as an indicator of local pollution, specifically, petroleum hydrocarbons, was confirmed by Walton *et al.* (1978) and Porter (1988) demonstrated the cunner's susceptibility to aquatic xenobiotics, particularly during its contracted reproductive cycle, as evidenced by MFO induction potentials.

The bioindicator strategy involves quantitative evaluation of stress responses representative of several levels of biological organization in order to assess the effect of a particular stressor imposed on a population, to provide an early warning signal of organismic compromise and to construct hypotheses concerning the dependence of the observed biological effects upon the toxic variable under scrutiny (Adams, 1990). Its underlying conjecture is that the manifestations of stress at each of the lower levels will be overt prior to any disturbances are realized at the population,

community or ecosystem levels. The ideal assessment design of a polluted system, then, would involve measurement of selected indicators for each major level of organization so that casual affinities between them could be determined (Adams, 1990). The major disadvantage of this approach is that any physiological compensation could minimize or disguise the true magnitude of a stress response. Stress may be defined as an environmental stressor that is intense enough to necessitate a neutralization response at the species, population or ecosystem level (Wedemeyer *et al.*, 1984). Sublethal xenobiotic pressure (i.e. chronic stress) is particularly noxious since its temporal pattern translates as exerting a gradual, but cumulative, effect over periods of weeks to years. As a result, this type of stress usually has long-term consequences, and, typically, affects the entire reproductive cycle of a species (Adams, 1990). A stressor may restrict physiological mechanisms, delay sexual events, depreciate growth and induce immunosuppression depending on its degree of severity (Adams, 1990).

The focus of the current study was to examine the link between effluent exposure in residential populations of cunners adjacent to a non-chlorinated, thermomechanical pulp and paper mill in the Humber Arm Estuary; an operation that has been discharging untreated pulp waste for approximately 70 years. As part of this mutidisplinary bioindicator approach, several bioindicators, reflecting several levels of biological organization, were examined, including somatic indices, macroscopic irregularities of the liver, histological tissue surveys, gill and intestinal parasite counts, otolith aging of populations and assay of hepatic detoxification enzymes. Response of these variables have been well-documented with regards to BKME exposure and thus can be used to evaluate toxicity of its unbleached couterpart.

Materials and Methods

I. Sample Site : The Humber Arm

Affected and reference sites were situated in the Humber River Estuary (49°32' N, 52°57') on the west coast of Newfoundland, at the entrance of the Bay of Islands (**Figure 2.**). This estuarine system is formed in the Humber Arm Fjord, a submerged channel derived from glacial mechanics, comprising a single arm of the Bay of Islands (SeaConsult Limited, 1993). It is 24 kilometres long and approximately 2 kilometres wide. The entrance of the Humber River is demarcated by an extensive shallow bar; the bottom substrate consists predominantly of mud with admixtures of sand and stone (SeaConsult Limited, 1993). This fjord estuary is characterized by a distinct, superficial brackish layer, over high salinity and cold water at greater depths. Freshwater is restricted to the upper 10 metres of the water column. Salinity approaches 20 to 25 parts per million and maximum temperatures of 15 ^{0}Celsius in the summer months (SeaConsult Limited, 1993). Average flow rate of the Humber Arm is approximately 265 m^3/second with a flushing time of fresh water from the Humber Arm about 7.5 days (Fraikin *et al.*, 1995). This temporal feature is relatively short due to the fact that most of this fresh water, originating from the Humber River, remains in the upper layers (few metres) with a net migration seawards. Minimum discharge time for the entire Humber Arm has been calculated at 38 days (Fraikin *et al.*, 1995).

Maps of general circulation patterns illustrate that the current originates at the head of the arm and progresses down the southern shore towards its mouth, accompanied by an influx of cold Labrador water flow along the northern coastline (SeaConsult, 1993). Tides are characteristically semi-diurnal in the estuary with a maximum range of 2 metres throughout (Fraikin *et al.*, 1995).

Cunner were sampled from two arbitrarily designated impacted sites, **Birchy Cove** (**BC**) and

Figure 2. Map of the Humber River Estuary illustrating location of Birchy Cove (✚), Summerside and Gillams sample sites for the 1993-1995 field collections of *Tautogolabrus adspersus*.


HUMBER RIVER
CORNER BROOK
HUMBER ARM
N

Summerside (**SS**) and from a reference location in the community of **Gillams** (**GM**). Birchy Cove is located approximately 2 kilometres downstream from the mill. Summerside and Gillams are both on the north side of the Humber Arm. The former site is approximately 3 kilometres northwest of the mill's outfalls; Gillams is an additional 9 to 9.5 kilometres northwest of Summerside.

Based on LeDrew and Bennett's (1988, 1989) sediment sample evaluation, **Birchy Cove** represents a region of moderate to heavy impact (**Type III-IV**) characterized by deposits of wood, fibre and few or none organisms. **Summerside** is designated as **Type II** : an area of slight effect (no wood/fibre and organisms present); and **Gillams**, **Type I** (no impact).

According to the Environmental Effects Monitoring (**EEM**) requirements, the zone of effluent mixing is to be specified to a limit of 1% (100:1 dilution) effluent concentration. Fraikin *et al.* (1995) conducted plume dispersion models for the proposed outfall of CBPP's secondary treatment facility scheduled for summer 1996. Model computation utilized future average flow of 47,500 m^3*day^{-1}, maximum flow of 58,000 m^3*day^{-1} and maximum monthly flow of 52,000 m^3*day^{-1} compared to current CBPP discharge of 89,639 m^3*day^{-1}. Delineation modelling confirmed that distribution of the effluent plume from, as well as contamination of ambient waters and sediment due to, CBPP are restricted to near shore areas of the Humber Arm. Mill and sewage effluents are generally discharged and confined to the top layer (i.e. 0-3 metres) of the water column due to the existence of a strong halocline (maximum difference in density between top and bottom layers is 18 $kg*m^3$) which inhibits vertical mixing of fresh and saline layers (Fraikin *et al.*, 1995). Consequently, dissolved contaminants are not typically available to bottom waters or benthos and strong freshwater flushing rates of less than 8 days ensure that this contact would be negligible. However, it is significant to report that during slack tide and minimum wind action suspended

particles, such as wood, fibre and bark, do settle to the substratum (Fraikin *et al.*, 1995).

The results of Fraikin *et al.*'s (1995) plume delineation study indicates that the maximum reach of CBPP's 1% effluent concentration zone (for its proposed secondary treatment outfall) may extend as far west as Petries Point, approximately4.5 km downstream of the mill . Maximum extent of dilution isopleths of this plume confirm that the **Birchy Cove** site is situated in a region of **20:1** effluent dilution, Summerside between **40:1** and **60:1**, and **Gillams** considerably beyond the **100:1** effluent dilution range. Therefore, location of impact and reference study sites pertaining to mill exposure in this thesis are appropriate based on sediment analysis and plume dispersion modelling.

Weighing the fact that these minimum dilution cases were based on future flow rates approximately 50% of present discharges, it would seem that effluent concentrations at the aforementioned sites were, in fact, greater during the course of the current study.

II. Collection and Necropsy Protocols

In order to assess the feasibility of sampling in the Humber Arm and to generate a working hypothesis regarding the extent of effluent impact, preliminary analyses were conducted in late June of 1993. Observations of CBPP discharge, which is quite distinct in colour, extending to Summerside during sampling indicated the necessity for a more pristine control site (R.Khan, personal communication). Intense field collections and necropsy in mid- to late-summer (1993) of other fish species resident to the Humber Arm, such as *Pleuronectes americanus,* confirmed that Gillams was an appropriate reference location (R. Khan, personal communication). With modifications to sampling design incorporated, an extensive collection was delineated for summer of 1994.

Cunner were collected by baited hoop nets (approximately 0.5 m in height) which resemble

commercial crab pots in design and dimension. Sampling and necropsy were conducted between the last week of June up to the first week of August, inclusive. Traps were deposited from community wharves between 3.5 to 4.5 metres in depth (minus 1 metre at low tide), monitored twice daily (early morning and late evening) for evidence of damage and rebaited with fresh herring (*Clupea harengus*). A maximum of 20 fish were processed in a given day. Two insulated coolers (a maximum of 10 fish per cooler) were filled to capacity with water from the specific net site and location. Transport coolers were each provided with 3 to 4 bags of ice to induce specimen semi-torpor. Green and Farwell (1971) demonstrated that torpor could be artificially simulated by decreasing water temperature below 5^{0} C and that there were no indications of adverse reactions induced by such rapid and significant temperature variations. Reducing metabolic function of these fish and depressing water temperatures (i.e. increasing oxygen saturation) minimized the danger of suffocation or respiratory distress during transit. Once deposited at laboratory facilities, air stones were added to ensure adequate oxygenation.

Prior to vivisection, fish were bled with 1 or 3 cc heparinized syringes (10, 000 units) fitted with a 25 G 5/8 needle by a cardiac puncture route. A blood smear for each individual was prepared and syringes refrigerated upright at 4^{0}Celsius to effect separation and extraction of the plasma layer. Fish were immediately sacrificed by cephalic dislocation. Necropsy procedures were conducted as quickly as possible after death occurred to minimize the compounding variable effects of typical post-mortem pathology. Morphological parameters, including body length (nearest 0.1 centimetre, caudal fin to upper lip), dressed and excised organ weights (nearest 0.01 gram) and sex were recorded on standardized necropsy sheets. Observations of any external or internal macroscopic abnormalities, such as lesions, were annotated. Evidence of gross pathology, including tumour-like

masses, were detailed and samples fixed in gluteraldehyde for future electron microscopic processing. A gill smear was prepared in 1994-1995 using the second gill filament of the right branchial arch. The first gill lamellae of the right branchial arch, the liver, spleen, kidney and gonads were pooled in a 10 ml scintillation vial for each fish and fixed in 10% neutral buffered formalin (**NBF**, pH 7.6). Individually tagged intestinal tracts were fixed in 10% NBF to assess enteric parasite loads. Carcasses were also labelled and placed in freezer storage so that the otoliths could be removed for aging at later date (1994).

A 1995 sample from Birchy Cove (n=25), Summerside (n=25) and Gillams (n=23) was collected for hepatic assay of 7 ethoxyresorufin O-deethylase catalytic activity. Typical experimental necropsy protocol was adhered to with the following exception : after recording total liver weight, approximately one symmetrical half was bisected; placed in labelled whirlpak storage bags and stored in liquid nitrogen (- 195. 8^{0}Celsius; Canadian Liquid Air, personal communication) for the duration of the field study (June 22 through to July 2, 1995). Samples weretransferred to dry ice storage (sublimation point - 78.5^{0}Celsius; Canadian Liquid Air, personal communication) for air transport (approximately 1 hour) to the Department of Fisheries toxicology facilities (St. John's) where they were placed in a -80^{0}Celsius industrial ultra freezer until mixed function oxidase activity could be assayed (within 2-3 weeks post-storage).

III. Histological Processing and Histopathological Assessment Surveys

Fixed tissue sections were processed and embedded in Tissue Prep (Fisher^{TM}) wax and cut to a thickness of seven (7) micrometers (μm) on American Optical 820 "Spencer Microtome" (conventional histological methodology; **Table A. 1.**). Duplicate sections of tissue were heat-fixed for (1) hour at 60^{0}Celsius. One slide per tissue section pair was stainedwith haematoxylin and eosin.

Unstained splenic and lamellar counterparts were localized with Perl's Prussian blue method for haemosiderin (Drury *et al.*, 1967) and the periodic Acid Schiff Reaction, respectively.

A. Gill

The distal 1/3 of each of five (5) primary filaments from sample lamellar sections representing each experimental field site (1993-1994) were histologically surveyed at 400X magnification and annotated for number of *Cryptocotyle lingua* metacercariae, degree of hyperplasia, interlamellar thickening, epithelial lifting and other structural departures (i.e., telangiectasias and chloride cell proliferation).

The extent of hyperplasia of the secondary (2^0) filament epithelium was expressed on a numerical scale, ranging from 1 through 3 (based on epithelial diameter), where :

1 : slight hyperplasia (number of nuclei < 2)

2 : moderate hyperplasia (2 < number of nuclei < 4)

3 : excessive hyperplasia (number of nuclei ≥ 4)

Epithelial lifting was defined as separation of basal epithelia from central blood sinus; interlamellar thickening was recognized as the occurrence of trough hyperplasia between adjacent secondary lamellae.

B. Liver

Sample hepatic sections from each experimental site (1993-1994) were histologically surveyed at 400X magnification for the presence or absence of melanomacrophage centers, cellular necrosis, clear cell foci and/or vacuolation, as well as, other cytoarchitectural aberrations.

Histological examination at oil immersion magnification (100X) was used to verify suspect or ambiguous anomalies.

C. Gonad

Sample gonad sections from each experimental field site (1993-1994) were histologically surveyed at 10x (low) through to 100X (high) magnification todetermine sex and stage of maturity. Three categories of female cunner maturity were included in this analysis, where:

1. **Prespawn : ≥ than 50% oocytes characterized by central (or slightly skewed from central position) germinal vesicle, perinuclear nucleoli and exogenous vitellogenin.**
2. **Spawn/postspawn : ≥ than 50% oocytes (comparatively larger) with peripheral germinal vesicle, late vitellogenic and/or post-ovulatory follicles.**

Males were easily defined as **pre-spermiating** or **mature** : mature sperm in testicular follicles were either absent (characterized by predominance of spermatozoa/spermatids) or present in sections.

IV. Splenic Melanomacrophage Quantification

Sample splenic sections from each experimental field station (1993-1994) were assessed for haemosiderin deposits by digital analysis (Khan and Nag, 1993) implementing Mocha V1.2 software (Jandel Scientific Video Analysis system or JAVA™) and a Truevision Targa+™ frame grabber board (E. Hatfield, personal communication).

The frame grabber converts the videotaped images to a set of digitized values and renders them as a set of discrete cells referred to as pixels (or picture elements). Each pixelis assigned a numeric value determined by image brightness at that location, and the data can subsequently be subjected to mathematical analysis and file storage. The frame grabber samples for gray levels at spatial coordinates; thus it can resolve each pixel's worth of continuous, possibly fluctuating gray

and assigns a single intensity value to the area represented by a pixel. A 512 by 400 pixel grid map supports 256 gray levels. Applications of image processing include removal of artifacts and background noise due to fixation inconsistencies that could yield poor and inaccurate data.

V. Endoparasitic Fauna Profiles

Sample intestines from each experimental field site (1993-1994) were longitudinally dissected into a sieve tray (0.025 millimetre mesh) and flushed with ambient seawater. The filtered residue was transferred in saline solution to a watch glass and observed under a compound microscope (1.2 to 4X magnification) to record prevalence and number of the following helminth taxonomic groups that have been documented to parasitize this labrid species: (i) Acanthocephala; (ii) Nematoda; (iii) Trematoda (Digenea) and (iv) Cestoidea (Scott and Scott, 1988). Parasites were pooled and fixed in 10% neutral buffered formalin according to site and year of sample.

VI. Otolith Aging

The otoliths of teleosts are complex polycrystalline bodies which act as balancing organs in the inner ear (Carlstrom, 1963; Gauldie, 1988). Primary constituents of this structure include crystalline calcium carbonate in the form of aragonite and a fibrous, collagen-like protein : otoline (Degens *et al.*, 1969; Morales - Nin, 1986a; 1986b; 1992). Otolith maturation proceeds by repetitive superficial precipitation of these mineral constituents-this process is dependent on physiological calcium metabolic activity (Simkiss, 1974) and on amino acid synthesis. This is architecturally manifested in the otolith as daily increases of growth (Pannella, 1971; Dunkelberger *et al.*, 1980). The diameter of these increments and the density of aragonite microcrystals is a function of stage of maturity and season (Irie, 1960). Substantial opaque zonation is indicative of summer growth. This band gradually becomes transparent with decreasing photoperiod and temperatures concomitant with

fall and winter season, (i.e. fish growth is decelerated) (Craig, 1987). This narrow transparent region is marked by a sudden shift to opacity in late spring (Craig, 1987).

Otoliths representing each experimental field site were extracted in pairs from frozen carcasses (1994). One otolith per specimen was mechanically sanded on its convex side with a grinding stone in order to facilitate age determination. Fish otoliths were mounted in glycerin and observed under a compound microscope at 1.2X up to 4X magnification. The pith was arbitrarily designated as the point of origin and was counted as Year 1 in all otolith chronological analyses. Ages were reported to the nearest whole number, and rings were counted in a minimum of two (2) trials to ensure accuracy.

VII. 7 Ethoxyresorufin O - Deethylase Assay Protocol

A. Homogenate Preparation

Each liver sample was slightly thawed from -80^{0}Celsius storage to facilitate dissection. A representative sample of liver (approximately 1 gram) was removed from the central lobe to ensure standardization among homogenates, as well as an adequate lipid substrate concentration. Homogenates were prepared (1 : 4, w : v) in ice-cold 50 mM Tris buffer, pH 7.5, using 10 passes of the pestle of a Ten Broeck hand tissue grinder. Liver homogenates were centrifuged at 9, 000 X g (centrifugal force) for 10 minutes at 4^{0}Celsius. Precipitated pellets were discarded and each S9 supernatant pipetted equally into four capped disposable Eppendorf 1.5 millimetre microcentrifuge tubes and cryogenically maintained at -80^{0}Celsius. A top fatty layer, characteristic of cunner, was also removed.

B. Mixed Function Oxygenase Activity - EROD

Ethoxyresorufin O-deethylase (**EROD**) activity was assayed fluorimetrically using a Perkin-Elmer LS-5 fluorescence spectrophotometer (Porter *et al.*, 1989). The reaction mixture, final volume 1.25 millimetres, consisted of 53 nmol Tris-sucrose buffer (50 mM, pH 7.5), 50µl of S9 hepatic homogenate and 2.25 nmol 7-ER (150µm ethoxyresorufin). The catalytic effect was started with the addition of 0.16 mgNADPH (1.25 mg/ml). The reaction was terminated after a 15 minute incubation at 27 ^{0}Celsius in a temperature-controlled water bath by the pipetting of 2.5 millimetres of ice-cold HPLC (spectro- analysed) grade methanol. Methanol blanks for each assay run contained the same reagent components as the sample tubes, with the important differentiating factor being the addition of alcohol before the addition of **NADPH**. An internal pooled standard was run with every incubation trial to maintain accuracy. Assay tubes were vortexed and the protein precipitate removed from suspension by centrifugation at 3600 X g for five (5) minutes. Resorufin fluorescence was measured in duplicate sets of fluorometric cuvettes (1 centimetre path length)at 585 nm with an excitation wavelength of 550 nm (slit width of 0.5 millimetres). Enzyme activity was linear with time (based on standard curve generation) and protein concentration (Lowry Protein Method). The rate of enzyme activity in pmol/min/mg protein was obtained from theregression of fluorescence against standard concentrations of resorufin.

C. Protein Determination

Protein was determined by the procedure of Lowry *et al.* (1951), using a Perkin-Elmer UV-Visible scanning spectrophotometer. Lowry reagent mixture consisted of 20 g of Na_2Co_3 (anhydrous) and 4 g NaOH dissolved in 950 ml ofdouble distilled water and diluted to 1 L. Five ml of a solution consisting of 1 ml of 1% $CuSO_4$, 1 ml of 2% Na K tartrate and 100 ml of the Lowry

reagent was added to 0.5 ml of the S9 suspension (20 µl of S9 to 480 µl of double distilled water). A series of bovine serum albumin (**BSA**) concentrations, ranging from 50 to400 µl, were used as standards and blanked against 0.5 ml of double distilled water blank (no protein). Following a 15 minute incubation at room temperature, 0.5 ml of 2N (normal) Folin-Phenol Ciocalteau reagent was added to the mixture, immediately vortexed and incubated for 30 minutes (room temperature). Absorbance was recorded at 620 nm. A linear standard curve for protein concentration versus absorbance was produced from the BSA standards and used to calculate S9 protein concentrations in mg/ml and mg/g liver.

VIII. Data Analysis

Somatic body indices were transformed from raw necropsy data in the following manner and recorded as ± standard error:

i. Condition (K) Factor = (dressed or eviscerated weight / (total body length)^3) X 100

ii. Hepatosomatic Index or HSI = (liver weight / dressed or eviscerated weight) X 100

iii. Splenosomatic Index or SSI = (spleen weight / dressed or eviscerated weight) X 100

iv. Visceralsomatic Index or VSI = (intestinal weight / dressed or eviscerated weight) X 100

v. Gonadosomatic Index or GSI = (gonad weight / dressed or eviscerated weight) X 100

Length and dressed weight data were tested by oneway analyis of variance (**ANOVA**).

Condition factor and somatic indices were examined for site significance using analysis of covariance (**ANCOVA**). Normality and data homogeneity were examined by residual analyses. Non-normal or heterogeneous data were log-transformed and retested prior to statistical tests. With reference to age, length and dressed weight, if parametric assumptions were not met by data transformation, the Kruskal-Wallis non-parametric analysis of variance was used. Least squares regression analysis examined relationships of carcass weight and body length to organ weights and age, in addition to, growth patterns.

Results

I. Morphological Statistics

A total of 421 cunner were collected in the Humber Arm Estuary from 1993 through 1995 summer field collections, inclusive. Twenty and 21 fish were necropsied for preliminary study in 1993 from Birchy Cove and Summerside sites respectively; in 1994, 110 cunner were trapped at Birchy Cove , 100 at Summerside and 98 at Gillams. Birchy Cove, Summerside (n = 25, each) and Gillams (n = 23) were sampled for biochemical analysis in 1995. Both numerical distinction field directives indicate that 1994 was the principal field collection period.

Length-frequency histograms generated from 1993 morphometric data illustrate that total length of pooled Birchy Cove residents ranged from 14.0 cm to 25.9 cm (mean= 20.6 ± 0.5 cm), with the greatest frequency in the 18-19, 20-21 (both males and females) and 22-23 (females) cm size classes (**Figure 3.**). Cunner from Summerside ranged in total body length from 12.0 cm to 25.9 cm (mean = 17.7 ± 0.6 cm), with the greatest numerical frequency of both males and females assigned to the 14-15, 16-17 and 18-19 cm size classes (**Figure 3.**). The mean total length from the 1993 Birchy Cove sample was significantly greater ($p < 0.001$) than that of Summerside (**Table 4.**). Mean dressed weights were significantly greater ($p < 0.001$) at Birchy Cove (149.4 ± 11.5 cm) than at Summerside (mean = 83.2 ± 8.6 g, **Table 4.**).

There were no sexually dimorphic differences in body lengths or eviscerated weights at either Birchy Cove or Summerside in 1993.

Length frequency histograms generated from the 1994 field season data indicated that total body length at Birchy Cove ranged from 10.0 cm to 25.9 cm, whereas at both Summerside and

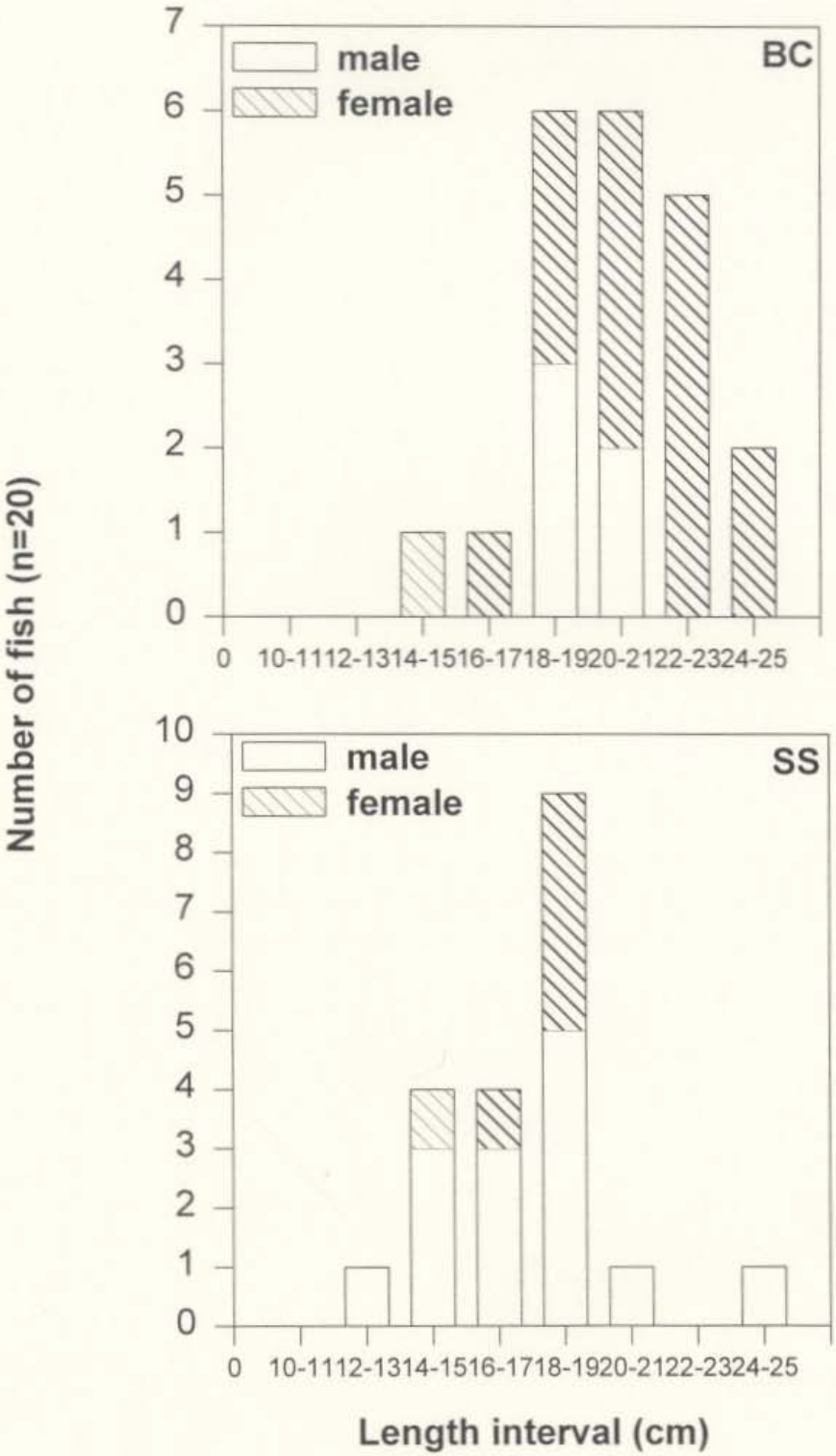


Figure 3. Length frequencies for male and female *Tautogolabrus adspersus* collected from Birchy Cove (BC) and Summerside (SS), 1993.

Table 4. Mean (± s.e.) length, dressed weight, condition factor (=K-Factor), and organ somatic indices (SI) of *Tautogolabrus adspersus* collected from Birchy Cove and Summerside (1993), where ♂ and ♀ denote males and females, respectively.

Variable		Summerside n=14(♂), 6(♀)	Birchy Cove n=5(♂), 16(♀)	
Length (cm)		17.65±0.6	20.57±0.5	b
Weight (g)		85.15±8.6	149.43±11.5	b
K-Factor		1.48±0.03	1.69±0.07	ns
Gonadosomatic Index	(♂)	6.05±0.62	7.22±1.07	ns
	(♀)	3.83±0.19	5.03±0.38	ns
Hepatosomatic Index		2.06±0.19	2.86±0.22	a
Visceralsomatic Index		2.98±0.18	3.97±3.97	a
Splenosomatic Index		0.16±0.01	0.17±0.02	ns

a Birchy Cove significantly greater than Summerside $0.001<p<0.05$
b Birchy Cove significantly greater than Summerside $p<0.001$

ns non-significant

Gillams, values ranged from 10.0 cm, with an upper maximumof 23.9 cm. However, this similarity was not evident in size class distributions. Pooled and male Birchy Cove fish were more frequently assigned to the 18-19 cm intervalthan any other size class, whereas Birchy Cove females exhibited greatest numerical frequency in both 16-17 and 20-21 cm size classes. Pooled Summerside fish were more frequently classed to the 16-17 cm size range and Gillams' samples to the 12-13 (male) and 14-15 (female) cm length interval (**Figure 4.**). **Table 5.** illustrates a decreasing trend in mean pooled body length, with Gillams (mean = 15.5, ± 0.3 cm) significantly different ($p < 0.001$) from Birchy Cove (mean = 18.4 ± 0.3 cm)and Summerside (mean = 18.0 ± 0.2 cm) samples. Since male and female body lengths at Gillams were significantly different from each other in 1994 ($p = 0.007$, **Table A. 2.**) statistical significance between sites also included sex as a factor. Male and female body length data reflected pooled statistical significance and numerical trends ($p < 0.001$). Body length analyses were reflected in dressed or eviscerated weights of pooled and gender populations. Cunners at the Gillams site weighed (mean = 52.3 ± 2.9 g) significantly less ($p < 0.001$) than either Birchy Cove or Summerside residents (mean = 88.9 ± 4.5 g and 79.0 ± 3.5 g, respectively; **Table 5.**). Again, sexual differences ($p = 0.004$, **Table A. 2.**) were observed at the Gillams' station which necessitated gender analyses between sites. Both male and female fish at Birchy Cove and Summerside were significantly heavier ($p < 0.001$) than Gillams fish (**Table 5.**).

Length frequency histograms generated from 1995 field collections illustrate that cunners at the Birchy Cove site supported a minimum body length of 14.0 to a maximum of 27.9 cm. Both Summerside and Gillams populations ranged in total body length from 14.0 cm up to 25.9 cm. A greater number of pooled fish at Birchy Cove were assigned to the 18-19 cm and 20-21 cm size classes, respectively (males were also more frequently assigned to the 16-17 cm interval), whereas

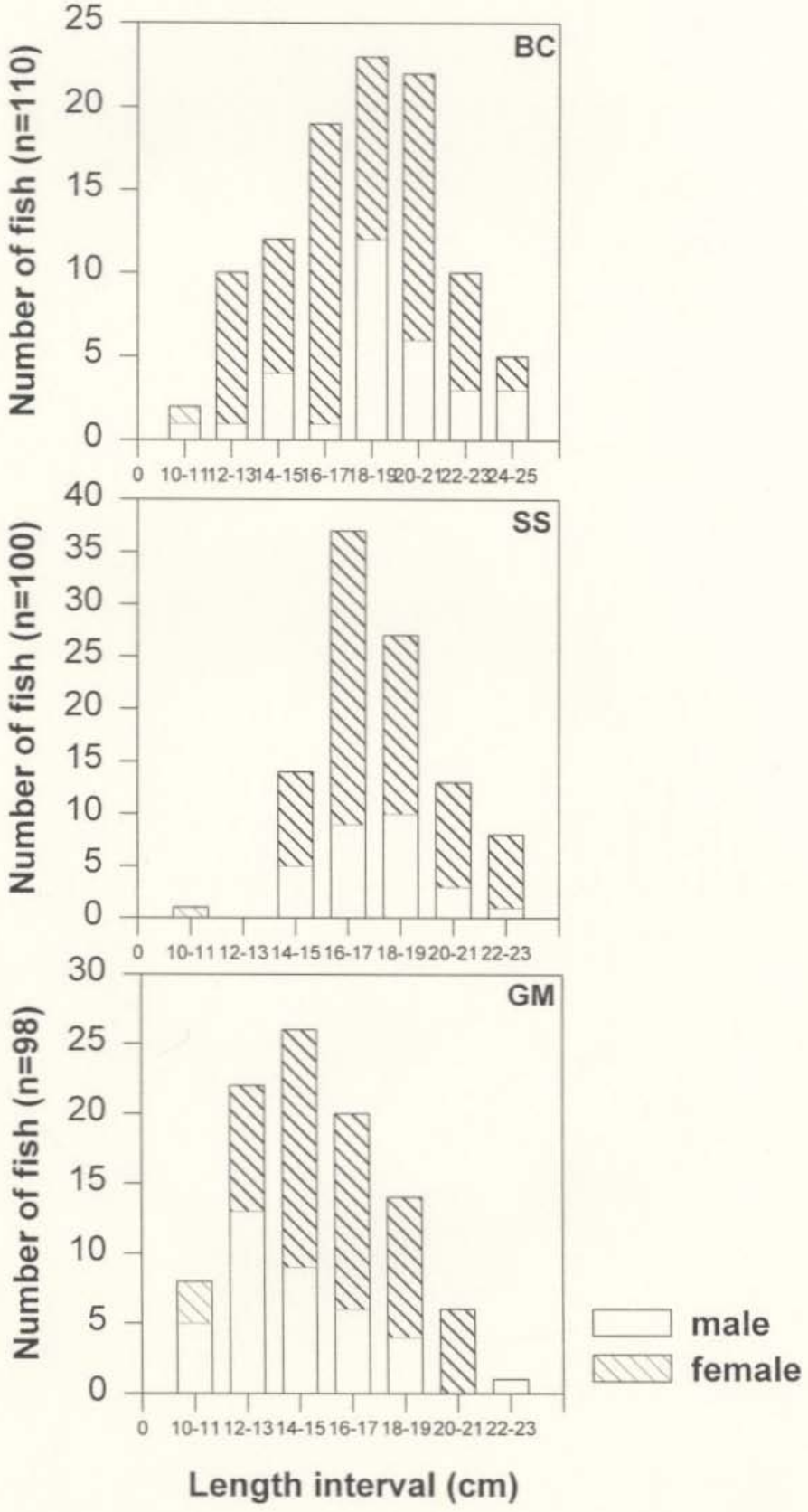


Figure 4. Length frequencies for male and female *Tautogolabrus adspersus* collected from Birchy Cove (BC), Summerside (SS) and Gillams (GM), 1994.

Table 5. Mean (±s.e) length, dressed weight, condition factor (=K-Factor), and organ somatic indices (SI) of *Tautogolabrus adspersus* collected from Birchy Cove, Summerside and Gillams (1994), where ♂ and ♀ denote males and females, respectively.

Variable		Birchy Cove n=31(♂), 79(♀)	Summerside n=28(♂), 72(♀)	Gillams n=38(♂), 59(♀)	
Length (cm)	(♂)	19.31±0.62	17.89±0.41	14.63±0.43	c,e
	(♀)	18.04±0.36	18.05±0.30	16.09±0.35	c,e
Weight (g)	(♂)	105.29±9.99	74.47±5.97	43.37±4.48	c,e
	(♀)	82.51±4.73	80.69±4.26	58.00±3.59	c,e
K-Factor		1.31±0.01	1.28±0.01	1.26±0.01	ns
Gonado-SI	(♂)	7.36±0.66	7.56±0.77	3.28±0.40	c,e
	(♀)	5.62±0.26	7.68±0.50	4.93±0.48	d^1,e
Hepato-SI	(♂)	2.05±0.11	1.79±0.82	1.78±0.08	d^1,ns
	(♀)	3.08±0.10	3.35±0.11	2.68±0.13	f^1,e^1
Visceral-SI		3.34±1.29	3.48±0.10	3.50±0.13	d^1,ns
Spleno-SI	(♂)	0.20±0.02	0.20±0.01	0.18±0.01	ns,e^1
	(♀)	0.21±0.03	0.21±0.01	0.19±0.02	ns,e^1

c Birchy Cove significantly different from Gillams, $p<0.001$
d Birchy Cove significantly different from Gillams, $0.001<p<0.05$

e Summerside significantly different from Gillams, $p<0.001$
f Summerside significantly different from Gillams, $0.001<p<0.05$

$(x)^1$ significant interaction, where (x)=(a,b,c, or d)

ns non-significant

the 16-17 cm and 18-19 cm length intervals at the Summerside sitecontained 9 fish each (**Figure 5.**). A similar trend was exhibited for Gillams samples; fish (pooled and female numbers) fell more frequently in the 16-17 cm and 18-19 cm size classes (males were most numerically assigned to the 14-15 cm length interval; **Figure 5.**).

Neither body lengths nor eviscerated weights at impact sites (Birchy Cove and Summerside) differed significantly from that at Gillams during the 1995 sampling season (**Table 6.**). There were no sexually dimorphic differences in body lengths or eviscerated weights at either field site in 1995. Yearly site differences with respect to body length and dressed weight were negligible with the exception of Birchy Cove (1993 vs 1994) and Gillams (1994 vs 1995, **Tables A. 3.-5.**).

A least squares linear regression between the dependent (predicted) variable, dressed weight, and the independent (predictor) variable, body length, for all three field seasons, grouped by site, generated (**Figure 6**) the following equations :

(i) Birchy Cove, Y (dressed weight) = 18.42X (length) - 229.59 {r = 0.84, p(tail) < 0.0005}.

(ii) Summerside, Y (dressed weight) = 15.13X (length) - 181.99 {r= 0.99, p(tail) < 0.0005}.

Slopes or intercepts did not differ significantly beyond chance between sites in 1993 ($p = 0.10$). In 1994, the following regression equations were generated (**Figure 7.-7a.**):

(i) Birchy Cove, Y (dressed weight) = 13.37X (length) - 157.15 {r = 0.94, p(tail) < 0.0005};

Male, Y (dressed weight) = 15.47X (length) - 193.47 {r= 0.96, p(tail) < 0.00005}.

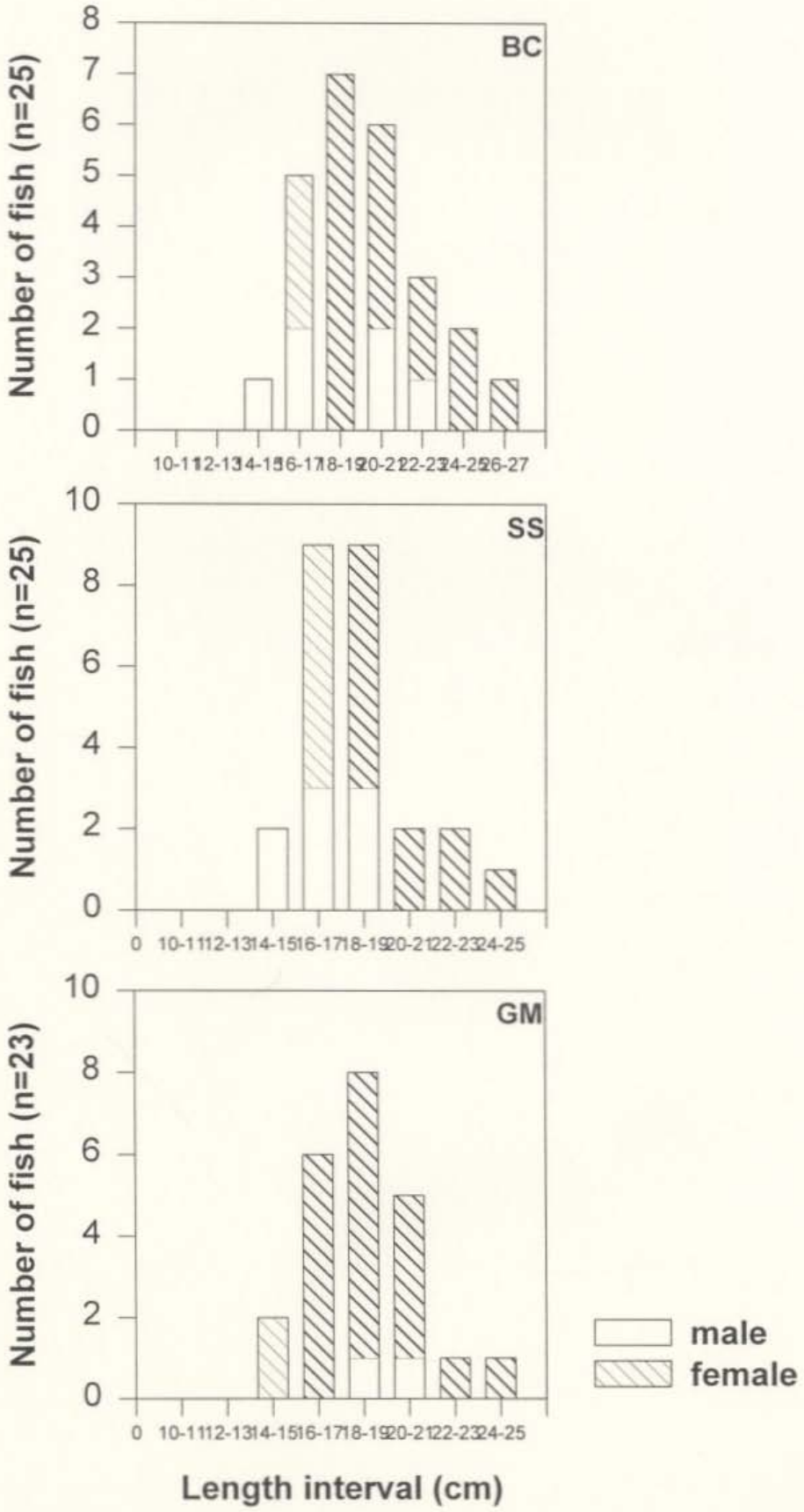


Figure 5. Length frequencied for male and female *Tautogolabrus adspesus* collected from Birchy Cove (BC), Summerside (SS) and Gillams (GM), 1995.

Table 6. Mean (±s.e.) , dressed weight, condition (=K-factor), and organ somatic indices (SI) of *Tautogolabrus adspersus* collected from Birchy Cove, Summerside and Gillams (1995), where ♂ and ♀ denote males and females, respectively.

Variable		Birchy Cove n=6(♂), 19(♀)	Summerside n=7(♂), 18(♀)	Gillams n=2(♂), 21(♀)	
Length (cm)		19.92±0.58	18.44±0.46	18.67±0.46	ns,ns
Weight (g)		117.10.58	82.25±6.80	91.26±6.66	ns,ns
K-Factor		1.39±0.03	1.25±0.01	1.36±0.03	ns,ns
Gonado-SI	(♂)	12.46±2.01	6.73±1.02	5.58±2.19	ns,ns
	(♀)	14.49±0.92	8.98±0.69	12.43±0.75	ns,ns
Hepato-SI		4.19±0.29	3.07±0.20	3.48±0.17	ns,f[1]
Visceral-SI		5.48±0.31	7.08±0.69	4.68±0.30	d[1],ns
Spleno-SI	(♂)	0.17±0.03	0.15±0.02	0.10±0.01	d[1],e[1]
	(♀)	0.18±0.02	0.13±0.01	0.18±0.05	ns,ns

c Birchy Cove significantly different from Gillams, $p<0.001$
d Birchy Cove significantly different from Gillams, $0.001<p<0.05$

e Summerside significantly different from Gillams, $p<0.001$
f Summerside significantly different from Gillams, $0.001<p<0.05$

$(x)^1$ significant interaction, where (x)=(a,b,c, or d)

ns non-significant

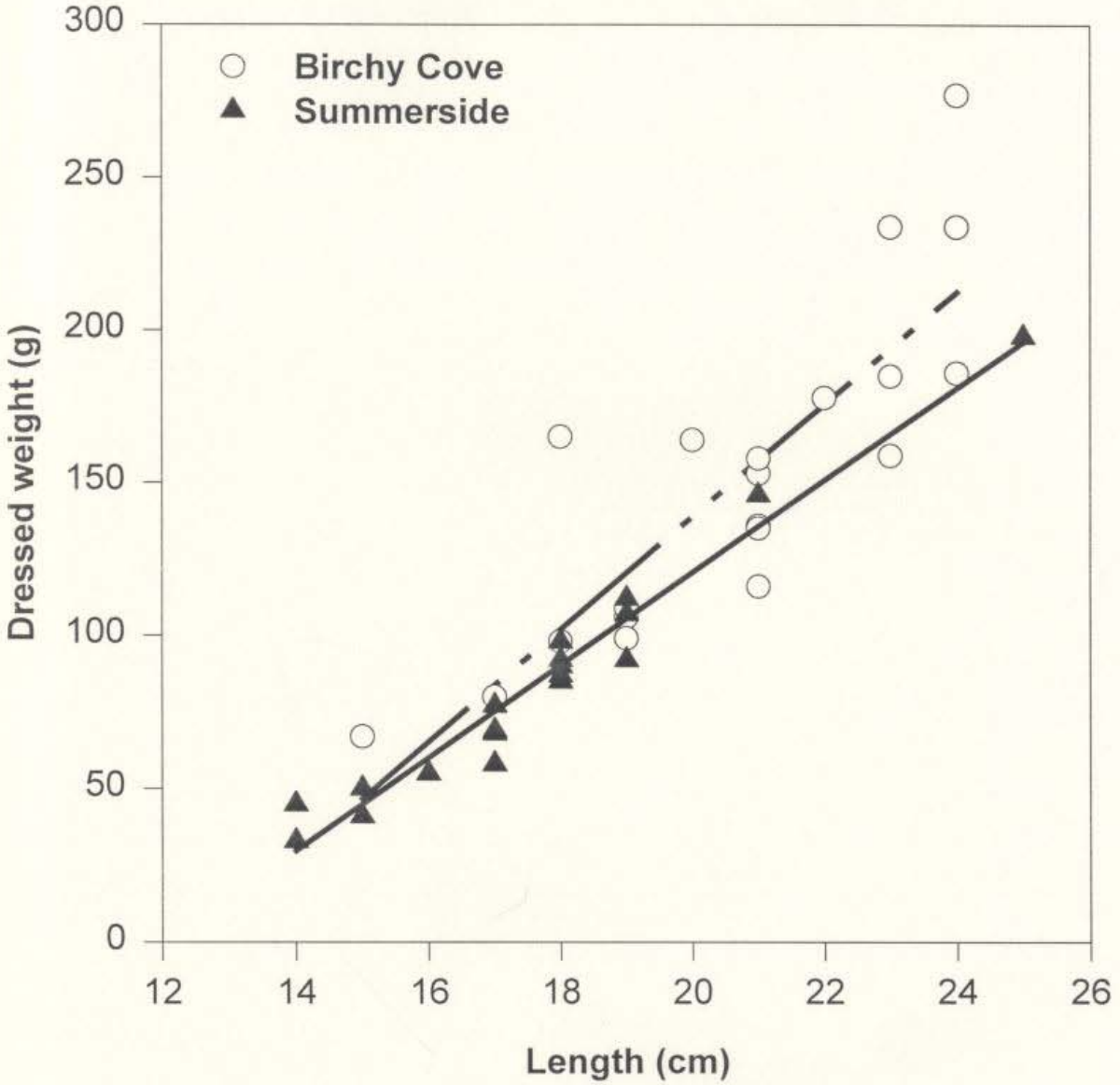


Figure 6. Length-dressed weight regression analyses for *Tautogolabrus adspersus* collected from Birchy Cove (BC) and Summerside (SS), 1993.

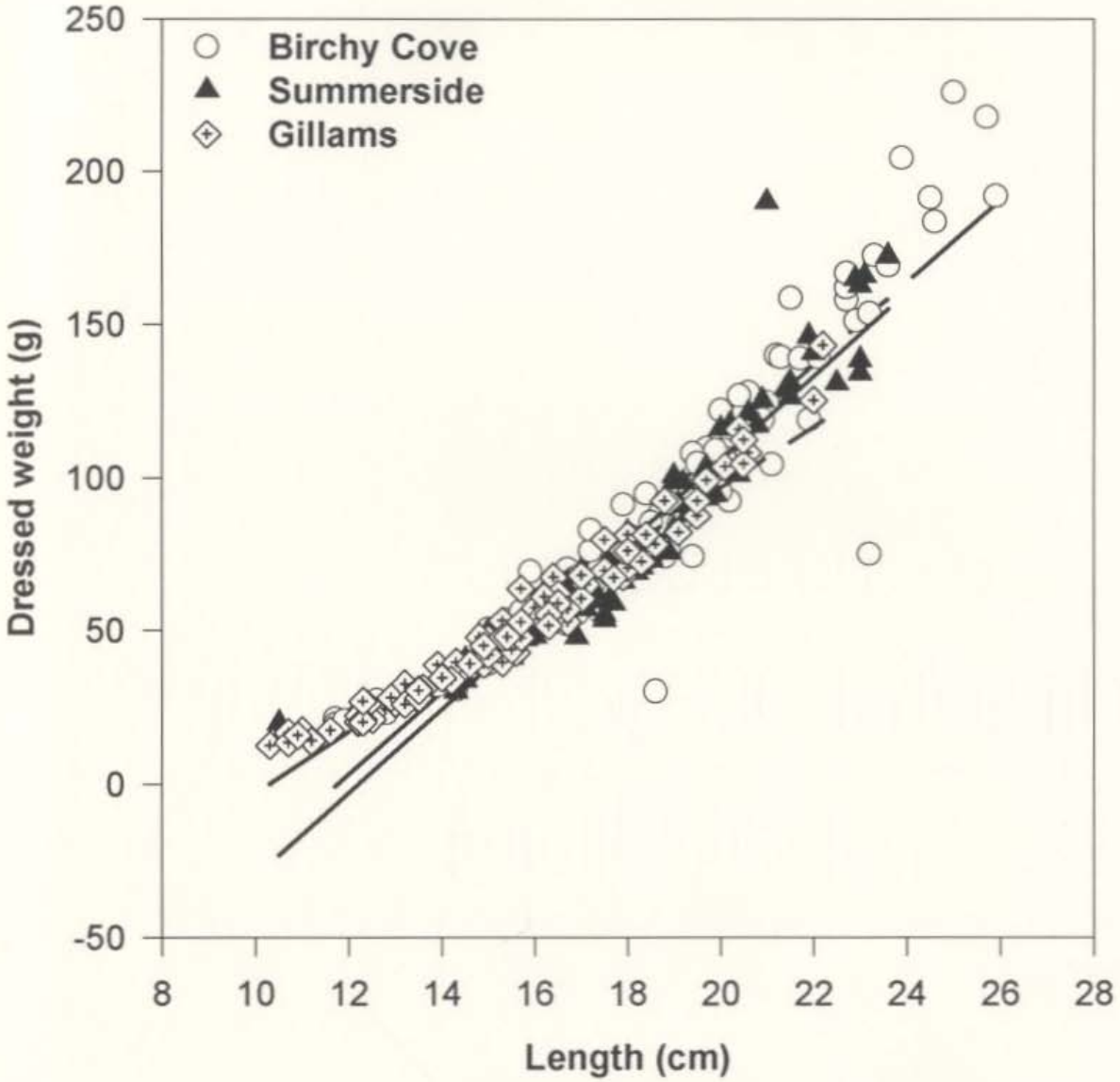
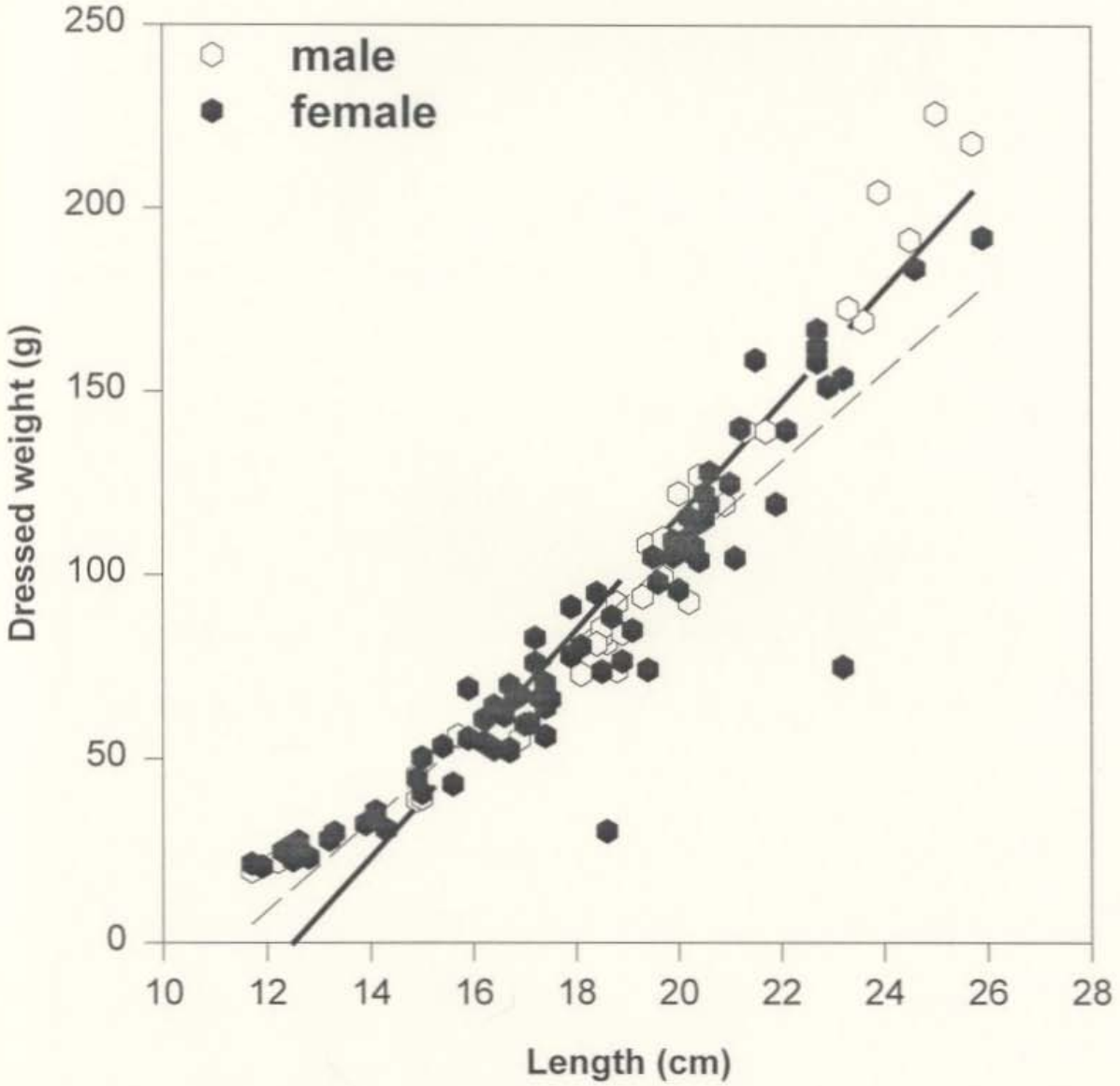


Figure 7. Length-dressed weight regression analyses for *Tautogolabrus adspersus* collected from Birchy Cove (BC), Summerside (SS) and Gillams (GM), 1994.



Male:Dressed weight=15.5 length - 193.5, $p < 0.00005$, $r = 0.96$
Female:Dressed weight=12.2 length - 138.1, $p < 0.00005$, $r = 0.93$
Regression over groups:p=0.001

Figure 7. a. Length-dressed weight regression of male and female *Tautogolabrus adspersus* collected from Birchy Cove, 1994.

Female, Y (dressed weight) = 12.22X (length) - 138.07 {r = 0.93, p(tail) < 0.00005}.

Regression over groups (male and female, 1994): p = 0.001

(ii) Summerside, Y (dressed weight) = 13.59X (length) - 165.74 {r = 0.94, p(tail) < 0.0005}.

(iii) Gillams, Y (dressed weight) = 9.98X(length) - 102.65 {r = 0.98, p(tail) < 0.0005}.

Slopes or intercepts differed significantly beyond chance between impact and reference site(s) in 1994 ($p < 0.0005$).

In 1995, the following linear regressions were calculated (**Figure 8**.):

(i) Birchy Cove, Y (dressed weight) = 17.84X (length) - 238.13 {r= 0.97, p(tail) < 0.0005}.

(ii) Summerside, Y (dressed weight) = 14.41X (length) - 183.38 {r= 0.98, p(tail) = 0.000}.

(iii) Gillams, Y (dressed weight) = 13.98X (length) - 169.87 {r= 0.96, p(tail) = 0.000}.

Slopes or intercepts differed significantly beyond chance between impact and reference site(s) in 1995 (**BC and SS vs Gillams, p = 0.006 and 0.04**, respectively).

Analysis of residual distribution met normality assumptions of the simple linear regression model. Note that pooled values are given except where sex was a significant factor. Seasonal differences in site growth patterns are summarized in **Tables A. 6.-8.**

Pooling of field seasons demonstrated that females numerically dominated their male counterparts approximately 3 : 1 in both Birchy Cove and Gillams' samples (females = 75%(112), 76%(80); males = 25%(42), 24%(40), respectively)). Although the females at Summerside also outnumbered male residents (females = 58%(104); males = 42%(51)), the sexual dichotomy was not

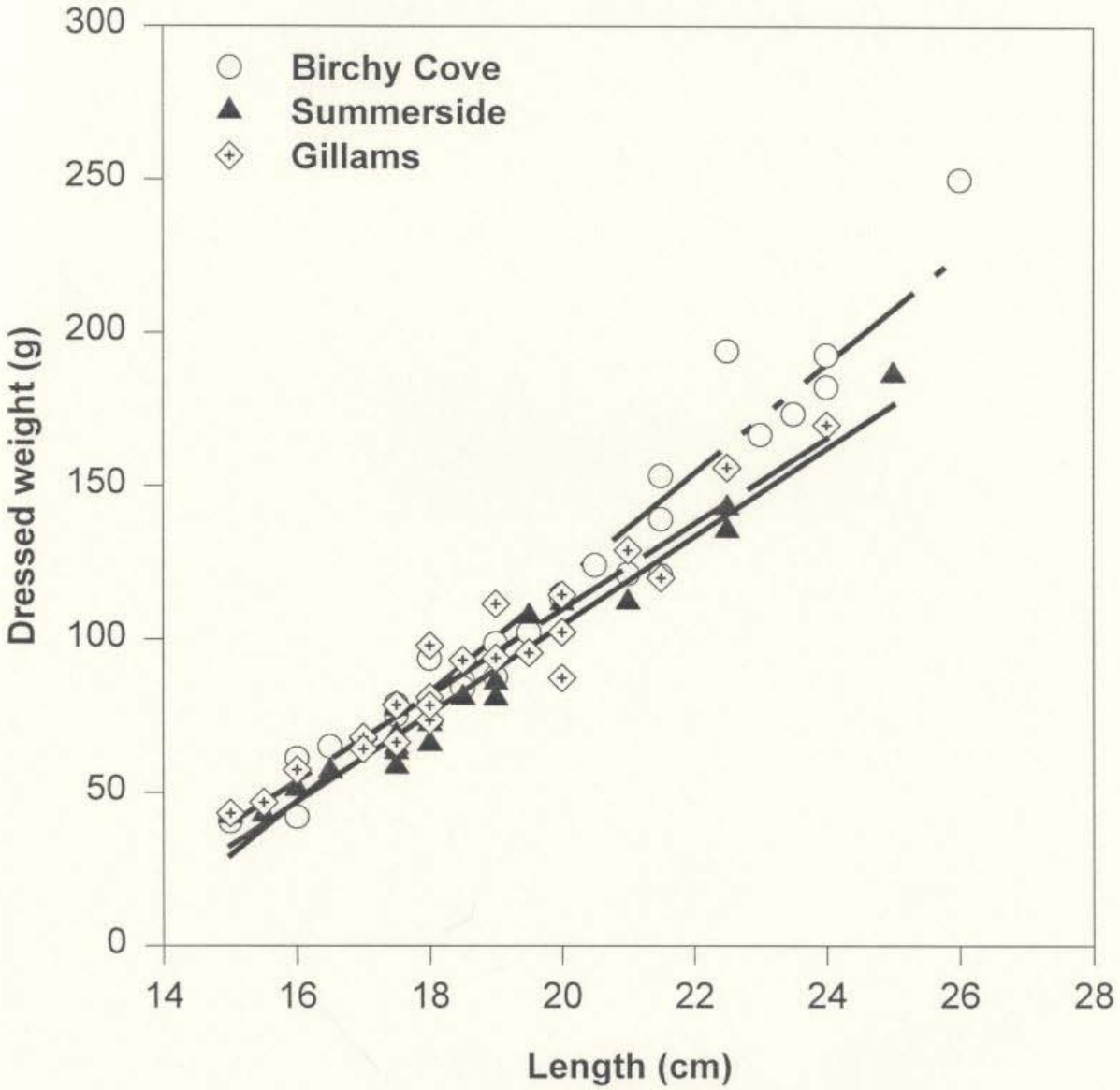


Figure 8. Length-dressed weight regression analyses for *Tautogolabrus adspersus* collected from Birchy Cove (BC), Summerside (SS) and Gillams (GM), 1995.

as distinct. This may be attributed to a numeric reversal in trend at the Summerside site in 1993, where approximately twice as many males (70%(14)) were collected as females (30%(6)). This sexual disproportionality is reflected in **Figures 3. to 5.**

II. Body and Organismic Index Parameters

Somatic indices in 1993 were greater in value at Birchy Cove than at Summerside (**Table 4.**). Statistically, site differences were restricted to hepato- ($p = 0.04$) and visceralsomatic indices ($p = 0.03$, **Table 4.**). Scatterplots of GSI and total body length by sex were constructed for 1993 field collections to demonstrate relative frequencies of mature to post-spawn/immature fish. **Figure 9.** illustrates that more male and female Summerside cunner have lower GSI values than Birchy Cove. Sexual differences within somatic indices were found to be non-significant in 1993.

Preserving tabular order of sample sites, pooled somatic indices reflected a decreasing trend in value in 1994, with the exception of both the hepato- and visceral-somatic indices (**Table 5.**). Sexual differences in SI values were observed at all three sites; namely Birchy Cove SSIs ($p= 0.002$), HSIs at Summerside ($p < 0.001$) and reference GSIs ($p < 0.001$, **Table A. 2.**). Gender analyses between sites were carried out with respect to these somatic indices. Condition factors did not statistically deviate between sites (**Table 5.**).

Ovarian and testicular indices at impact sites were significantly different from that of reference fish (**Table 5.**). 1994 scatterplots of GSI versus total body length illustrated that, in general, both sexes at the Gillams site had smaller gonadosomatic indices than male or female residents at Birchy Cove and Summerside (**Figure 10.**).

Male and female hepato-SIs at Birchy Cove site were significantly different ($p = 0.03$; 0.01, respectively, **Table 5.**) from reference somatic values in 1994. Statistical significance between HSIs at Summerside and Gillams was limited to the female population ($p < 0.001$). Site differences ($p = 0.003$, **Table 5.**) in pooled visceralsomatic indices were only observed between Birchy Cove and Gillams. Both male and female splenosomatic indices at Summerside were significantly different

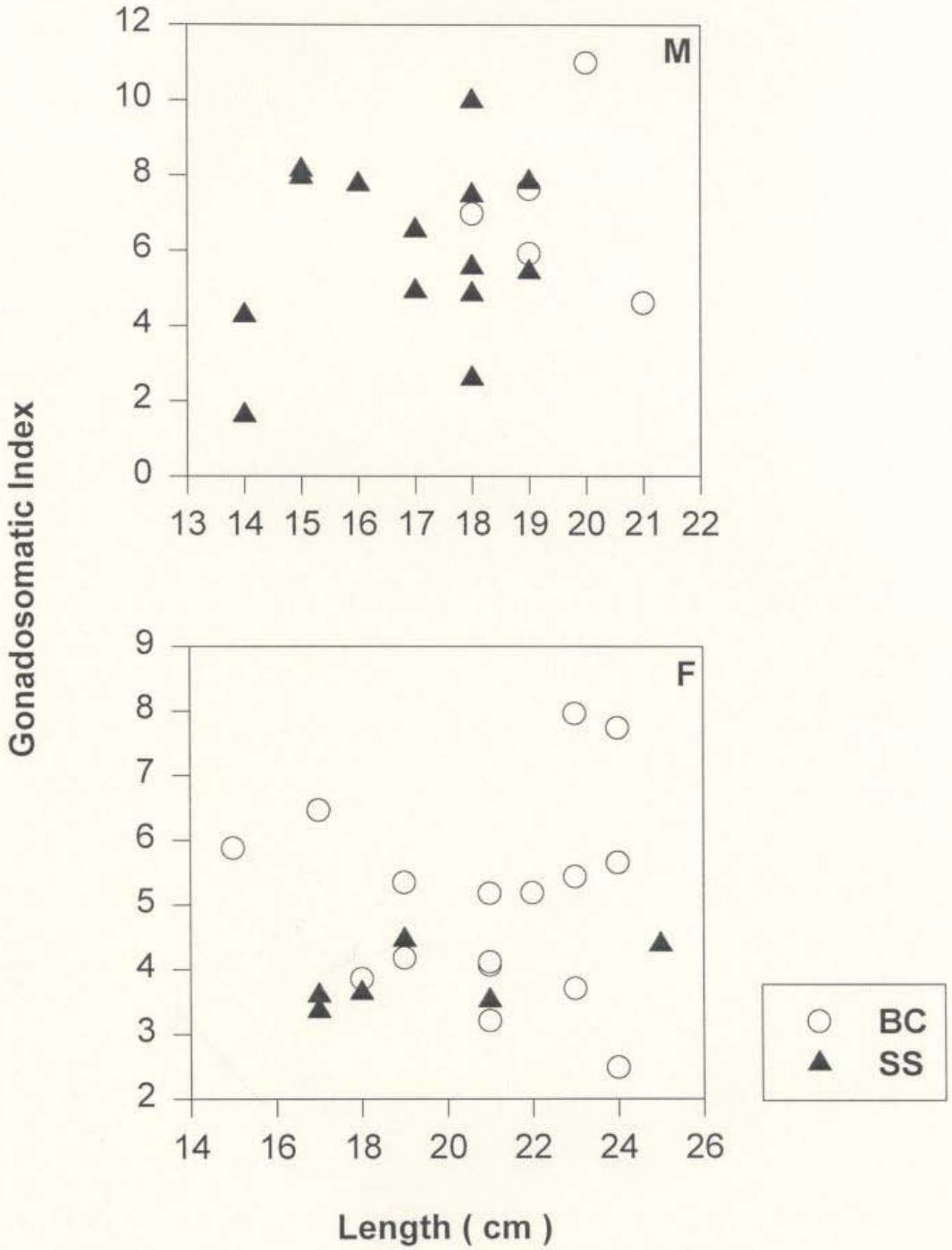


Figure 9. Scatterplot of GSI vs length (cm) for male (M) and female (F) Birchy Cove (BC) and Summerside (SS), samples 1993.

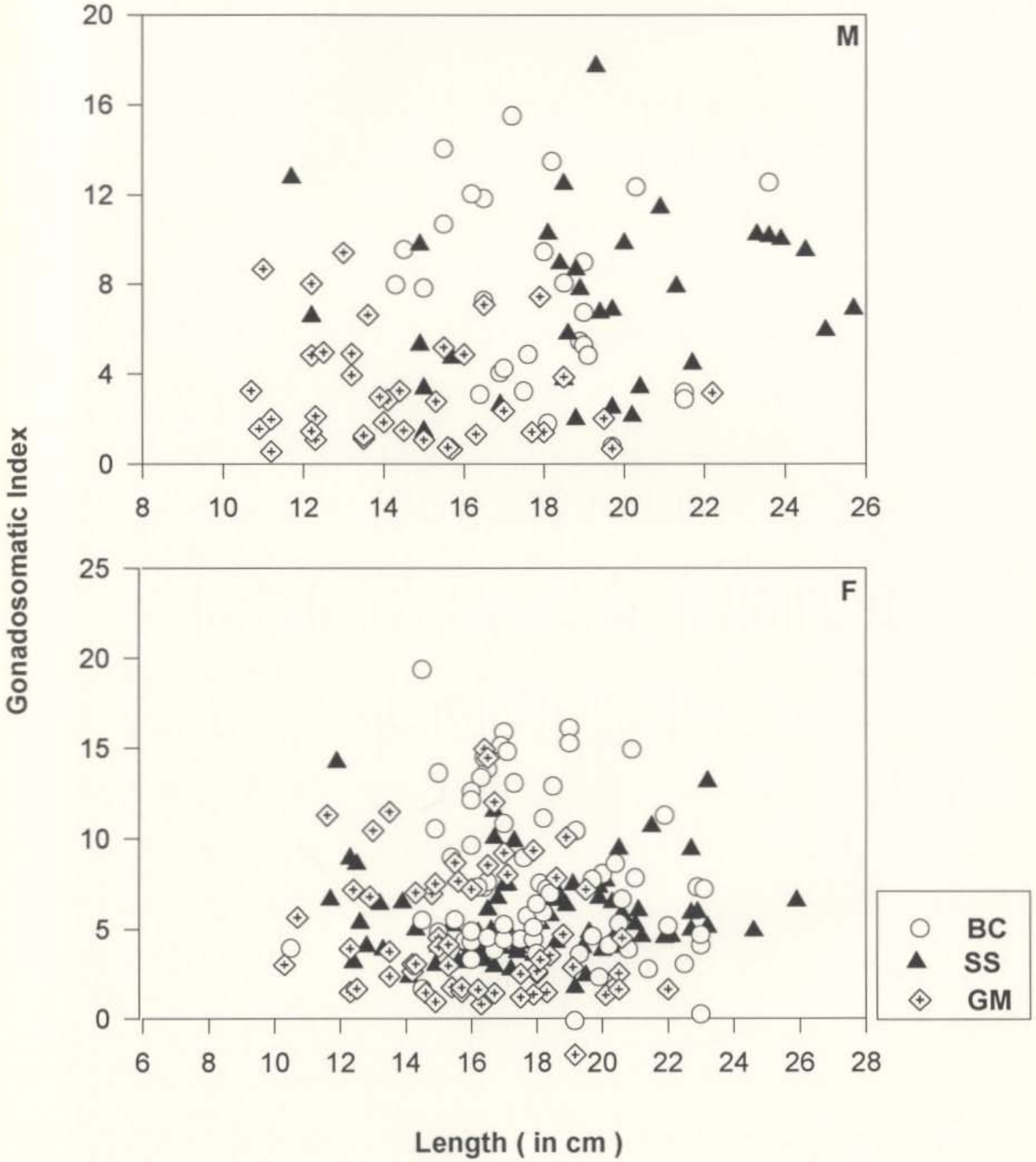


Figure 10. Scatterplot of GSI and length (cm) for male (M) and female (F)*Tautogolabrus adspersus* collected from Birchy Cove (BC), Summerside (SS) and Gillams (GM) for the 1994 field season.

($p < 0.001$, **Table 5.**) from control counterparts in 1994.

Transformation of 1995 necropsy data illustrated a slight departure from value trends characteristic to the 1993 and 1994 field seasons. With the exception of the visceral- and male splenosomatic indices, pooled Birchy Cove populations supported the greatest numerical values followed by Gillams and Summerside, respectively (**Table 6.**). Sexual differences in spleno-SI values were observed at Gillams and gender analyses between sites were carried out with respect to this somatic index (**Table A. 9.**). Site differences in pooled condition factor and ovarian/testicular (gonado-) -SIs were non-significant (**Table 6.**). Scatterplots of gonadosomatic indices versus body length were sexually inconsistent. Male Birchy Cove residents had greater gonadosomatic indices compared to Summerside or Gillams' samples in 1995, whereas the majority of female cunner at both Birchy Cove and the reference site were characterized by larger GSIs than at Summerside (**Figure 11.**). Pooled hepatosomatic indices were significantly different ($p = 0.02$, **Table 6.**) between Summerside and Gillams. Visceralsomatic indices (pooled data) were significantly greater ($p = 0.007$, **Table 6.**) at Birchy Cove than at the Gillams site. In 1995 site significance of splenosomatic indices was limited to the male population, specifically, fish sampled at impact stations (Birchy Cove and Summerside) and were characterized by significantly greater ($p = 0.01$; < 0.001, respectively, **Table 6.**) indices than their control counterparts at Gillams.

To summarize somatic index results, the principal field season (1994) demonstrated a statistical difference between control cunner sampled at Gillams and impact site(s) (Birchy Cove and/or Summerside), with the exception of site non-significance of condition factors.

Yearly site differences with respect to somatic indices were variable among sample stations.

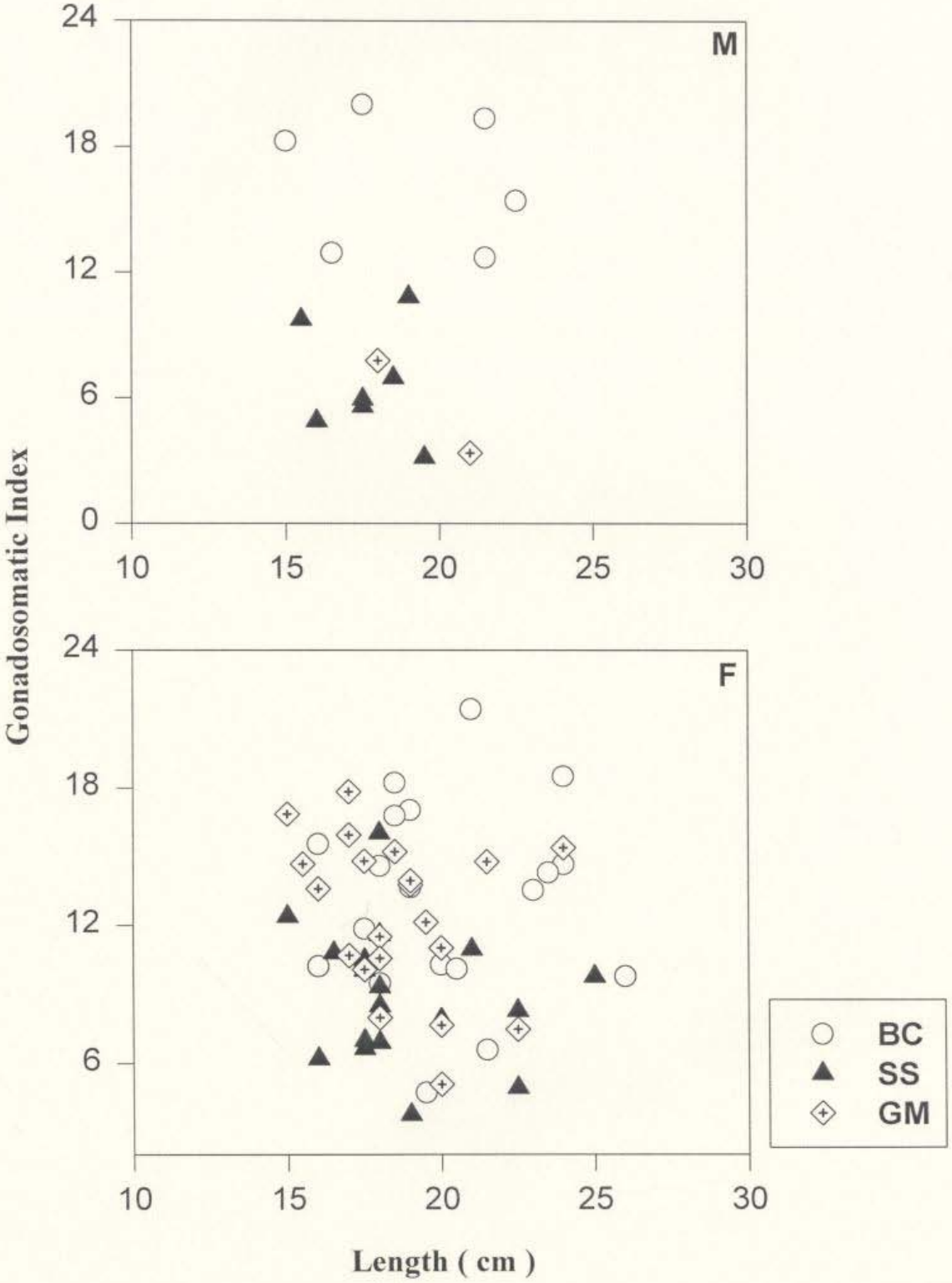


Figure 11. Scatterplot of GSI and length (cm) for male (M) and female (F) *Tautogolabrus adspersus* collected from Birchy Cove (BC), Summerside (SS) and Gillams (GM) samples 1995.

Seasonal SI differences are summarized in **Tables A. 3.-5.**

Seasonal regressions of body size (eviscerated weights) against organ weights grouped by site can be referenced in **Figures A. 2** through to **A. 13**.

III. Macroscopic Hepatic Anomalies

Evidence of external lesions and deviations from typical macroscopic appearance were noted during all field season necropsies. Liver spots were macroscopically characterized by a white, stipple-like pattern on the dorso-ventral surface. "Necrotic" areas or opaque foci were visible within the liver and defined as small (approximately 1-3 mm) opaque nodules, gray/white to yellowish in colour; whereas, tumour-like masses were easily differentiated from other anomalies as raised, dense matter that could be excised from surrounding tissue. In 1993, Birchy Cove supported greater incidences of observed hepatic spots and necrosis (n=17, 81%; n=12, 57%; respectively) compared to Summerside (n=2, 10%; n=7, 35%; **Table 7.**). Tumour-like masses were a pathological manifestation exclusive to Summerside samples (n=4, 20%; **Table 7.**).

Site differences in hepatic macroscopic anomalies were deemed to be significantly more frequent at impact(s) compared to the liver appearance at Gillams (Chi-squared, $p < 0.05$) .

In 1994, this trend extended to hepatic spots only, whereas "necrosis" was more prevalent at Summerside (n=56, 56%) compared to Birchy Cove residents (n=33, 33%) and Gillams (n=1, 1%; **Table 7.**). Tumour-like aggregates were restricted to a single occurrence at both impact sites (**Table 7.**).

The 1995 necropsies exhibited more spotty livers in Summerside fish (n = 6, 24%) compared to Birchy Cove (n=3, 12%), whereas the reverse was true of hepatic foci (n= 6, 24%; n=20, 80%, respectively; **Table 7.**). Tumour-like masses were again exclusive to Summerside (n=3, 12%; **Table 7.**). Gillams livers showed minimal external pathology (n=1, 4%; **Table 7.**) for all anomalous categories in 1995.

Both 1994 and 1995 results illustrate that external macroscopic (hepatic) lesions occurred

Table 7. Number of cunner, *Tautogolabrus adspersus*, and percentage of total sample demonstrating external macroscopic hepatic lesions for stations collected during the 1993-1995 field seasons.

Pathological Criteria	Site		
Liver	**Birchy Cove (1993)**	**Summerside (1993)**	
Spots	17 (81%)	2 (10%)	
Necrosis/Opaque Foci	12 (57%)	7 (35%)	
Tumour-like Masses	0	4 (20%)	
Total Anomalies	**29 (138%)**	**13 (65%)**	
Liver	**Birchy Cove (1994)**	**Summerside (1994)**	**Gillams (1994)**
Spots	20 (18%)	3 (3%)	5 (5%)
Necrosis/Opaque Foci	33 (30%)	56 (56%)	1 (1%)
Tumour-like masses	1 (<1%)	1 (1%)	0
Total Anomalies	**54 (49%)**	**60 (60%)**	**6 (6%)**
Liver	**Birchy Cove (1995)**	**Summerside (1995)**	**Gillams (1995)**
Spots	3 (12%)	6 (24%)	1 (4%)
Necrosis/Opaque Foci	20 (80%)	6 (24%)	1 (4%)
Tumour-like Masses	0	3 (12%)	1 (4%)
Total Anomalies	**23 (92%)**	**15 (60%)**	**3 (13%)**

more often in cunner captured at impact sites than at Gillams, the reference sample.

IV. Histopathological Assessment Surveys

A. Gill

Typical teleost gill cytoarchitecture consists of two sets of four holobranchs which construct the lateral boundaries of the pharynx; each holobranch is further comprised of two hemibranchs (Roberts, 1989). The hemibranchs embody the primary filaments or lamellae; the surface area of each single primary is increased by dorso-ventral semilunar evaginations - the secondary lamellae (Roberts, 1989). A "normal' gill structure was identified as the absence of pathology outlined in the materials and methods (Mallatt, 1985).

In 1993 branchial tissue of impact samples deviated from typical gill cytoarchitecture. None of the stained sections examined could be classified as normal due to the presence of gill lesions (**Table 8.**). Metacercariae of *Cryptocotyle lingua,* encysted in both the primary filaments and gill arches, were approximately 2.5 fold greater in Birchy Cove (n=77) than in Summerside samples (n=31; **Table 8.**). Slight hyperplasia of secondary lamella was much more prevalent at Summerside (n=10, 63%) versus Birchy Cove (n=3, 25%; **Table 8.**). However, Birchy Cove exhibited greater numbers and, correspondingly, greater percentages of immoderate hyperplasia than did branchial sections examined from Summerside. Specifically, 50% (n=6) of Birchy Cove gills illustrated moderate hyperplasia compared to 38% (n=6) at Summerside. Excessive cellular proliferation was observed exclusively at Birchy Cove (n=2, 17%) and 33% of lamellar sections (n=4) were characterized by one-fourth to one-fifth interlamellar thickening (as a proportion of total secondary filament length) versus 19% (n=3) at Summerside (**Table 8.**).

In 1994, none of the gill sections microscopically examined at Birchy Cove and only one (7%) of the Summerside sections appeared normal (**Table 9.**). The majority of Gillams' fish (n=16,

Table 8. Histopathological survey of necropsied tissue wax sections of *Tautogolabrus adspersus* stained with haemotoxylin and eosin for the 1993 field collection, indicating number of fish and percentage of sample illustrating microstructural criteria.

Tissue Criteria	Site	
Gill	**Birchy Cove**	**Summerside**
Normal	0	0
Slight Hyperplasia	3 (25%)	10 (63%)
Moderate Hyperplasia	6 (50%)	6 (38%)
Excessive Hyperplasia	2 (17%)	0
Interlamellar Thickening(1/4-1/5)	4 (33%)	3 (19%)
Total cryptocotyle	77	31
Total Examined	12	16
Liver		
Normal	7 (41%)	16 (88%)
Slight Vacuolation	1 (6%)	1 (6%)
Excessive Vacuolation	5 (29%)	0
Melanomacrophage Centers	5 (29%)	1 (6%)
Total Examined	17	18

Table 9. Histopathological survey of necropsied tissue wax sections of *Tautogolabrus adspersus* stained with haemotoxylin and eosin for the 1994 field collection, indicating number of fish and percentage of sample illustrating microstructural criteria.

Tissue Criteria	Site		
Gill	Birchy Cove	Summerside	Gillams
Normal	0	1 (7%)	16 (76%)
Slight Hyperplasia	5 (28%)	9 (64%)	1 (5%)
Moderate Hyperplasia	9 (50%)	3 (21%)	0
Excessive Hyperplasia	4 (22%)	1 (7%)	0
Interlamellar Thickening(1/4-1/5)	3 (17%)	1 (7%)	0
Clubbing	0	1 (7%)	0
Total cryptocotyle	60	83	89
Total Examined	18	14	21
Liver			
Normal	10 (59%)	13 (76%)	16 (94%)
Necrosis	2 (12%)	0	0
Slight Lipid Vacuolation	1 (6%)	1 (6%)	1 (6%)
Excessive Lipid Vacuolation	4 (24%)	0	0
Melanomacrophage Centers	2 (12%)	2 (12%)	0
Total Examined	17	17	17

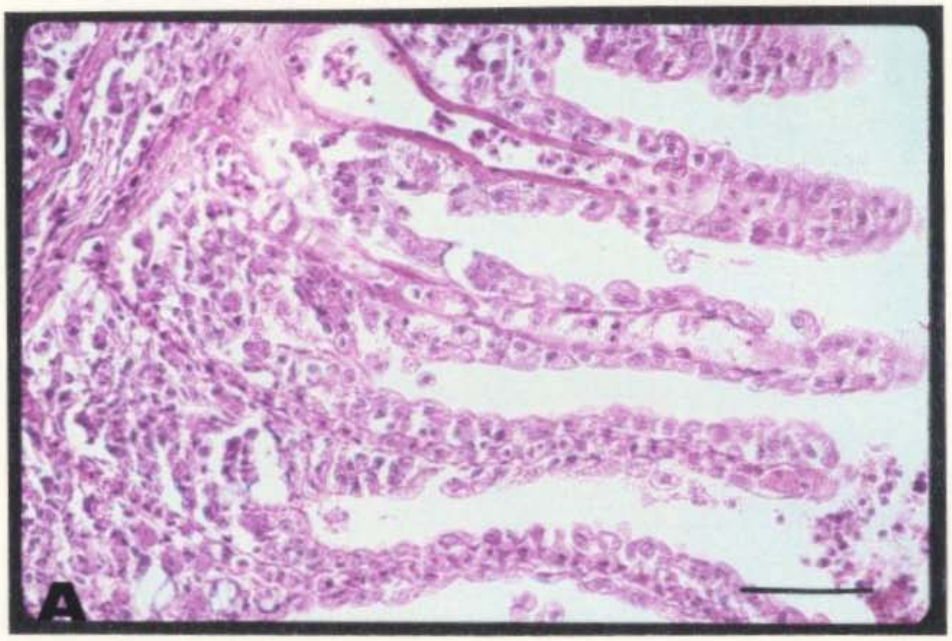
76%) possessed normal branchial ultrastructure (**Table 9.**; **Figure 12.**). Parasite counts for both Summerside (n=83) and Gillams' (n=89) specimens were similar and were greater than those from Birchy Cove site (n=60, **Table 9.**). Reflecting 1993 histology, slight hyperplasia of secondary lamellae was more prevalent at Summerside (n = 9, 64%) than at Birchy Cove (n=5, 28%). Only one incidence was annotated in Gillams sections (5%; **Table 9.**). And, moderate to excessive hyperplasia were more prevalent in Birchy Cove gills (n=9, 50%; n=4, 22%, respectively; **Figure 12.**) than in Summerside samples (n=3, 21%; n=1, 7%, respectively). No evidence of the aforementioned lesions were observed at the reference site (**Table 9.**). This trend also extrapolated to interlamellar thickening, with approximately twice as much cellular infiltration of secondary filament troughs (on the order of one-fourth to one-fifth) at Birchy Cove (n=3, 17%) than at Summerside (n=1, 7%; **Table 9.**). Clubbing or telangiectasia was restricted to a single occurrence at Summerside in 1994 (7%; **Table 9.**).

To summarize, both seasons of histological observation illustrated a greater frequency of gill lesions at impact sites than in the reference sample in 1994 (Fisher's Exact Test, $p < 0.001$), and more extensive categories of epithelial hyperplasia were more evident in samples from Birchy Cove than Summerside. However, in 1994, metacercaria cysts were greatest in gills from Gillams than either impact sample.

A. Liver

The teleost liver differs from its mammalian counterparts in thatthere is less tendency for hepatocytic arrangement to be cord or lobule-like, but rather positioned in tubules (Roberts, 1989; Hinton and Laurén, 1990). The basal pole of hepatocytes isdirected towards sinusoids and collectively their tapered apices construct the bile canaliculi membrane. Typically, hepatocytes are

Figure 12. Cross sections of the first branchial arch of the cunner, *Tautogolabrus adspersus*, stained with haemataxylin and eosin to illustrate differential morphology. (A) Cross section of Birchy Cove gill. Note hyperplasia in the secondary lamellae and in the interlamellar trough. (B) Cross section of Gillam gill exhibiting typical lamellar microstructure. (Scale bar = 250 μm).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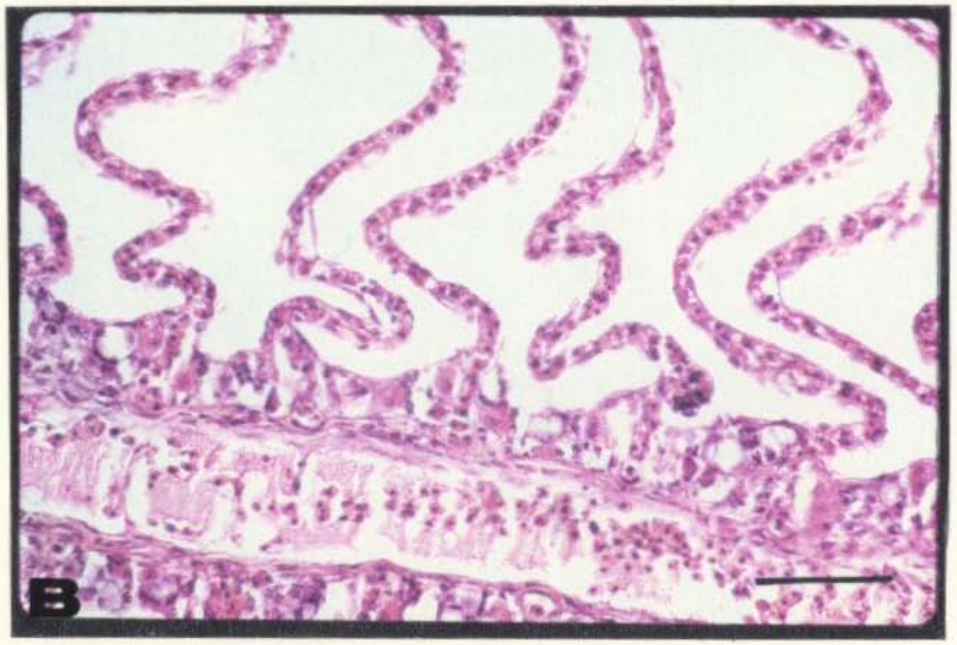
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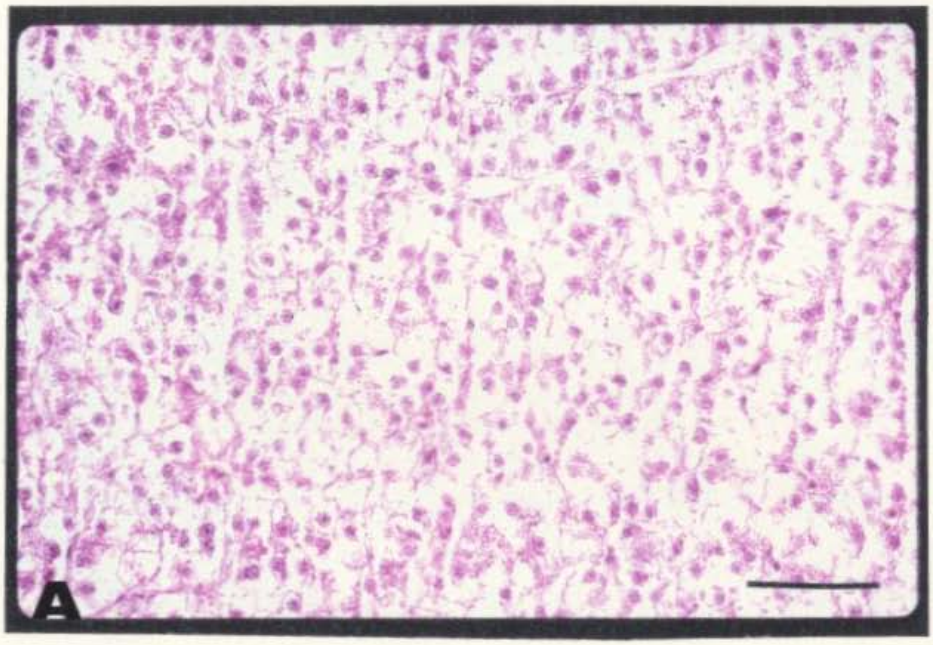
polygonal in configuration and are characterized by a distinct central nucleus with dense staining chromatin (Roberts, 1989). Intensity of haematoxylin and eosin cytoplasmic staining can be a diagnostic tool; symptomatic of toxicant exposure (Hinton and Laurén, 1990).

In 1993, approximately twice as many Summerside hepatic sections (n=16, 88%) were normal compared to their Birchy Cove counterparts (n=7, 41%; **Table 8.**). Instances of slight vacuolation (defined as < 10% of section examined) were minimal and comparable at both sites (n=1, 6%; **Table 8.**). Only Birchy Cove sections recorded considerable and more severe pathologic categories; specifically, excessive hepatic vacuolation (defined as > 90% of section examined; n=5, 29%). Only one liver section from Summerside exhibited melanomacrophage centers or pigment aggregates in 1993 (6%, **Table 8.**). Site difference in hepatic lesions was found to be significant in 1993 (Chi-squared, p=0.01).

Liver tissue surveys in 1994 revealed that 94% of Gillams' samples were free of pathological anomalies, compared to only 76% and 59% at Summerside and Birchy Cove sites, respectively (**Table 9.**; **Figure 13.**). Necrosis was a minimal occurrence and confined to Birchy Cove sections (n=2, 12%; **Table 9.**). As in 1993, slight vacuolation was equal at the three sites in the following season (n=1, 6%) and excessive fatty infiltration was again confined to Birchy Cove sections (n= 4, 24%; **Table 9.**; **Figure 13**). In both seasons, proliferative vacuolation was observed histologically as pale-staining hepatocytes compared to a more concentrated stain of liver cells in control sections (**Table 9.**). Melanomacrophage centers were observed in two of the impact sections (n=2, 12%; **Table 9.**). Overall, Birchy Cove supported a significantly greater (Fisher Exact Test, p=0.01) percentage of hepatic lesions than control (Gillams) counterparts in 1994.

Histological observations for both seasons were evident of more pronounced liver pathology

Figure 13. Cross section of the liver of cunner, *Tautogolabrus adspersus*, stained with haematoxylin and eosin to illustrate differential morphology. (A) Cross section of Birchy Cove liver showing less intense staining of cytoplasm and vacuolation of hepatocytes. (B) Cross section of Gillam liver. Note basophilic-staining of cytoplasm and typical hepatic microstructure. (Scale bar = 250 μm).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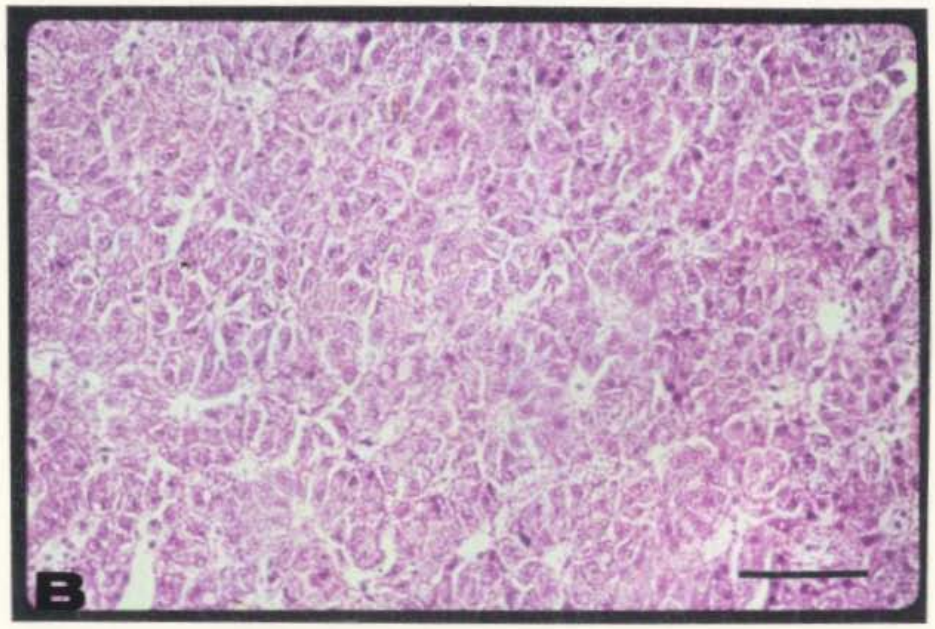
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at impact sites, particularly Birchy Cove, compared to negligible lesions at Gillams.

C. Gonad

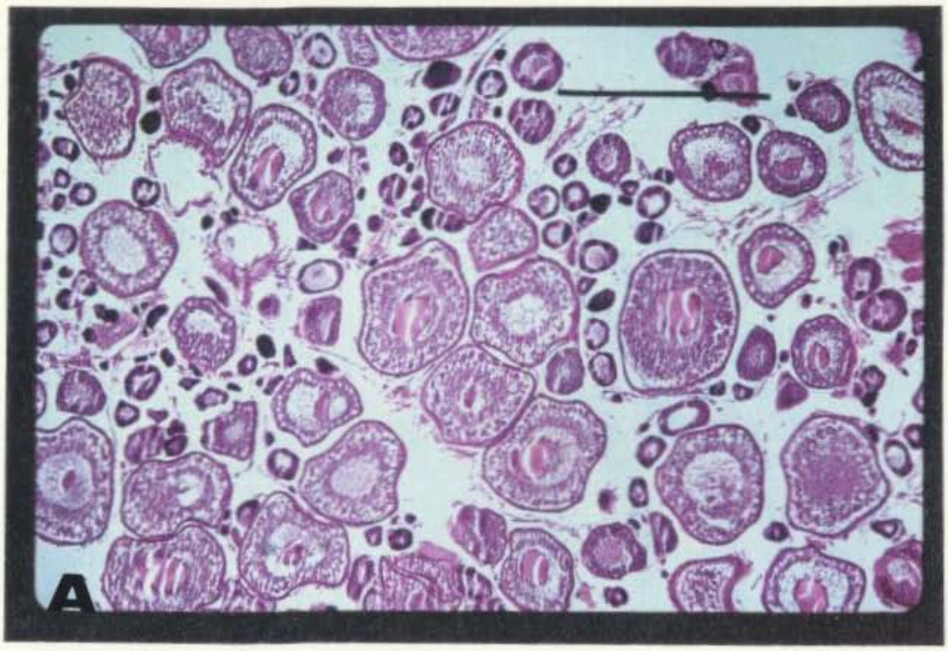
In 1993, 100% of ovaries examined at both Birchy Cove (n=15) and Summerside (n=8) sites were characterized by oocytes with central germinal vesicles, perinuclearnucleoli and exogenous vitellogenin (**Table 10.**; Wallace and Selman, 1981). Testicular surveys revealed 14% (n=2) of sampled Birchy Cove males exhibited secondary spermatocytes, spermatids and few sperm, whereas 86% (n=12) were void of mature sperm (**Table 10.**). No males at Summerside possessed mature sperm; this site difference was statistically different (Chi-squared, p=0.01). In summary, 100% (n= 29; 12) of both Birchy Cove and Summerside gonads appeared to be in the pre-spawn stage.

In 1994, 100% (n=14; 12, respectively) of impacted site ovaries displayed oocytes with central germinal vesicles, perinuclear nucleoli and exogenous vitellogenin, in contrast to only 50% (n=5) at the reference site (**Table 10.**). Oocytes with peripheral germinal vesicles, late vitellogenic and/or post-ovulatory follicles were restricted to and present in half (n=5) of ovaries examined at Gillams in 1994 (**Table 10.**; **Figure 14.**). The spawn histological category was more frequently observed at Gillams than at either impact site (Birchy Cove or Summerside) (Fisher's Exact Test, p=0.01; 0.02, respectively). Mature sperm was more frequently observed in 75% (n=6)of male samples at Gillams, in contrast to 40% (n=2) at the Birchy Cove site. Significant differences (Fisher's Exact Test, p=0.03) in testicular stage were found between Summerside and Gillams only, the sections from the former site were devoid of sperm (**Figure 15.**). The remaining percentages were attributed to testicular presence of both spermatids and few sperm, i.e. an earlier stage of spermatogenesis (**Table 10.**). Based on histological inspection, reproductive delays were observed

Table 10. Histopathology of necropsied gonadal sections of *Tautogolabrus adspersus* stained with haemotoxylin and eosin (1993-1994), indicating number of fish and percentage of sample.

Gonad	Site		
Ovary	**Birchy Cove (1993)**	**Summerside (1993)**	
Prespawn	15 (100%)	8 (100%)	
Total Examined	**15**	**8**	
Testes	**Birchy Cove (1993)**	**Summerside (1993)**	
2^0 spermatocytes/ spermatids/sperm	2 (14%)	4 (100%)	
2^0 spermatocytes/ spermatids	12 (86%)	0	
Total Examined	**14**	**4**	
Ovary	**Birchy Cove (1994)**	**Summerside (1994)**	**Gillams (1994)**
Prespawn	14 (100%)	12 (100%)	5 (50%)
Spawn/post-spawn	0	0	5 (50%)
Total Examined	**14**	**12**	**10**
Testes	**Birchy Cove (1994)**	**Summerside (1994)**	**Gillams (1994)**
Sperm	2 (40%)	0	6 (75%)
Spermatids/Sperm	3 (60%)	4 (100%)	2 (25%)
Total Examined	**5**	**4**	**8**
Pooled Total Examined	**19**	**16**	**18**

Figure 14. Cross section of the ovary of cunner, *Tautogolabrus adspersus*, stained with haematoxylin and eosin to illustrate differential morphology. (A) Cross section of Birchy Cove ovary showing perinuclear, vitellogenic ooycytes. (B) Cross section of Gillams ovary showing post-ovulatory oocytes. (Scale bar = 500 μm).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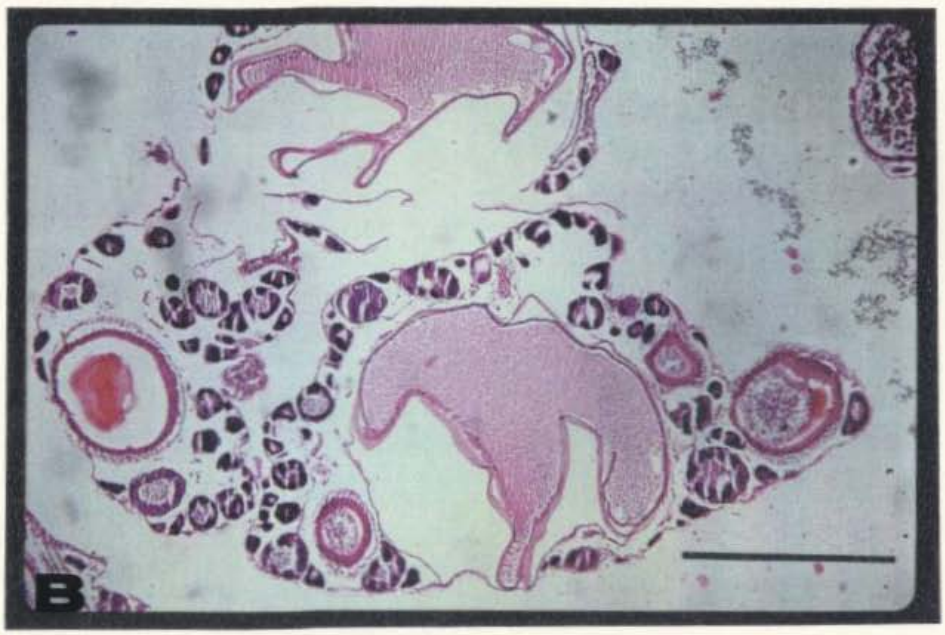
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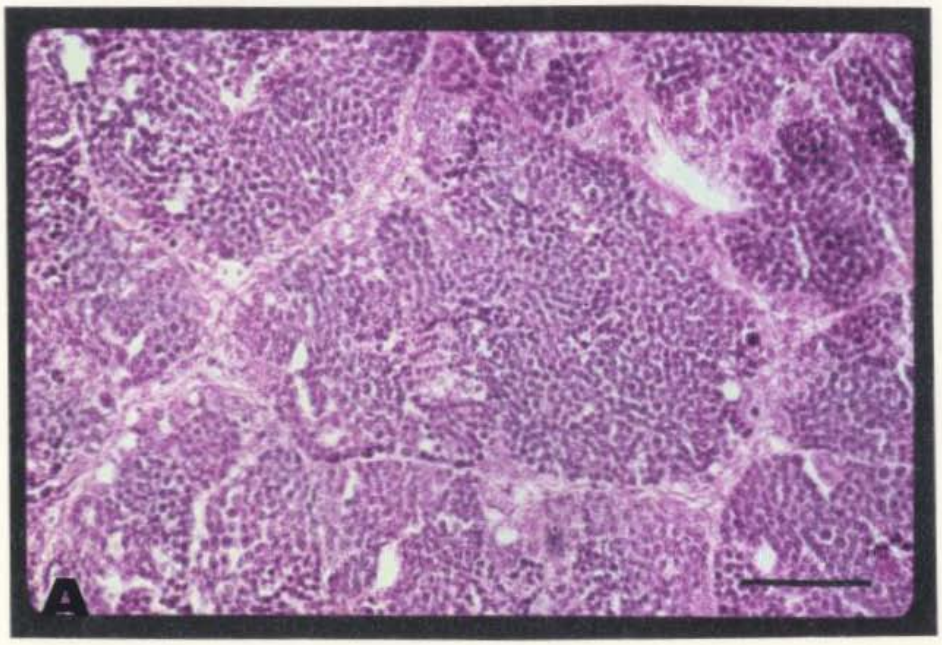
Figure 15. Cross section of the testis of cunner, *Tautogolabrus adspersus*, stained with haematoxylin and eosin to illustrate differential morphology. (A) Cross section of Birchy Cove testis containing primary and secondary spermatocytes. (B) Cross section of Gillams testis demonstrating mature sperm. (Scale bar = 250 μm).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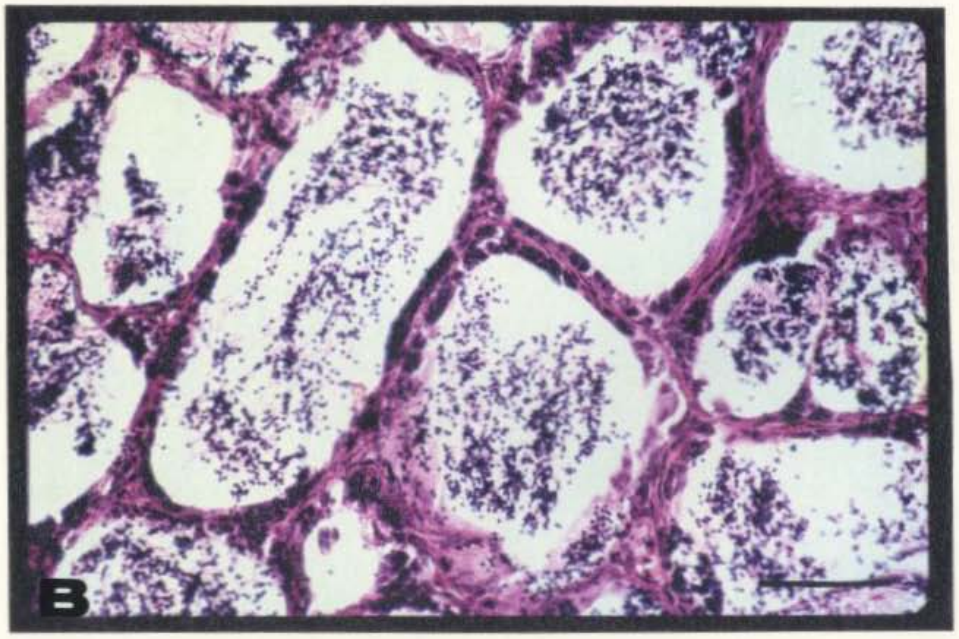
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in 89% (n=17), and 100% (n=16) and Birchy Cove and Summerside gonads,respectively (**Table 10.**) compared to Gillams' fish.

This paralleled field observations of a majority of fish at impact sites (Birchy Cove and Summerside) to possess gravid/ripe gonads that had not yet spawned, in contrast to a spent majority of reference cunner.

V. Splenic Melanomacrophage Quantification

Digital image analysis of splenic tissue sections visualized bythe Perl's Prussian Blue reaction indicated numerical differences in percent haemosiderin (as a percentage of non-symptomatic cellular architecture) among 1993-1994 field season venues.

In 1993, Birchy Cove had a higher percent of pigment deposition (n=15.24 ± 1.20, **Table 11.; Figure 16.**) compared to Summerside splenic analysis (n=12.14 ± 1.14, **Table 11.; Figure 16.**), but the difference was non-significant (**Table 11.**).

In 1994, percent haemosiderin was numerically greatest at the Birchy Cove site (n=14.73 ± 1.60), followed by reference and Summerside samples, respectively(n=11.98 ± 0.75 and n=10.12 ± 1.36; **Figure 19**; **Figure 17.**). In 1994, Summerside percent haemosiderin was significantly less than Birchy Cove sections (**Table 11.**).

In summary, percent splenic hemosiderin at impact sites was not significantly different from Gillams' tissue. Sex and year differences within sites were non-significant.

Table 11. Digital image quantification analysis of percent splenic haemosiderin (of non-pigmented tissue) in sample sections (Perl's Prussian Blue method) of *Tautogolabrus adspersus* collected from Birchy Cove, Summerside and Gillams, for the 1993-1994 field collections.

YEAR	SITE			Significance
	Birchy Cove	Summerside	Gillams	
1993	15.24 ± 1.20	12.14 ± 1.14		ns
1994	14.73 ± 1.60	10.12 ± 0.75	11.98 ± 1.36	ns

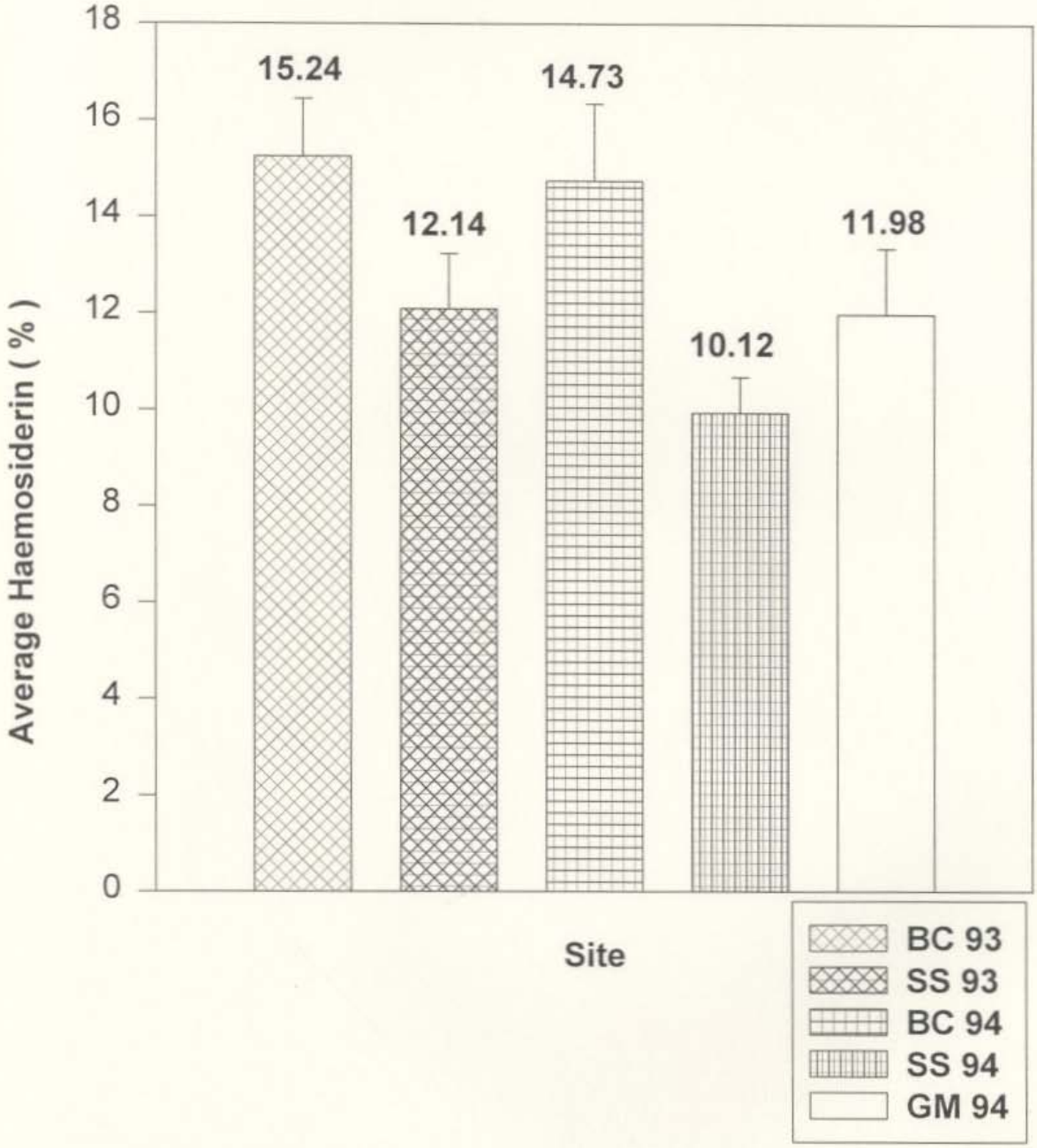
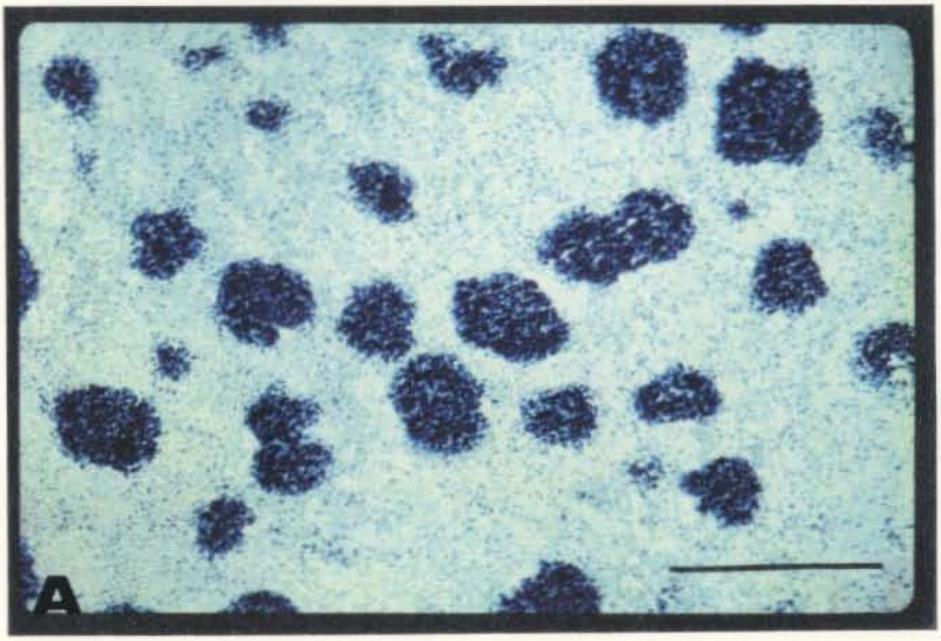


Figure 16. Average haemosiderin (%) for Birchy Cove (BC), Summerside (SS) and Gillams (GM) in sections of the spleen of cunner (1993-1994).

Figure 17. Cross section of the spleen of cunner, *Tautogolabrus adspersus*, (Perl's Prssian Blue method) to illustrate differential morphology. (A) Cross section of Birchy Cove spleen showing excessive deposition of the pigment. (B) Cross section of Gillam spleen demonstrating typical haemosiderin deposits. (Scale bar = 500 μm).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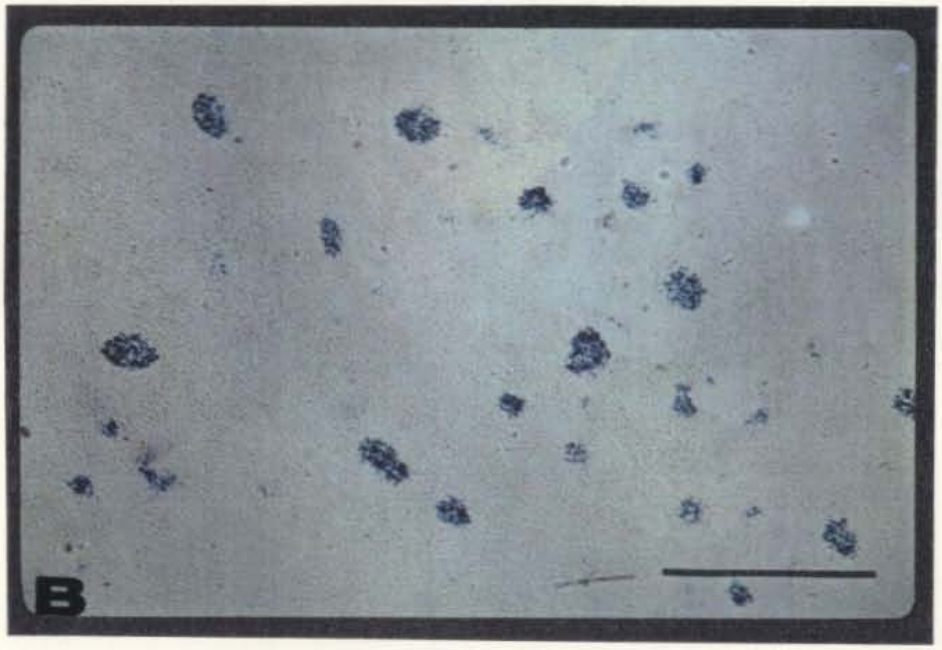

B

VI. Endoparasitofaunal Profiles

In 1993, intestinal analyses of Birchy Cove and Summerside identified only two helminth groups: cestodes and nematodes. A total of 18 and 16 Cestoidea were preserved from Birchy Cove and Summerside gut exudates, respectively (**Table 12.**). A single nematode species was exclusive to Summerside dissections, for a total enteric count of 18 parasites at Birchy Cove and 17 helminths at Summerside in 1993. Site differences in enteric fauna were non-significant.

Endoparasitic profiles in 1994 demonstrated an increase in total enteric intensity at impact sites from 1993, with the exception of the cestodes which decreased in number at impact sites (**Table 12.**). Cestode and nematode profiles were not site significant (**Table 12.**). Trematodes were significantly greater ($p=0.02$; **Table 12.**) in Birchy Cove guts than reference tissue. A total of 104 acanthacephalans were counted in the Gillams' sample. These were easily distinguished by their characteristic anterior proboscis, therefore identification was accurate. Acanthacephalan profiles at the reference site were statistically greater ($p < 0.001$; **Table 12.**) in intensity than at impact sites. Total enteric counts for the 1994 season were 26 at Birchy Cove, 11 at Summerside and 118 at the reference site (**Figure 18.**).

Table 12. Intestinal parasite frequency (number of helminths per sample) and taxon distribution for *Tautogolabrus adspersus* collecte

Helminth Taxon	Site		
	Birchy Cove (1993)	Summerside (1993)	
Cestoda	18	16	
Nematoda	0	1	
Total	18	17	
Helminth Taxon	Birchy Cove (1994)	Summerside (1994)	Gillams (1994)
Cestoda	4	3	0 ns
Nematoda	1	5	7
Trematoda	21	2	7 d
Acanthacephala	0	1	104 c,e
Total	26	11	118

c Birchy Cove significantly different from Gillams, $p < 0.001$

d Birchy Cove significantly different from Gillam $0.001 < p < 0.05$

e Summerside significantly different from Gillams, $p < 0.001$

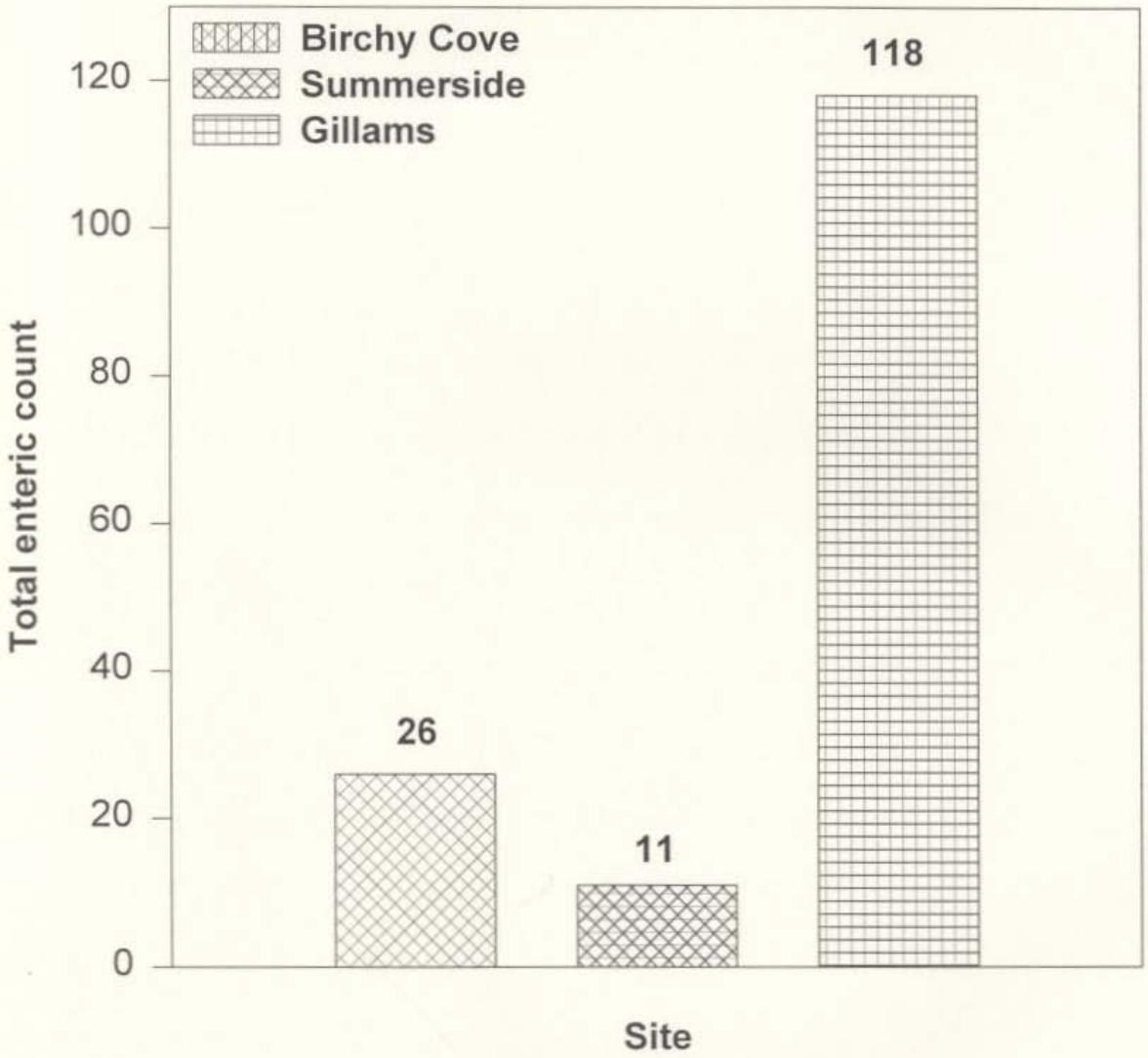


Figure 18. Total enteric parasite frequencies collected from *Tautogolabrus adspersus* at Birchy Cove (BC), Summerside (SS) and Gillams (GM) intestinal profiles, 1994.

VII. Otolith Aging

The average age of Birchy Cove residents was determined to be 6.71 ± 0.4 years, 7.44 ± 0.5 years at Summerside and 9.31 ± 0.5 years at Gillams (**Figure 19.**) Mean age at the control site deviated significantly from that at both Birchy Cove and Summerside stations ($p < 0.001$; **Table 13.**). Overall, 1994 collections demonstrated a significantly older population at Gillams by 2.6 and 1.87 years compared to Birchy Cove and Summerside, respectively.

Regressions of age against eviscerated (dressed) weight and length were generated to examine the dependence of cunner chronology on morphological parameters. As expected impact slopes of regression lines (for both body weight and length) were significantly different ($p < 0.0001$) from that of reference cohorts, reflecting Kruskal-Wallis age analyses. Only the reference population demonstrated any significant relationship between age and dressed weight ($p=0.0002$, $r=0.64$; **Figure 20.**) or length ($p=0.0003$, $r=0.68$; **Figure 21.**).

Site differences in ages of males and females were not statistically significant.

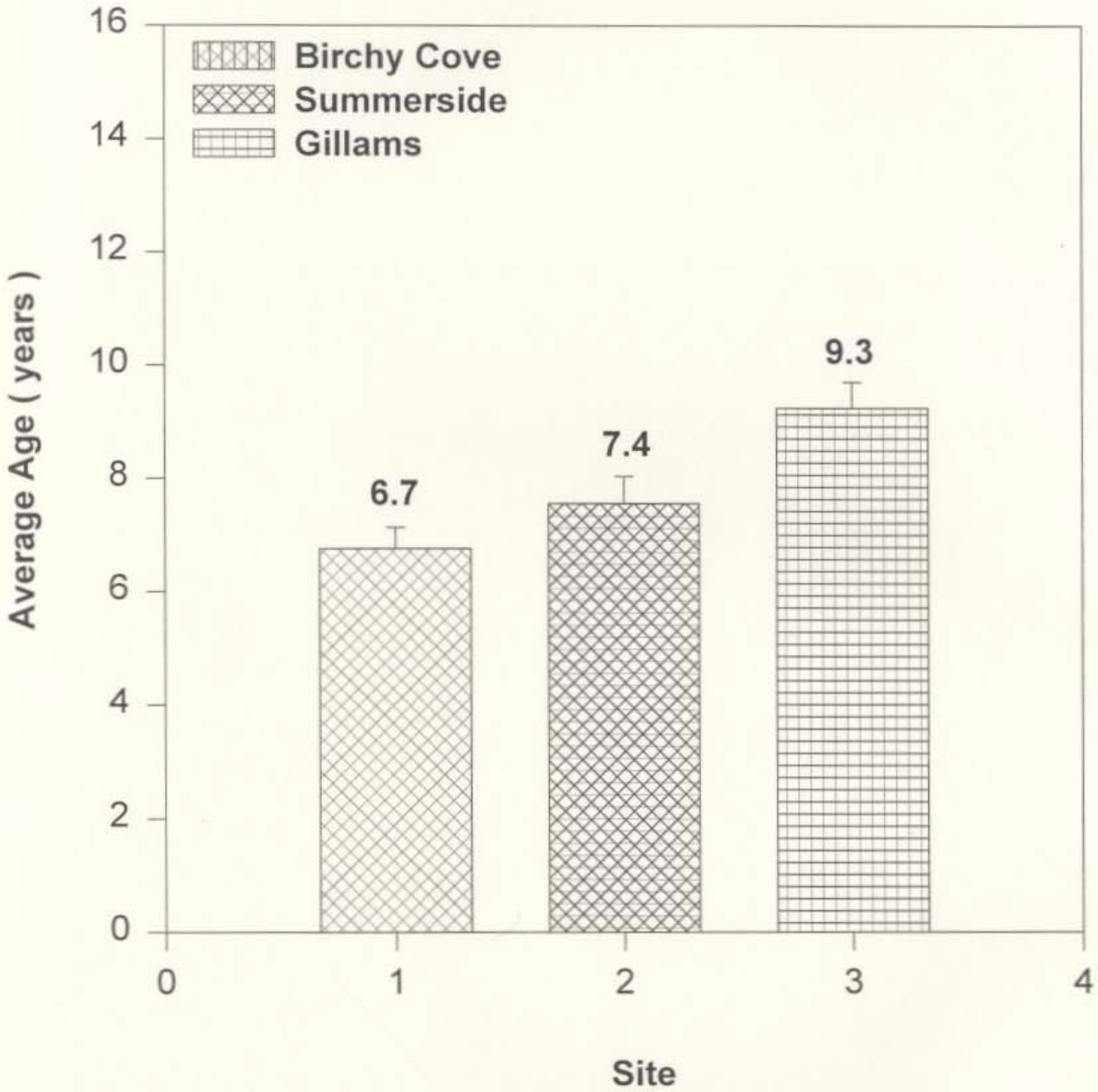


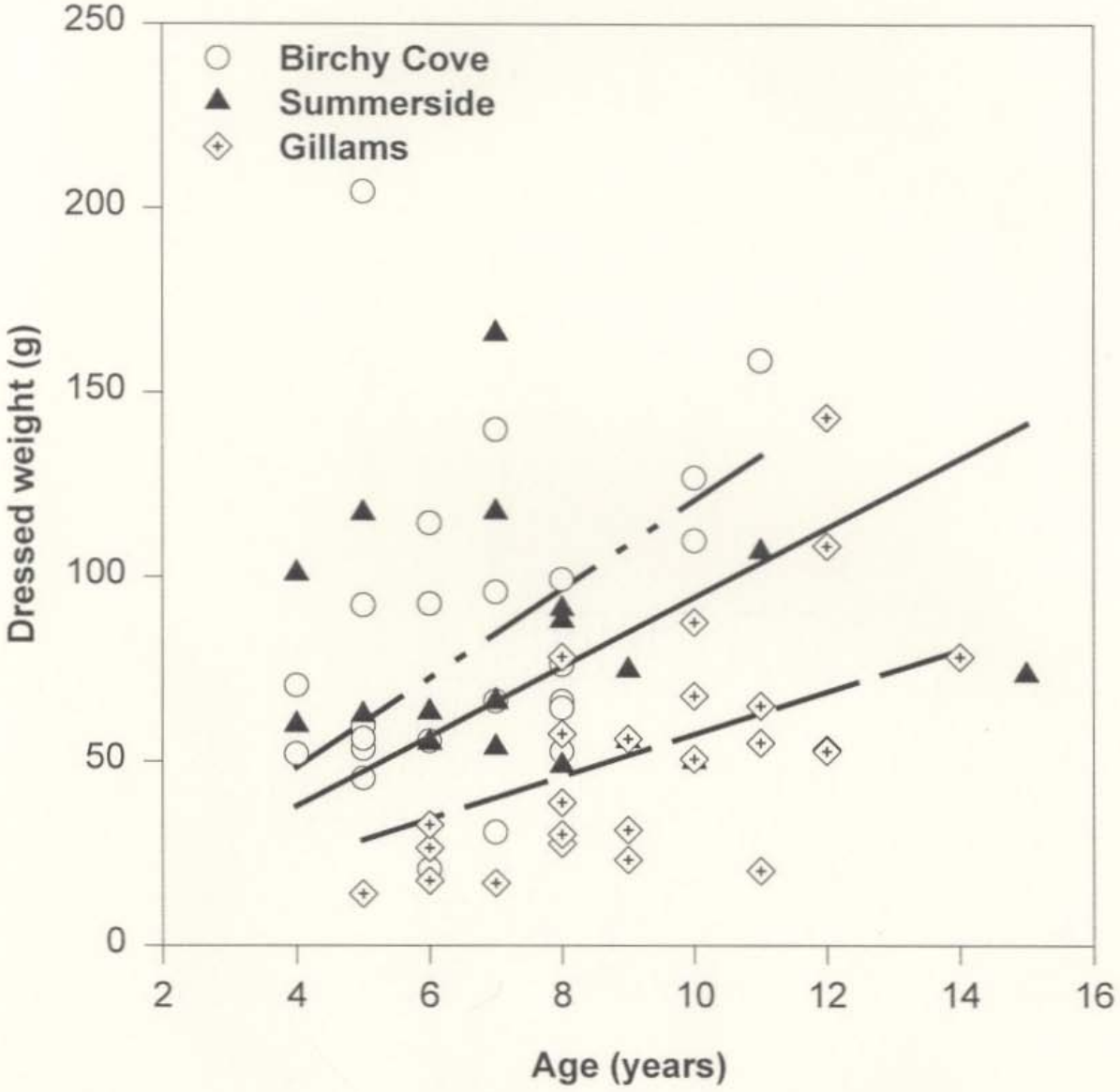
Figure 19. Chronological Summary of aged otoliths sampled from Birchy Cove (BC), Summerside (SS) and Gillams' (GM) populations, 1994.

Table 13. Average otolith age (in years) of cunner, *Tautogolabrus adspersus*, for Birchy Cove, Summerside and Gillams samples, 1995.

SITE		
Birchy Cove (n=2)	Summerside (n=25)	Gillams (n=25)
6.71 ± 0.39	7.44 ± 0.46	9.31 ± 0.45 d,f

d **Birchy Cove significantly different from Gillams, $0.001 < p < 0.05$**

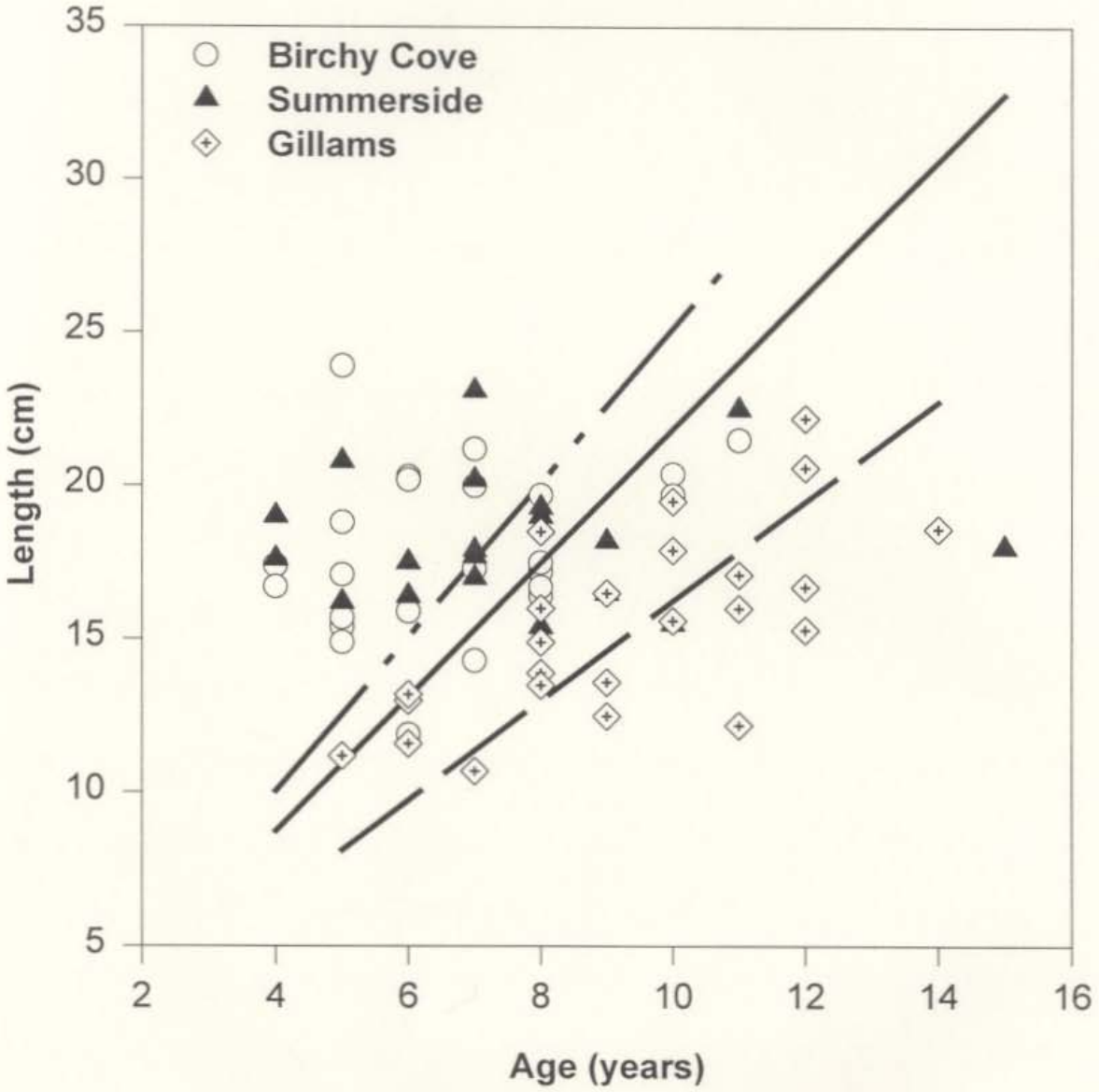
f **Summerside significantly different from Gillams, $0.001 < p < 0.05$**



BC:Dressed weight=2.52age + 22.86, p=0.12, r=0.33
SS:Dressed weight=6.22 - 0.64age, p=0.77, r=0.07
GM:Dressed weight=8.62age -28.40, p=0.001, r=0.64

Regression over groups:BC vs GM, p=0.0002
Regression over groups:SS vs GM, p=0.0002

Figure 20. Age vs dressed weight regression analyses for Birchy Cove (BC), Summerside (SS) and Gillams (GM), 1994.



BC:Length=0.24 age + 16.72, p=0.49, r = 0.15
SS: Length=0.02 age + 18.12, p=0.91, r = 0.03
GM:Length=0.88 age + 7.27, p=0.0003, r=0.68

Regression over groups:BC vs GM, p=0.00003
Regression over groups:SS vs GM, p=0.00001

Figure 21. Age vs length regression analyses for Birchy Cove (BC), Summerside (SS) and Gillams (GM), 1994.

VIII. Mixed Function Oxygenase (7 Ethoxyresorufin O-Deethylase) Activity.

Hepatic assays of 7 ethoxyresorufin O-deethylase induction illustrated mean specific activity to be highest in pooled Summerside liver homogenates (n=0.060 ± 0.03 nm/mg/min), followed by samples from Birchy Cove (n=0.054 ± 0.02 nm/mg/min)and Gillams (n=0.020 ± 0.005 nm/mg/min; **Figure 22.**). Specific activity of control liver samples (pooled and female) was significantly less than Birchy Cove cohorts (Kruskal-Wallis, p=0.01; **Table 14.**). This pattern was reflected in female specific activity at the three sites, however, male induction was greatest at Birchy Cove (n=0.132 ± 0.05 nm/mg/min) followed by Summerside (n=0.053 ± 0.01 nm/mg/min) and Gillams (n=0.027 ± 0.003 nm/mg/min; **Table 14.**). Within-site significance of sex differences was restricted to Birchy Cove; male specific activity was statistically greater (p=0.004;) than that of females. Male and female site differences were non-significant.

Liver size did not correlate with EROD induction at any of the three sites assayed.

A linear standard curve for protein concentration versus absorbance (read at 620nm) produced from bovine serum albumin (**BSA**) standards is depicted in **Figure A.1.** of the **Appendix**.

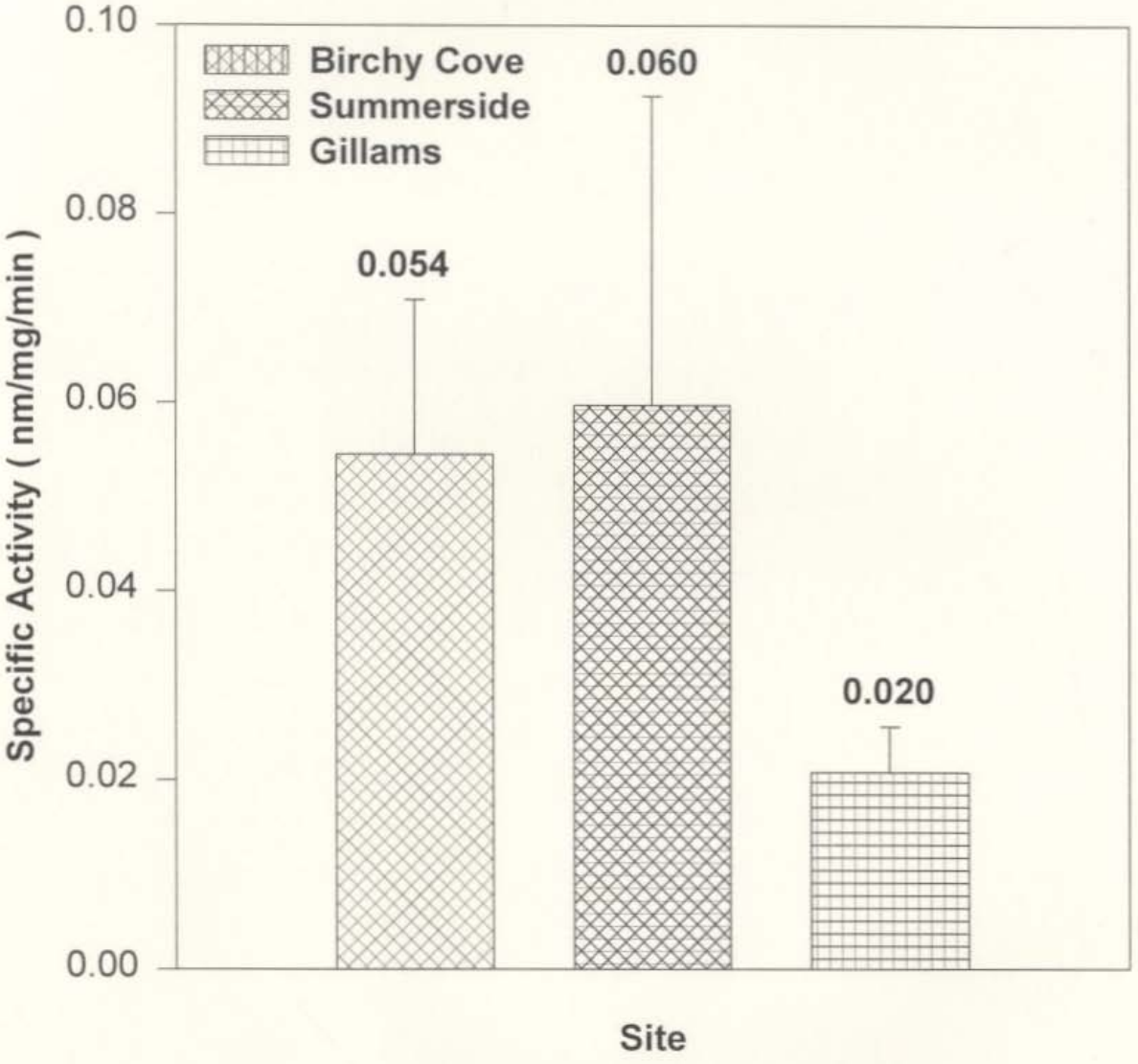


Figure 22. EROD (7 Ethoxyresorufin O-deethylase) specific activity (nm/mg/min) of pooled Birchy Cove (BC), Summerside (SS) and Gillams (GM) liver homogenates, 1995.

Table 14. Average Mixed Function Oxidase (7 Ethoxyresorufin O-deethylase) specific activity (nm/mg/min) in pooled, male (♂) and female (♀) cunner, *Tautogolabrus adspersus*, liver homogenates, 1995.

SITE		
Birchy Cove (n=24)	**Summerside (n=24)**	**Gillams (n=23)**
0.054 ± 0.02	0.060 ± 0.03	0.020 ± 0.005 d
(♂) 0.132 ± 0.05	(♂) 0.053 ± 0.01	(♂) 0.027 ± 0.002 ns
(♀) 0.027 ± 0.006	(♀) 0.063± 0.05	(♀) 0.020 ± 0.005 ns

d Birchy Cove significantly different from Gillams, $0.001 < p < 0.05$

Discussion

I. Morphological Statistics

The cunner is a seasonally abundant inhabitant of shallow, inshore Newfoundland waters, with the exception of the Northern Peninsula's northeastern coast (B. Hooper, personal communication; Green and Farwell, 1971; Scott and Scott, 1988). Interpretation of the results generated in the current study assumed torpid and non-migratory behaviours in this species. Prior to 1971, controversy persisted as to the winter habits of this temperate representative of the labrid family; however, certain Newfoundland studies have indicated that cunners enter a depressed metabolic state in November or December and remain inactive until May or June of the following year (Green and Farwell, 1971; Green, 1975; Valerio *et al.*, 1989). This quiescent condition usually coincides with water temperatures below 5^{0} C (Green and Farwell, 1971). The cunner's behaviour and physiological profile during the winter months can be interpreted as survival mechanisms in the face of freezing conditions. Since ice contact is potentially fatal to fish in a supercooled state, cunners tend to group under rock formations to reduce the possibility of freezing. Cessation of feeding activity and metabolic torpor translates into a decreased need for oxygen. As such, ventilation across the gill lamellae replete with ice crystals would be minimized under conditions of lowered respiratory rates (Valerio *et al.*, 1989). Secretion of copious quantities of mucous provides a physical barrier between frozen water and epithelial tissue fluids (Valerio *et al.*, 1989). Group aggregation, absence of food in the intestinal tract and viscosity of mucopolysaccharide secretions (i.e., evidence of torpor) were observed by SCUBA and during necropsy procedures in the current study indicating that fish were baited soon after torpor emergence.

A temporal delay in cunner activity among field sites was particularly noticeable during the

principal collection season (1994). Netting at Birchy Cove proved initially successful in mid-June followed by Summerside and Gillams, respectively, with procurement of cunners at the latter two sites extending into late July/early August. Asynchronous activity (i.e., feeding behaviour) among sites may be related to population density and/or site habitat. Itsappeared that cunner were in greater abundance at Birchy Cove than at Summerside or Gillams and may explain early success in netting quotas at the southern arm station. Bottom substrate in the vicinity of the man-made wharf at Gillams was more sandy and even-textured relative to the other two sites sampled in the estuary and may partially explain the difficulty and lag in netting fish at this site since cunner tend to aggregate about submerged structures and/or rocky bottoms (Scott and Scott, 1988). In addition, heavy boat traffic and gill netting at the control site may have contributed to offshore displacement of resident populations observed at Gillams (R. Billiard, personal communication). However, inthe absence of density studies and comprehensive SCUBA investigation, discussion of delays in netting between sites is limited in this thesis.

Sampling in 1993 and 1994 demonstrated a decrease in pooled body lengths and dressed weights in order of initial trapping dates. In 1993, total body lengths and eviscerated weights at Birchy Cove were significantly greater than at Summerside. In 1994, the reference site was characterized by smaller lengths and dressed weights than at Birchy Cove and Summerside. Thus, a distinct population shift in size dass frequencies between impact and reference sites was observed in 1993 and 1994. In 1995, the slight departure intrend for both lengths and eviscerated weights, as well as, site non-significance of these parameters may be due to small sample size. Total length ranges were similar to that of other studies (Johansen, 1925; Naidu, 1966) with no comparative reports in the literature with respect to the eviscerated or body weight spectrum.

In 1993, length-dressed weight regressions were not statistically significant between impact sites. Significantly decreased growth rates were observed at the reference site compared to both impact sites in 1994. Slower growth rates in Birchy Cove females relative to male counterparts in 1994 may reflect observed trends for the latter sex to have longer age specific lengths than females (Chiasson, 1995), especially in light of strong positive correlation coefficients between body length and size. However, this was the only instance of sexually dimorphism with respect to growth rate and can not be generalized to other sites or seasons. This trend was replicated in 1995's least squares linear regression. Seasonal site differences were shown to be variable with respect to significance, however, based on field directives it could be anticipated that annual differences might exist - a small collection might have a greater probability of having larger individuals, for example. This scenario might play itself out if low quotas and short netting periods were characterized by larger individuals outcompeting smaller counterparts for access to bait. Sample sizes in 1993 and 1995 were small ($20 < n < 25$) due to respective objectives (as outlined in the material and methods section), thus seasonal disparity was not unexpected.

Growth can be considered one of the most conclusive bioindicators of fish health and body condition because, as a parameter, it incorporates both biotic and abiotic factors and quantitatively reflects secondary manifestations of prolonged or chronic stress (Goede and Barton, 1990). A battery of bleached kraft mill effluent (**BKME**) studies originating from Sweden also reported increased juvenile and adult growth rates in perch (***Perca fluviatilis***) proximal to mill discharges (Sandström, 1987; Sandström *et al.*, 1988; Södergren, 1989). Munkittrick *et al.* (1994) was the first study to illustrate increased growth rates in female white sucker (***Catostomus commersoni***) adjacent to a themomechanical/sulphite mill in Kapuskasing, Kenora. Owens (1991) reviewed several theories

relating to the relationship observed between BKME and stimulated fish growth. In summary, it has been postulated that : (a) growth is inversely related to lowpopulation densities (LeCren *et al.*, 1977; Thorpe, 1977; Hansson, 1985), (b) exaggerated growth is induced by eutrophic conditions (Hartmann, 1978; 1982; Hansson and Westin, 1985), and (c) increased rate of growth is due to reduction of reproductive output in some individuals. While the first hypothesis does not seem applicable to the current study since general observations suggested considerable population numbers at both exposed sites, the latter two theories may be more relevant. Cunner are omnivorous scavengers and aggregate in areas where sufficient food resources exist or the potential for accretion would be increased (Olla *et al.*, 1975). The trap sites at Birchy Cove and Summerside were characterized by intense recreational fishery, disposal of fish offal from adjacent processing plants and a high frequency of refuse dumping (i.e. sewage outfall and community garbage) in the sampling area, all of which can augment food resources. Ledrew and Bennett (1988, 1989) reported an elevated organic:inorganic carbon capacity in a gradient towards the pulp mill discharge from the mouth of the Humber Arm (1:1) toward the north side(2:1, 3:1), the centre (3;1, 4:1) and toward the south side (4:1, 5:1). Samples taken directly adjacent to the mil reported ratios of 28:1 and sediment phosphorus levels exceeded Environment Canada guidelines. This may indicate opportunity for increased secondary productivity (heterotrophy) at impact sites compared to Gillams (R. Knoechel, personal communication; Hodson *et al.*, 1992). Since cunner populations are characteristically shallow and inshore (maximum trap depth was approximately 4 metres) adverse consequences of estuarine over-enrichment, i.e. anoxic fish kills etc, would be avoided. However, since feeding behaviour in the cunner ceases during its torpid state (approximately 7-8 months), it may be unrealistic to attribute growth acceleration entirely to increased feeding (Lehtinen, 1990). In

contrast, Adams *et al.* (1992) observed **decreased growth** in conjunction with **MFO** induction in redbreast sunfish (***Lepomis auritis***) downstream of polychlorinated biphenyls (**PCBs**) and mercury-contaminated industrial outfall in conjunction with low lipid levels. This growth disruption was also observed in a study of white sucker (***Catostomus commersoni***) populations adjacent to a bleached kraft mill receiving primary treatment (McMaster *et al.*, 1991). Reproductive efficiency may be affected in impacted populations in the Humber Arm and could explain enhanced growth as a result of increased energetic commitments to somatic processes. Lehtinen (1990) concluded that secondary stimulation effects on fish growth adjacent a **BKME** outfall were the consequence of a chronically-induced detoxification system (**MFO**) effecting steroidogenic hormonal imbalances. Significant induction of hepatic enzymes was observed at Birchy Cove compared to reference livers; however, correlations between steroid levels and **MFO** activity have proven to be ambiguous with regards to literature reviews. Nevertheless, differential growth rates in cunner exposed to non-chlorinated wastewater suggest that chlorinated compounds are not necessarily responsible for toxicity in fish adjacent to pulp mills.

Growth patterns observed in exposed fish may indicate a pathological disruption to normal lipid and protein metabolism. " Bigger" fish at both impact sites should not be misinterpreted as older populations. On the contrary, cunners at the control site were older than their Birchy Cove and Summerside counterparts based on otolith aging of 1994 samples. Indeed, control fish were the only sample to exhibit a significant and positive correlation between morphological data used to derive growth rate equations (eviscerated weight and total body length) and age. This is further evidence for conclusions by this authour that significantly greater growth rates at impact sites is not a chronological, but perhaps an exposure, effect. The absence of chronological data in the literature

can lead to false conclusions regarding sexual maturity of stressed and healthy fish populations if merely based on morphometric data. In contrast, Munkittrick *et al.* (1992) did observe considerably older male and female longnose sucker (***Catostomus catostomus***) at BKME sites. Older and reproductively impaired sucker at **BKME** sites suggested a delayed age to sexual maturity.

Overall, sex ratios illustated female dominance at all three sites and were reflected in length frequency histograms (1993-1995). In 1994, although numerically disproportionate, males represented the same range of intervals as did females. This was not the case in 1993 or 1995 and may reflect their small sample sizes. Gender inequality of collections suggested male-established territorial behaviour following emergence from winter torpor (Pottle and Green, 1979b). Male-defended territories were confirmed in this system by SCUBA observations.

II. Body and Organismic Index Parameters

The condition, or K-Factor, is one of the most common condition-related indices employed in stress assessment of fish (Goede and Barton, 1990). Quantitative departures from baseline or reference index values are usually interpreted as an interruption of energy metabolism, such as, mobilization of hepatic glycogen or somatic lipid reservoirs.

Both 1993 and 1994 necropsy results illustrated an increase in pooled condition factors in a gradient towards the pulp mill's outfalls. This was based upon the assumption that Summerside represented a transitional impact zone between Birchy Cove and the reference population at Gillams as evidenced in plume delineation models conducted for this mill site in 1995. Increased K-factors at Birchy Cove and Summerside reflected a similar trend in differential growth rates discussed in the preceding section. This pattern was expected since this length-weight relationship (estimated from regression of these variables) is similiar to condition factor (estimated from the ratio of these variables) (LeCren, 1951; Carlander, 1969; Everhart and Youngs, 1981). Site differences in condition factors (1993-1995), however, were statistically non-significant in contrast to significant differences in growth rates between impact site(s) and Gillams for all three field seasons. This also seems to contradict significantly greater body length and eviscerated weight results at impact site(s) in 1993-1994. Khan *et al.* (1996) also reported non-significant differences in K-factors between sites (Birchy Cove and Summerside) in the Humber Arm and a reference location at Norris Point ($40^{0}32'$ N, $57^{0}52'$ W). Hodson *et al.* (1992) observed non-significant deviations in condition factor downstream of a BKME plant, but with significantly greater lipid levels of whole fish homogenates at all sites compared to reference samples. This apparent contradiction between lipid estimates and K-factors suggests a disturbance in energy metabolism, possibly an interruption in somatic storage

(Hodson *et al.*, 1992). Non-significant differences between effluent-exposed and control k-factors in fish have also been reported in other studies (Barker *et al.*, 1994; Khan *et al.*, 1996). Lower condition indices were reported in winter flounder (***Pleuronectes americanus***) exposed to sulphite-treated effluent concomitant with non-statistical differences in length-weight regression between stressed and reference populations (Barker *et al.*, 1990). Perch (***Perca fluviatilis***) populations downstream from a bleached kraft mill in Norrsundet, Sweden exhibited both increased K-factor and stimulated growth (Owens, 1991).These results suggest a positive correlation between length : weight ratios (K-factors) and rate of fish growth adjacent to pulp mill discharges. In contrast, McMaster *et al.* (1991) attributed increased condition factors and slower growth rates to a disruption in metabolic efficiency and modified energy in white suckers exposed to**BKME**. It may be plausible that fish collected post-torpor would not reflect increased rate of growth with respect to body condition early in the active season. Chiasson (1995) demonstrated that torpor or winter development was comparatively slow compared to that of summer growth based on otolith ring zonation.

Somatic value trends in 1995 were somewhat ambiguous, with Gillams numerically intermediate in several pooled/gender indices (with the exception of the visceral-SI, testicular index and male spleno-SI) between Birchy Cove and Summerside. This deviation from trends observed in previous field seasons may be attributed to small sample sizes obtained in one netting effort at the reference site in 1995. Therefore, one could be looking at a non-representative sample of this population regarding length and dressed rate frequencies (i.e., in the upper limits of this species' range). This may explain a significant increase in several body parameters from 1994. Extensive sampling during this field season suggests that trends observed in 1994 may be more accurate to

interpretation of site pathology.

The gonadosomatic index, or **GSI**, is utilized as a morphological indication of sexual status and maturation in fish populations; specifically, higher GSI values are usually characteristic of mature individuals preparing to spawn or spermiate (Goede and Barton, 1990). Preliminary field work illustrated negligible site differences in male or female gonadosomatic indices; however, gonad weights at Gillams were significantly smaller in value than at impacted sites in 1994, indicative of differential maturity. In 1995, this numerical trend was applicable to meantesticular weight (%) with average ovarian values at the reference site intermediate between Birchy Cove and Summerside, respectively, however, there were no significant differences in GSI values among sites. Site non-significance in 1995 may be attributed to significantly larger-sized individuals collected at the Gillams' reference station compared to the previous season. Field collections in 1995 (targeted for assay of mixed function oxygenases) were designed to sample cunnerat all three sites as soon as they were responsive to bait. This was meant to avoid thepeak spawning season and potential depression of EROD induction, particularly in female cunner populations (Porter*et al.*, 1989). Compared to 1994, then, significantly greater GSI values in 1995 could merely be the result of collections taken during that period of maturation characterized by peak gonadosomatic indices (Walton *et al.*, 1983).

Fish at all sites (1993-1995) were considerably larger than the 8 to 11 cm range cited as a morphometric criterion of cunner sexual maturity (Johansen, 1925). This might infer a delay in spawning rather than persistence of juvenile stage, i.e. sexually immaturity, or suspension of reproductive development (Barker *et al.*, 1994). Observations during necropsy procedures (1993-1994) were indicative of sexual maturity at both impact sites (i.e., enlarged gonads) that had not spawned or spermiated at the time of collection, in comparison to a spent majority of reference

cunner (hence, lower mean GSI values). In general, necropsy observations were consistent with scatterplots of GSI (by sex) versus length (1993-1994). Fish collected at impact sites during the reproductive season were younger than effluent-exposed fish. Otolith and GSI results, then, are contradictory with several studies reporting a delayed age to reproduction at **BKME** sites rather, than, an interruption to spawning behaviour (McMaster *et al.*, 1991; Adams *et al.*, 1992; Munkittrick *et al.*, 1992; Gagnon *et al.*, 1994; Kloepper-Sams *et al.*, 1994; Munkittrick *et al.*, 1994; van den Heuvel *et al.*, 1994).

Parenchymal cells of hepatic tissue mediate several crucial physiological functions such as intermediate metabolism of proteins, carbohydrate and lipid, synthesis of plasma proteins and steroidogenic hormones, and biliary secretory processes (Roberts, 1989). The liver's role in detoxification of endogenous waste products, as well as environmentally-derived xenobiotic contaminants, is paramount. Since a diverse range of stimuli can adversely affect this organ and because of its numerous metabolic functions, the ratio of liver weight to body weight (**hepatosomatic index or HSI**) is commonly utilized as a pathological indicator (Roberts, 1989).

In 1993, pooled hepato-SIs were significantly greater at Birchy Cove than at Summerside. Male and female reference HSIs were significantly less than Birchy Cove and than both impact sites with respect to the latter sex in 1994. In 1995, liver indices at Gillams were intermediate in value between Birchy Cove and Summerside, but again site differences were not statistically dissimilar. This trend of exaggerated liver somatic indices at polluted site(s) is consistent with a host of studies investigating morphological consequences of bleached mill effluents on fish populations (McMaster *et al.*, 1991; Adams *et al.*, 1992; Bucher *et al.*, 1992; Hodson *et al*, 1992; Munkittrick *et al.*, 1992; Kloepper-Sams *et al.*, 1994). Munkittrick *et al.* (1994) investigated receiving areas in close

proximity to a large number of Canadian pulp mills. They observed that liver size increases were most dramatic at mills that did not employ either the kraft process or chlorine as a bleaching agent. This reinforces a theme common to all of the bioindicator results in this study. Although the identification of the chemicals responsible for the observed biological activity is unknown, evidence of stress in cunner population adjacent to this non-chlorinated mill in Newfoundland implies that the chemical composition of the inducing compound(s) does not include organochlorides as a prerequisite. Reports of increased liver SI values concomitant with pulp mill discharges have been attributed to hyperplastic activity of hepatic smooth endoplasmic reticulum - a cellular symptom of atypical oxidative metabolism (Bucher *et al.*, 1992; Hodson *et al.*, 1992). The smooth endoplasmic reticulum (**SER**) is the site of action for both **Phase I - (oxidative transformation)** and **Phase II - (conjugation)** catalysts of xenobiotic detoxification (Bucher *et al.*, 1992). Activity of hepatic mixed function oxidases (7-ER) was assayed for 1995 liver samples only and based on somatic trend deviations compared to previous seasons it would be highly speculative to attribute organ exaggeration entirely to cellular elaboration (SER). Liver size increases could be due to disruption of parenchymal fat synthesis and/or export into systemic circulation, hence, hepatocytic accumulation of lipid. A sexual dimorphic trend pertaining to liver weight was also apparent for all three field seasons; specifically, females exhibited greater HSI values than male counterparts, albeit non-significant except for Summerside samples in 1994. Differences in HSI between males and females have been attributed to reproductive maturity and vitelbgenin synthesis in livers of the latter (Hodson *et al.*, 1992). Consistent with GSI data, hepatosomatic indices numerically increased from 1994 to 1995. Walton *et al.* (1983) observed that peak HSI values in female cunner were relatively synchronous with that of gonadosomatic SIs; thus hepatic exaggeration may be partially due to

vitellogenesis in the current study. Female recrudescence is characterized by oocyte accumulation of lipid and protein stores. Delahunty and de Vlaming (1980) demonstratedthat peak lipid stores preceded the height of ovarian maturation inferred mobilization of hepatic reserves during egg development. These authours concluded that the gonadosomatic index may not be an accurate indicator of reproductive activity and emphasized the importance of ovarian/testicular histology (Delahunty and de Vlaming, 1980).Although male liverswould not be expected to enlarge during the reproductive season, pooled indices would reflect female hepatic exaggerations due to sexual disproportionality of samples. In contrast, Barker *et al.* (1994) observed depreciated HSI values in effluent-exposed (sodium hyposulphite + 10% chlorination of pulp) winter flounder. However, HSI values in this study were pooled and excluded samples taken in spring (Barker, 1993). Seasonal HSIs demonstrated significantly greater values at effluent exposed sites in two of the four months sampled and site differences appear negligible when spring SIs are included. The authour attributed significantly greater HSIs to larger fish collected, however, greater body lengths and eviscerated weights in mill adjacent populations were characteristic of all sampling efforts and were not limited to months when the hepatosomatic index was elevated at the impact site. Therefore, seasonal fluctuations in HSI appear significant and it would appear beneficial to examine such variability. In certain field studies, a drop in HSI has been correlated with a reduction in liver glycogen during fasting or starvation. Goede and Barton's (1990) assertion that this may not be universal was substantiated in this study in which the indicator species (***T. adspersus***) were collected post-torpor. This torpid state lasts approximately 7 to 8 months and is characterized by total cessation of feeding behaviour. However, an accompanying metabolic dormancy could possibly curtail hepatic depletion of glycogen stores in the cunner.

Mean visceral somatic indices (**VSI**) were significantly greater at Birchy Cove than at Summerside in 1993. In 1994, pooled Gillams' samples recorded comparatively greater values than Birchy Cove. This trend invisceral indices, based on intestinal food volume or satiation, suggested increased food consumption (by weight) at the reference site (Barker *et al.*, 1994) during the principal field season. However, in 1995, site comparison of visceral weight (as a percentage of eviscerated weight) results were reversed. These findings suggest that there is no direct relationship between VSI and effluent exposure.

Site differences were non-significant in 1993, however, both male and female SSIs at the Summerside site were elevated compared to the reference gender counterparts in 1994. Male SSIs at Gillams were significantly smaller in value than that of either impact site in 1995. Immunosuppressed fish periodically display swollen spleens or **splenomegaly**; a morphological manifestation of leukocytic proliferation (Anderson, 1990) and thus elevated splenic somatic indices at impact site, particularly in 1994, may reflect this condition.

To reiterate the summary of SI results : based on intensive field sampling in 1994, somatic indices were significantly different at impact site(s) than in reference collections, with the exception of condition factors, which may be attributed to differential non-chlorinated effluent exposure.

Yearly site differences were variable within sample stations, however, it seems somewhat inappropriate to place much emphasis on inconsistent seasonal variability considering the directives of each field season. Preliminary collections in 1993 did demonstrate significant differences between both Birchy Cove and Summerside. This season (1993) also reflects major trends in 1994 pertaining to the transitional nature of Summerside between Birchy Cove and Gillams. This parallels physical

data reviewed in the materials and methods section of this thesis. Variation between this initial sample and 1994 can be attributed to comparatively smaller sample size of the former effort. The objective of sampling in 1995 was to acquire livers for assay of MFO and thus was carried out earlier in the season than 1994. Trend deviations were noted from previousyears and again, this may be attributed to small sample size and, particularly, timing of collections.

The underlying assumption when employing somatic indices for comparative or descriptive purposes is that there is no disproportionality regarding the relationship between size of fish and the actual somatic ratio. This appears to be species-specific and even intra-specifically speaking, the constancy of a particular index can be variable (Goede and Barton, 1990). This asymmetry may occur because the organs and body, as a whole, grow at different rates (Delahunty and de Vlaming, 1980). This is the rational in using the**ANCOVA** for statistical analysis of SIs - designating dressed or eviscerated weight (in the current study) as the covariate circumvents potential problems of differential growth rates between ratio variables. Significant and positive correlations of organ weight regressions against body size (in the majority of analyses) reflect that these ratio variables are not mutually exclusive.

III. Histopathology/Macroscopic Anomalies

Toxicopathic influences on biochemical and physiological mechanisms are eventually manifested as alterations to typical cellular and subcellular architecture. Therefore, histopathological changes may be expressed as a the culmination, or the net sum of, molecular irritations. If contaminant concentrations within a given population reach toxicity thresholds, sublethal injury to cellular structures can be induced, particularly in situations of chronic and persistent pollution (Hinton and Laurén, 1990). These structural modifications can be utilized as bioindicators of stress and can predict probable consequences at higher levels of biological organization.

A. Gill

Normally, the respiratory lamella the gill is bordered by an epithelial layer, two cell (squamous) layers in width (Mallatt, 1985). Internal to this epithelial tissue is the lamellar blood sinus, lined by contractile pillar cells. A marginal blood channel is sandwiched between endothelial cells and is located at the secondary filament apices (Mallatt, 1985). A thick, stratified epithelium lines the filament of each lamellar trough and is comprised of two cellular types : chloride and mucous cells (Mallatt, 1985).

Lamellar structures are multifaceted in that they mediate a diverse array of crucial physiological functions that include respiration, osmoregulation and nitrogenous waste excretion (Hinton and Laurén, 1990). The fragility of this system makesit particularly sensitive to toxic injury induced by dissolved or suspended xenobiotics introduced in a system (Roberts, 1989).

Slight hyperplasia of lamellar epithelium occurred more frequently in Summerside gills, whereas more extensive categories of cellular proliferation (i.e., moderate and excessive hypertrophy) were numerically more pronounced or restricted to Birchy Cove residents. This

pathological trend was consistently observed in both 1993 and 1994 histological surveys. Gill cytoarchitecture at the control site demonstrated comparatively negligible cellular deviations. Hyperplasia of undifferentiated cells of the gill and branchial alteration are, in general, a non-specific response reflecting a stereotyped physiological reaction or defense mechanism of fish to toxicant-induced stress. Lamellar hyperplasia is usually a response of malphigian cells to chronic, often less concentrated, exposure to irritants (Roberts, 1989), as would be expected with the historical discharge profile of the Corner Brook Pulp and Paper Mill. Couillard *et al.* (1988) observed increased prevalence and severity of the aforementioned lesions in fish exposed to sublethal concentrations of **BKME**. Cellular hypertrophy and hyperplasia were the most common gill lesions in winter flounder adjacent to sulphite pulp mill discharges (Khan *et al.*, 1994). Epithelial lifting, swelling and hyperplasia of the lamellar epithelium may serve as a mechanism of defense in fish exposed to waterborne contaminants since these morphological reconstructions act to increase the diffusion distance to the bloodstream (Mallatt, 1985). However, the trade-off may be a depreciation in respiratory efficiency particularly, if oxygen saturation is low.

Metacercaria infestations of the marine digenetic trematode, ***Cryptocotyle lingua***, were observed as pigmented cysts within the primary and secondary lamella. This was the only ectoparasite detected in the gill filaments and/or basal epithelium of cunner. Determination of larval frequency of the pectoral, pelvic and caudal fins was impractical because of heavy infections at all three sites, although the authour would recommend this technique in ectoparasitic assessment of other species, i.e. ***Pleuronectes americanus*** (Barker, 1993). Digenetic trematode species are characterized by the complexity of their life cycles, characterized by multiple (and sequential) larval generations, alternation of asexual and sexual phases, and a number of intermediate hosts preceding

maturation within its definitive host (Post, 1987). Eggs are fertilized more commonly in the intestinal tract, gall or urinary bladder of black-back gulls indigenous to the North Atlantic *(**Larus argentatus**)* and are ultimately voided in the faeces or urine. They are subsequently ingested by the first intermediate host, the common periwinkle (***Littorina littorea***; Sinderman, 1990). Within the digestive gland of the snail, fertilized eggs metamorphose into **rediae** and, ultimately, free-swimming **cercaria** are released and encyst as **metacercaria** within the tissues of the second intermediate host. A definitive host ingests the encysted larva along with the fish and the lifecycle is completed (Post, 1987). Thus, ***Cryptocotyle lingua*** infestations are more common in species which are inshore residents, at least for part of their lifecycle. This criterion for parasitization intensity would account for the degree and severity of integument digeneans at all sites since cunner establish permanent inshore territories. Therefore, lamellar infection of ***C. lingua*** may be more related to habitat than effluent concentration in the current study.

Site differences in lamellar digeneans were negligible and demonstrated no apparent relationship with effluent exposure. Population site estimates of periwinkles, in addition to experimental studies, might delineate if gill infections are a function of habitat or influenced by xenobiotic profiles.

Overstreet (1993) proposed that parasitic summaries be incorporated into the bioindicator approach, particularly since the multi-host life cycles of certain parasites can be exclusively symptomatic of certain toxicants, as well as specific to habitats and geographical ranges. A review of the applicability of parasites as pollution monitors by Khan and Thulin (1991) demonstrated that, in general, the prevalence and intensity of ectoparasites increased in pollutant-exposed fish. Barker *et al.* (1994) observed increased prevalence and intensity of ***C. lingua*** metacercariae in gill filaments

of sulphite-exposed winter flounder. Frequencies of gill ciliates were found to be higher in three-spined stickleback experimentally exposed to high effluent doses (Axelsson and Norrgren, 1991). In contrast, Kloepper-Sams *et al.* (1994) reported significant reductions in gill flukes exposed to chlorinated discharges. Hypothetically, ***Cryptocotyle*** load should temporally increase since excystment is completely dependent upon ingestion by the definitive host (Barker *et al.*, 1994). Thus, greater ***Cryptocotyle*** gill loads in reference fish (which were significantly older than exposed cohorts) in 1994 may be the result of longer exposure histories than that of younger, impacted fish. Resolution of this issue necessitates further sampling of the control site (and impact sites) for ectoparasitic enumeration since infection was recorded only once (1994) at Gillams. Observations of excessive proliferation of mucoid cells in polluted cunner compared to control gill sections would suggest a pathological response. Copious mucous secretions in fish are considered a defence mechanism in response to toxic irritation, and may provide an ideal environment for parasitic invasion (Roberts, 1989). Initial efforts suggest that quantification of mucoid cells could be successfully resolved by digital image analysis. This would facilitate accurate and objective reports of structural modifications to branchial tissue.

B. Liver

Parenchymatous hepatocytes of the teleost liver are cytologically grouped as tubules or cords (Roberts, 1989). In this unique arrangement, liver cells are basally directed toward sinusoids, and their tapered apices shape the wall of the bile canaliculi (Hinton and Laurén, 1990). Thus, deviation from typical hepatocyte and biliary epithelial cell structure can provide information on essential liver functions. Alterations in liver morphology can be used as bioindicators of a population's exposure history to environmental toxicants. An estimated 85% of liver volume in fish is occupied by

hepatocytes, the predominant cell type (Hinton and Laurén, 1990). Stressor-induced modification of liver cells can be manifested in the nucleus and/or cytoplasm (Hinton and Laurén, 1990).

A higher frequency of macroscopic, opaque foci at both Birchy Cove andSummerside (1993-1995) in contrast to virtually unblemished reference livers at Gillams was observed. Bucke and Feist (1993) observed that clear cell foci in dab (***Limanda limanda***) liver sections corresponded to small glassy nodules on the surface of the liver (similar to the opaque foci in the current study). Foci of clear cells in hepatocyte staining is often a symptom of tumour formation. Focal change in hepatocytic staining is a component of a collection of cytological alterations involved in tumour development (Hinton and Laurén, 1990). However, cellular necrosis, usually a concomitant effect of liver carcinoma, was demonstrated only twice in 1994 Birchy Cove sections and their were no indications of pre-neoplastic morphology (i.e., clearing or necrotic tissue) in anyof the histologic preparations. Hepatocellular necrosis was also reported to be a rarefinding in dab livers sampled from the North Sea (Bucke and Feist, 1993). Tumour-like masses were a rare macroscopic occurrence, but more frequent to Summerside hepatic sections. A firm diagnosis of hepatoma was not possible in the absence of electron microscopic analysis. Histopathological surveys of flatfish livers by Bucke and Feist (1993) also noted that mottled or "spotty" surfaces correlated to either depletion or repletion of storage substances in hepatocytes. Intra-cytoplasmic vacuoles denoting high lipid content (Bucke and Feist, 1993) were histologically observed at all sites. Hepatic cell tumours are recognized in the initial stages as whitish or yellowish nodules on the liver surface (Roberts, 1989). Histologically, these may appear to be comparatively more contoured and typical than adjacent tissue, which is characteristic of fatty hepatocytic inclusions in rapidly growing fish (Roberts, 1989). Therefore, seemingly "normal" liver cells of such individuals are vacuolated and

stain weakly (Roberts, 1989). Thus, there may be an underestimaton of pre-neoplasia in histological examinations, particularly since fish at impact sites exhibited faster rate of growth, due to interpretation of pale-stained tissue as exclusively due to high lipid content. More pronounced categories of hepatic vacuolation were restricted to Birchy Cove livers, a pattern that was consistent to both survey seasons (1993 and 1994). This parallels findings by Khan *et al.* (1994) who observed a progressive increase in focal vacuolation coincident with chronic exposure of flounder to sulphite pulp wastewaters. A disruption in parenchymal fat synthesis and its rate of export into systemic circulation may account for lipid accumulation by hepatocytes and increasesin hepatosomatic indices reported by several authours and observed in the present study (Andersson *et al.*, 1988; Axelsson and Norrgren, 1991; Owens, 1991; Bucher *et al.*, 1992; Kloepper - Sams *et al.*, 1994). Pigment aggregates or melanomacrophage centers (**MMCs**) were restricted to Birchy Cove and Summerside sites (1993-1994), and represent a by-product of effete red blood cell degradation that has been filtered from the lymphoid-macrophage system (Khan *et al.*, 1994). The liver, however, appears to be secondary to the spleen with respect to the extent of hameosiderin deposition. Review of the significance of melanomacrophage aggregates of the latter haemopoietic organ will follow.

Necropsied reference livers in 1995 demonstrated negligible external lesions. This substantiates the theory that appreciated somatic indices at Gillams compared to 1994 may be due to trapped specimens representing the upper end of the size range (> 18cm); thus, normal liver histology would be.

Seasonal variability of hepatic lesions within sites were noted with respect to spottiness and liver foci. This was particularly evident for the former criteria at the Birchy Cove site. Because livers exhibited such morpholological aberration in populations adjacent to themill, inexperience

in criteria recognition may have been a source of error. However, the author's working definition of opaque foci was easily observed at necropsy since these "necrotic" lesions are quite distinct. Thus, the frequency of these seasonal anomalies is reported with confidence by the authour. However, it is acknowledged that percentages of liver marks (spots) may have been initially reported with some degree of inaccuracy.

C. Gonad

In general, any pollutant-induced perturbation to homeostatic mechanisms that results in reproductive dysfunction can be employed as a bioindicator of sexual impairment. Reproductive development is a continuous process throughout ontogeny (Donaldson, 1990). It represents a progressive course of interrelated events that is subject to toxicant pressures at all life cycle phases, including fertilization, embryonic development, sexual differentiation, gametogenesis, final maturation, ovulation or spermiation, as well as, spawning behaviour (Donaldson, 1990). It is critical; therefore, to select reproductive indices that are appropriate to the particular life cycle stage potentially affected by an environmental.

In general, categories of gonadal histology demonstrated that the pre-spawn condition (both male and female) occurred more frequently at impact site(s) than evidence of more advanced development at Gillams. This cytological assessment was consistent with the higher frequency of gonads at impact sites with visible granulation (i.e., enlarged) versus those at the control site which appeared fully ripe (i.e., discharged eggs or sperm upon handling) or spent (1993-1995) hence, their lower GSI values (1994). Gonadal histology support hypotheses of impaired reproductive physiology in fish populations exposed to pulp mill effluent, particularly a non-chlorinated mode of action, in the current investigation. Such disruption may be more extensive than previous

suggestions (refer to discussion of GSIs) of a temporal delay in spawning behaviour. Although cunner were collected during the period of maximum spawning activity for this species, and exceeded minimum lengths required for sexual maturity, gonad architecture showed delayed gametogenesis at impact site(s) (Johansen, 1925; Scott and Scott, 1988). Cunner exhibit comparatively short or contracted reproductive cycles. Unlike other species, such as male winter flounder, which might mature as early as November/December but do not spawn until the following spring/summer, cunner usually undergo the stages of gonadal maturation, spawning and regression within the months of July and August (Walton *et al.*, 1983), i.e. post-torpor. Therefore, impact cohorts at Birchy Cove and Summerside may have been chronologically mature but were collected during initial stages of gametogenesis. According to maturity indices defined by Walton *et al.* (1983), fish at effluent-exposed sites were necropsied during early stages of maturation in contrast to spawning individuals at the reference site. In 1994, both Summerside and Gillams' sampling temporally overlapped each other and the former collection occurred later in the season compared to 1993. However, histologic comparison of Summerside 1993 and 1994 ovaries, for example, demonstrates that both seasons found 100% of their ovaries in the pre-spawn condition. It would appear then, that retarded oocyte development in impact fish is more than a function of collection timing and may be due to effluent exposure. There has been little investigation concerning the cunner within this geographical range, thus further studies documenting reproductive behaviour and physiology in the Humber Arm are warranted to delineate the impact of non-chlorinated effluent on this parameter.

Several authours have reported negligible differences in gross estimates of reproductive capacity of **BKME**-exposed fish (Khan *et al.*, 1994; Kloepper-Sams *et al.*, 1994). The results of

Hodson *et al.* (1992) on **BKME**-induced effects on sexual maturation, and specifically on levels of serum hormones and gonadosomatic index, were inconclusive. This ambiguity was attributed to interspawn collections of white sucker (***Catostomus commersoni***). In contrast, a number of studies have demonstrated reduced levels of sex steroids and decreased gonad weights in **BKME**-exposed populations (McMaster *et al.*, 1991; Munkittrick *et al.*, 1994; Servos *et al.*, 1994). This reproductive impairment has also been exhibited in close proximity to non-chlorinated or sulphite mills (Munkittrick *et al.*, 1994; Servos *et al.*, 1994). A direct relationship between depressed steroid concentrations and gonadal size, however, is not universally accepted. Several authours have reported hormonal suppression in conjunction with negligible site differences in GSI of stressed and non-stressed populations (Owens, 1991; Munkittrick *et al.*, 1992; Gagnon *et al.*, 1994). These results suggest that the relationship between steroid perturbations and impaired reproductive status is somewhat tenuous (Gagnon *et al.*, 1994).

Pottle (1979) refers to cunner (both sexes) as capable of multiple spawns. Gonadal histology at the control site demonstrated post-ovulatory follicles and late-vitellogenic oocyte populations in the presence of what appeared to be primary oocytes. Thus, this may provide cytological evidence of serial spawning, however, more histological and behaviourial studies are necessary to confirm this aspect of cunner reproduction

IV. Splenic Melanomacrophage Quantification

Under of conditions of toxaemia, melanomacrophage centers of haemopoietic tissue are typically herniated and the pigment granules dispersed (Roberts, 1989). Specifically, haemosiderosis describes a pathological or diseased state characterized by abnormally exaggerated deposition of the yellow-brown pigment, **haemosiderin**, in vertebrate tissues. This condition results from immoderate destruction of effete erythrocytes following trauma-induced haemorrhages, chronic congestion, haemolytic disease, parasitic proliferation and xenobiotic toxins (Khan and Nag, 1993). The presence of haemosiderin can be readily demonstrated by the Perl's Prussian blue staining method, a monospecific biochemical reaction that selectively distinguishes it from haemoglobin, biliary pigments (i.e., bilirubin, haemotoidin), malarial granules and porphyrin constituents (Drury and Wallington, 1967).

Roberts (1989) cites evidence that melanomacrophages are intimately involved in the piscine immune response since lymphocytes appear to home to these centers. Thus, greater percentages of haemosiderin at Birchy Cove support earlier discussion of elevated impact SSIs as possibly reflecting **splenomegaly**; a morphological manifestation of leukocytic proliferation.

Although differences were not significant, Birchy Cove typically had higher haemosiderin levels than their Summerside counterparts in 1993. The following year revealed pigment quantification at Gillams to be significantly lower than splenic granules in Birchy Cove samples, but greater than that of Summerside samples; again this observation was not statistically relevant. Otolith analyses verified that reference cunner were of a significantly older age class than both Summerside and Birchy Cove, respectively. The higher levels of reference (Gillams) haemosiderin relative to Summerside in 1994 may simply be a chronological effect. In addition to pathological

expression, moderate pigment aggregation is a typical and functional response to removal of effete erythrocytes from the bloodstream and increases naturally with age (Khan*et al*., 1994). Thus, greater percentages of haemosiderin in younger, Birchy Cove fish (1994), suggests a toxic response at this site.

Ideally it would be beneficial to standardize age classes with respect to site quantification of haemosiderin deposits. This would present a more confident and, perhaps significant, interpretation of the causative agent of melanomacrophage exaggeration in this system. Unfortunately, insufficient age data of fish used for splenic analyses prevented such investigation. In view of the weak correlation between age and body length at impact sites, haemosiderin standardization with respect to length class might increase the probability of statistical error and was not utilized.

V. Endoparasitofaunal Profiles

The helminth fauna of marine and/or euryhaline fish includes monogeneans, digenetic trematodes, cestodes, acanthacephalans and nematodes, a number of which caninduce significant disease in their hosts (Sinderman, 1990). The larval stages of trematodes, cestodes and nematodes are notably significant as the etiologic or causative pathological agent, whereas adult acanthacephalans inflict host injury (Sinderman, 1990). Fish typically harbour a diverse assemblage of both ecto- and endo-parasites without apparent deleterious effects. Both parasites and their hosts may be influenced by toxic xenobiotics; thus, pollutants may directly or indirectly modify the prevalence, intensity and pathogenicity of a parasite (Khan and Thulin, 1991). Hence, a synergistic effect may develop if parasitic infection and pollutant exposure are synchronous events.

A. Cestoda

Enteric cestodes constituted the major helminth taxon at Birchy Cove and Summerside in 1993. Intestinal profiles in 1994 reported fewer cestodes at both impact sites and no evidence of reference site parasitization.

Marine species may serve as definitive or intermediate hosts for cestodes, although adult stages are more common to the digestive tract of fish. All cestodes are oviparous. The eggs are expelled in the faeces of the primary host, and may hatch in the aquatic medium to liberate a motile larval form (Roberts, 1989; Sinderman, 1990). In a series of papers, Valtonen and co-workers showed that enteric cestodes of roach (***Rutilus rutilus***) were unaffected by effluent exposure and similar in prevalence to control fish (Valtonen and Koskivaara, 1987, 1989; Valtonen *et al.*, 1987a, b; Valtonen and Taskinen, 1988).

B. Nematoda

Nematodes were lower in sample number at both Birchy Cove and Summerside than at Gillams (1993-1994), but these differences were non-significant.

The majority of piscifaunal nematodes are oviparous in nature, and their eggs, which may or may not be embryonated, are defecated by their host (Roberts, 1989). Fertilized eggs metamorphose into a free-swimming larva which must be ingested by an intermediate host, usually an arthropod, where further development of the juvenile phase occurs (Roberts, 1989). Adult nematodes typically proliferate and invade the digestive tract with minimal pathological consequences, unless infection is quite severe (Sinderman, 1990). The metabolites of alimentary nematodes are discharged with the host's wastes and thus their toxicity is significantly diluted, in contrastto the byproducts of tissue parasites which concentrate and elicit an inflammatory response (Grabda, 1991).

Barker *et al.* (1994) attributed high prevalence and intensity of third stage anisakid larval nematodes in effluent-exposed winter to increased susceptibility to parasitic infection. In contrast, negligible site differences were observed in the current study.

C. Trematoda

Digenetic trematodes were exclusive to 1994 intestinal profiles samples; intensities were significantly greater at Birchy Cove. The life cycle and generational stages for this helminth group have been discussed elsewhere (refer to lamellar ectoparasitization in histopathology section). Conversely, Barker *et al.* (1994) observed no difference in either the prevalence or intensity of gastrointestinal trematodes in winter flounder from reference and effluent-impacted areas. Valtonen and Koskivaara (1987) reported 0 % prevalence of adult digeneans in the intestines of roach, ***Rutilus rutilus***, residing in a freshwater lake exposed to **BKME**. Enteric trematodes have demonstrated a

reduction in infestation when subjected to oil-contaminated sediment and water-soluble petroleum hydrocarbon fractions in both pelagic and benthic marine hosts (Khan and Kiceniuk, 1983; Khan, 1991).

However, the reverse trend observed in the current investigation is not necessarily conflicting evidence, since many endoparasites do increase in population distribution and host number when subjected to sublethal concentrations of xenobiotic toxins (Khan and Thulin, 1991). This suggests that certain contaminants may potentiate the deleterious consequences of piscine enteric taxa. **D. Acanthacephala**

Similar to trematode profiles, acanthacephalans were restricted to 1994 gastrointestinal analyses and were significantly greater in Gillams' samples.

Thulin *et al.* (1986; 1988) noted the absence of enteric fauna in perch (***Perca fluviatilis***) adjacent to unbleached pulp outfalls compared to reference localities. Barker *et al.* (1994) also observed acanthacephalan infestations to be more prevalent in winter flounder unaffected by **BKME** emissions. This distribution pattern was also demonstrated in Atlantic cod (***Gadus morhua***) exposed to sublethal concentrations of petroleum crude extracts (Khan and Kiceniuk, 1983). Conversely, Valtonen and Koskivaara (1989) showed increased prevalence of this helminth taxon in roach (***Rutilus rutilus***) from a freshwater lake contaminated with chlorinated pulp residues.

It was not an objective of this study to delineate potential mechanisms mediating the differential helminth parasitization of cunner populations. Endo- and ecto-parasitic (as previously discussed) profiles were constructed in order to critically evaluate the deployment of parasites as indicators of chronic exposure to **non-chlorinated** pulp. Total enteric counts were significantly exaggerated in the control sample versus impact sites. This pattern is consistent with an impressive

body of literature citing depreciations of enteric fauna in fish from degraded habitats (e.g. Khan and Kiceniuk, 1983; Khan, 1990; 1991; Khan and Thulin, 1991; Barker *et al.*, 1994). The results in the current study are particularly significant as they holistically infer that enteric remission in fish adjacent to pulp mill wastewaters may be independent of its organochlorine constituents. However, since this relationship was only observed with respect to acanthacephalan distribution, caution should be exercised in generalizing to the helminths as a group. This distribution may be attributed to nutrient deficiency in the host's intestinal tract, direct toxicity of effluent compounds to parasitic fauna, and/or outfall-induced modification of enteric physiology resulting in an inferior environment for helminth survival and reproduction. Although the tegument of digenean, acanthacephalan and cestode species are intimately involved in the absorption of digested food within the host's digestive tract, 1994 differences in VSIs do not appear to reflect the remarkable disparity in parasitic intensities between impact and control site(s). Marine fish must drink seawater in order to maintain osmoregulatory processes; therefore, parasites may be voided from the gut as a consequence of toxic absorption or alteration to intestinal cytoarchitecture. The latter could be tested by histological surveys in future studies.

VI. Mixed Function Oxygenase (7 Ethoxyresorufin O-Deethylase) Activity

The term mixed-function defines those enzymes that mediate reactions in which one atom of molecular oxygen is reduced to water while the other is incorporated into the catalytic substrate (Payne, 1984; Payne *et al.*, 1987). Mixed function oxygenases perform a critical role in detoxification by depreciating the lipid solubility of organic toxins to facilitate excretion (Jimenez and Stegman, 1990). This pollutant immobilization is biphasic. During **Phase-I**, a polar reactive group is inserted into the relatively insoluble organic compound to yield a substantially more water-soluble configuration (Payne, 1984; Jimenez and Stegman, 1990). In **Phase-II**, organic constituents (the majority of which are **Phase-I** metabolites) merge with an endogenous substrate to produce a conjugated isomer that is readily excreted in the bile or urine of fish (Payne *et al.*, 1987). The **MFO** system contains iron-containing haemoproteins as the terminal oxidases. These are unique in that induction by xenobiotic chemicals to levels often several fold higher than typical baseline specific activities, are a notable feature of this enzyme scheme in vertebrate tissues (Payne, 1984; Payne *et al.*, 1987; Jimenez and Stegman, 1990; Payne *et al.*, 1994). A great advantage of employing enzyme biological activity as an indicator of aquatic stress is the sensitivity of enzymes to specific pollutant inducers (Jimenez and Stegman, 1990). Seven ethoxyresorufin O-deethylase activity has also been cited as an extremely robust measure of hepatic mixed function oxygenase or P4501A induction, minimally degraded by variation in assay methodology, sample preparation and handling; thus, it facilitates interlaboratory comparisons (Munkittrick *et al.*, 1993). A multitude of studies have reported significant inductions in hepatic MFOs of **BKME**-exposed populations relative to reference piscine cohorts (e.g. Rogers *et al.*, 1989; McMaster *et al.*, 1991; Munkittrick *et al.*, 1992; Balk *et al.*, 1993; Gagné and Blaise, 1993; Ahokas *et al.*, 1994; Gagnon *et al.*, 1994). Of particular consequence

to, and consistent with the current study, is a growing body of evidence pertaining to **MFO** activity in response to non-chlorinated wastewaters (Lehtinen, 1990; Smith *et al.*, 1991; Kloepper-Sams and Swanson, 1992; Lindström-Seppä *et al.*, 1992; Martel *et al.*, 1994; Munkittrick *et al.*, 1994). In the Humber estuary, **EROD** activity was significantly greater in pooled Birchy Cove samples compared to the reference assay. These results indicate that organochlorides are not necessarily the sole prerequisite for exaggerated MFO stimulation. However, there is ambiguity over whether the inducing capacitor(s) in unbleached effluents are generated during the pulping process or the result of material(s) added during the pulp or washing protocol (Lindström-Seppä et al., 1992). Martel *et al.* (1994) postulated that natural wood extracts or compounds manufactured during the kraft cooking procedure could be the origin of non-chlorinated MFO inducers. Conclusions pertaining to the former were not possible due to data limitation; however, spent cooking (black) liquor from the latter process demonstrated an unequivocal **EROD** induction in fish populations (Martel *et al*, 1994). Lindström-Seppä *et al.* (1992) concluded that acute exposure of rainbow trout hepatocytes to unbleached effluent fractions significantly induced P4501A-dependent 7 ethoxyresorufin O-deethylase specific activity in low concentrations. However, in contrast to their bleached counterparts, higher concentrations of the non-chlorinated fraction squelched **EROD** induction entirely (Lindström-Seppä *et al.*, 1992). Lehtinen (1990) asserted that unbleached kraft- pulp processes are likely to induce inhibitory, and bleached pulp production stimulatory, consequences to the hepatic detoxification systems of fish. Chronic exposure to the former; however, is indicative of MFO animation in the present system and is consistent with more pathologically-relevant effects observed with recurring exposure to high dilutions of wastewaters (Lehtinen, 1990). However, activity at impact sites demonstrated only a 2.7 (Birchy Cove) to 3.0 (Summerside) fold increase

from reference sites and may indicate inhibitory unbleached effect, although Otto *et al.* (1994) interpreted a 3-4 fold increase in rainbow trout exposed to contaminated sediments as "strong inductive potential" of unbleached effluent on EROD activity. Martel *et al.* (1994) demonstrated a slight but, statistically significant EROD activity of unbleached effluent (2.3-2.5 fold). The authours concluded that chlorine use in pulp bleaching was not an exdusive determinant factor in the capacity of discharged effluents to induce MFO activity in exposed fish populations (Martel *et al.*, 1994). Walton *et al.* (1983) observed that when specific activity of arylhydrocarbon hydroxylase in cunner was expressed as total hepatic AHH activity (/100g body weight), induction increased 166 fold and 155 fold for males and females, respectively. In their review of MFOs in biological monitoring, Payne *et al.* (1982) cited several field trials where negligible specific, but significantly increased total, activity was observed in contaminant-exposed fish This is particularly of interest to the current study where enlarged impact livers were a concomitant effect as in the aforementioned examples. Thus, MFO induction may have been interpreted as more significant at CBPP-adjacent sites, if total enzyme activity had been calculated, i.e. mathematical elimination of any bias contributed by liver weight differences.

Liver samples were collected in 1995 during the last week of June when cunner began responding to baited nets. Sampling was designed to advance the spawning season of this labrid species so as to avoid any potential EROD suppression due to reproductive "noise" (Payne and Fancy, 1982). The Birchy Cove site was the only sample to exhibit sexual dimorphism with respect to specific activity. However, male sample size at Gillams was quite small (n=2); a function of both sexual territoriality (refer to discussion on sex ratios) and temporal limitation of site collections (i.e. to avoid the spawning season). Although significant induction was observed at Birchy Cove, these

data limitations and hormonal influences may have resulted in underestimation of activity. Ideally, it would be beneficial to assay livers of immature males to avoid the confounding variables introduced by maturation/spawning.

Effluent mediated liver dysfunction and histological departures have been correlated with active hepatic detoxification processes. Lehtinen (1990) proposed several **BKME**-governed mechanisms effecting hepatic incapacitation :

1. exaggerated concentrations of intermediate metabolites (i.e. free radicals) comparatively more toxic than parental progenitors, eliciting membrane damage;

2. natural wood extracts (i.e. resin acids) suppress conjugating or **Phase-II** enzymes, resulting in substance hypertoxicity in the liver;

3. original constituents inhibit **Phase I** enzymes, inducing cytotoxic consequences.

This chlorinated effect is consistent with significant **EROD** induction in the present study concomitant with hepatic anomalies (i.e., hyperplastic and vacuolated response). Again, this is indicative of effluent toxicity independent of its organochloride profile. Pesonen and Andersson (1992) demonstrated that unbleached effluent extracts exert more potent effects on plasma membrane integrity and cellular glutathione content (an indicator of oxidative stress and reactive metabolite formation) than do bleached components.

Since steroidogenic hormones are naturally-occurring, endogenous substrates for **MFO** catalysts, the biological potential for affecting critical organismic functions due to exposure to significant concentrations of inducing xenobiotics may have ecological consequences (Payne *et al.*, 1987). Several studies have demonstrated that fish livers may be less responsive to **MFO** induction during gonadal maturation and/or spawning, including cunner (Walton *et al.*, 1983; Payne *et al.*,

1987). Analysis of specific activity illustrated male induction to be approximately 5-fold greater at Birchy Cove than for female assays. Subsequently, this gender difference may have been reflected in lower pooled specific activity at the aforementioned site due to disproportionately fewer males in homogenate samples. However, **EROD** stimulation was readily resolved in this study and significantly exaggerated in exposed pooled populations. Porter *et al.* (1989) illustrated the feasibility of employing **MFO** enzymes for biomonitoring protocols even during the reproductive season of this species.

Summary

(i) Birchy Cove and Summerside sites, postulated to be impacted by the Corner Brook Pulp and Paper Mill, western Newfoundland, since the late 1920s, supported territorial populations of cunner characterized by greater body lengths, eviscerated weights and exaggerated growth rates relative to control counterparts at Gillams (1993-1994), approximately 12 kilometers downstream of effluent outfalls and on the northern shore. These somatic disparities might be attributed to eutrophication of the Humber River Estuary and or posible diversion of energetic committment from reproductive efforts. Trend departures in 1995 for the majority of indices could be the morphometric manifestation of larger fish captures at the control site.

(ii) Sex ratios skewed in favour of females at each site were evidence of male territoriality at each site (confirmed by SCUBA). This substantiates non-migratory behaviour of this labrid species and its potential as a reliable bioindicator of perturbations symptomatic to a particular environment.

(iii) Condition factors illustrated non-significant differences with respect to sites.

(iv) Gonadosomatic indices demonstrated significantly smaller values at Gillams than at impacted areas during the principal field season in 1994. This, in conjunction with histological surveys, suggested delayed gametogenesis and/or spawning in effluent-exposed populations. Fish at the aforementioned sites may have been necropsied during an earlier maturational phase than were control counterparts. Therefore, ambiguity about unbleached outfall induction of reproductive pathology remains.

(v) A general pattern of exaggerated liver indices at polluted sites compared to cunner somatic profiles at Gillams may indicate hepatic hyperplastic activity of smooth endoplasmic reticulum (**SER**). Sexual dimorphic responses, specifically comprartively greater HSI values in females, concur withr reports correlating reproductive maturation and vitellogenic synthesis with increased hepatosomatic indices. Histologically, categorically more pronounced cellualr inclusions (i.e. lipid vacuolation), were restricted to Birchy Cove tissue and could partially account for a corresponding increase in HSI values.

(vi) Mean visceral indices did not establish any tangible relationship with effluent exposure in the current study.

(vii) Swollen spleens (i.e. splenomegaly?) was demonstrated in Birchy Cove and Summerside samples relative to control tissue (1994). Reference fish in 1995 demonstrated considerably larger indices, secondary only to Birchy Cove. However, older populations at Gillams (1994) would suggest a chronologically-mediated effect, rather than, pathological induction. Splenic melanomacrophage quantification of haemosiderin also exhibited greater, albeit non-significant, aggregate percentages in Birchy Cove sections than at Summerside or Gillams (1993-1994). This pathological architecture in younger fish adjacent to the mill may suggest a toxic response, then.

(viii) Lamellar histopathology (i.e. secondary filament hyperplasia), a non-specific toxicant stress response, was more pronounced in Birchy Cove and Summerside residents, compared to negligible cellular deviations in Gillams' counterparts. Increased diffusion distances across these gas exchange

surfaces, particularly in the case of excessive hyperplasia, may effect adverse effects on respiratory efficiency. *Cryptocotyle lingua* was the only branchial ectoparasite observed in the current study, however, data was insufficient for seasonal or site-specific comparisons. Thus, its reliability as a bioindicator in this particular study is open to debate.

(ix) Endoparasitofaunal profiles exhibited variable site-specific prevalence and intensity with respect to invidual helminth taxa. However, as a general assemblage, intensities (total number of parasites per sample) were significantly exaggerated in control tracts versus Birchy Cove and Summerside, respectively. This concurs with reports of enteric depletion in polluted fish populations and may substantiates its potential value as a reliable bioindicator of aquatic degradation (i.e. in the vicinity of pulp waste).

(x) Specific activity of 7 ethoxyresorufin O-deethylase exhibited significantly greater induction at Birchy Cove compared to assay of control liver homogenates. Although the current study demonstrated that activity was readily resolved in both males and females, a sexually dimorphic trend established greater activity in the former gender. This may be attributed to damping of female sensitivity to MFO induction during gonadal maturation and/or spawning. Female dominance of samples may have minimized pooled specific activity. Mixed function oxygenase induction may support SER hyperplasia as a possible cytoarchitectural response effecting corresponding exaggerated hepatosomatic indices at Birchy Cove.

(xi) The current study has provided evidence of chronic stress in residential populations of cunner

impacted by unbleached pulp mill wastewater by using several bioindicators and supports hypotheses that the bleaching process may be a minimal prerequisite in toxic manifestations in effluent-exposed fish populations.

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Table A. 1. A conventional schedule for processing tissue sections to wax.

1. 70% ethanol for 2 hours
2. 80% ethanol for 2 hours
3. 95% ethanol for 2 hours
4. 95% ethanol for 2 hours
5. absolute (100%) ethanol for 2 hours
6. absolute (100%) ethanol for 2 hours
7. chloroform for 1 hour
8. chloroform for 1 hour
9. wax for 2 hours
10. Vacuum wax for 2 hours

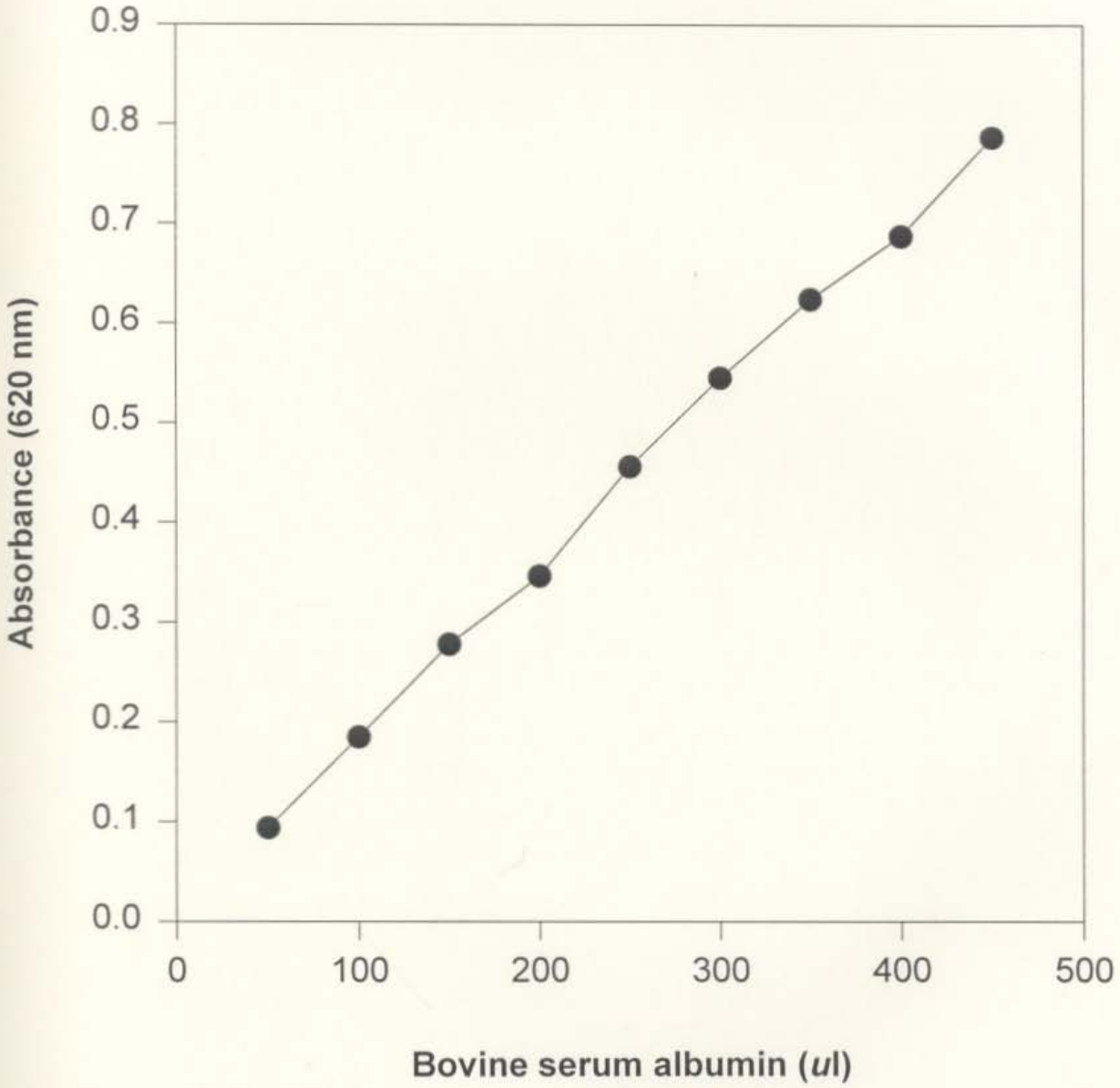


Figure A.1 . Standard curve of bovine serum albumin (BSA; Lowry Protein Determination) for 7 Ethoxy resorufin O - deethylase (EROD) assay.

Table A.2. Site sexual differences between male and female *Tautogolabrus adspersus* for body parameters and somatic indices (SI) collected at Birchy Cove, Summerside and Gillams (1994), where ♂ and ♀ denote male and female, respectively.

Variable		Birchy Cove 31(♂), 79(♀)	Summerside 28(♂), 72(♀)	Gillams 38(♂), 59(♀)
Length (cm)	(♂)			19.50±1.50
	(♀)			18.59±0.49 h
Weight (g)	(♂)			103.79±25.27
	(♀)			90.07±7.04 h
Gonado-SI	(♂)			5.58±2.19
	(♀)			12.43±0.75 h
Hepato-SI	(♂)		1.95±0.15	
	(♀)		3.51±0.20 g	
Spleno-SI	(♂)	0.17±0.03		
	(♀)	0.18±0.02 h[1]		

g Male and female significantly different, $p<0.001$
h Male and female significantly diffreent, $0.001<p<0.05$

(x)[1] significant interaction, where (x)=(h)

Table A.3. Yearly differences for body parameters and somatic indices of *Tautogolabrus adspersus* collected at Birchy Cove (1993-1995), where ♂ and ♀ denote male and female, repectively.

Variable		Birchy Cove 1993	Birchy Cove 1994	Birchy Cove 1995	
Length(cm)		20.57±0.5	18.40±0.32	19.92±0.58	j, ns
Weight(g)		149.43±11.8	88.93±4.49	117.17±10.58	i, ns
K-Factor		1.69±0.07	1.31±0.01	1.39±0.03	ns, ns
Gonado-SI	(♂)	7.22±1.07	7.36±0.66	12.46±2.01	j^1, k^1
	(♀)	5.03±0.38	5.62±0.26	14.49±0.92	ns, ns
Hepato-SI		2.86±0.22	2.79±0.09	4.19±0.29	ns^1, ns
Visceral-SI		3.97±0.41	3.34±1.29	5.48±0.31	ns, ns
Spleno-SI	(♂)	0.20±0.12	0.20±0.01	0.17±0.03	ns, ns
	(♀)	0.16±0.02	0.21±0.03	0.18±0.02	ns, ns

i Birchy Cove 1993 significantly different from Birchy Cove 1994, $p<0.001$
j Birchy Cove 1993 significantly different from Birchy Cove 1994, $0.001<p<0.05$

k Birchy Cove 1994 significantly different from Birchy Cove 1995, $p<0.001$
l Birchy Cove 1994 significantly different from Birchy Cove 1995, $0.001<p<0.05$

$(x)^1$ significant interaction, where (x)=(i,j,k, or l)

Table A.4. Yearly differences for body parameters and somatic indices of *Tautogolabrus adserpsus* collected at Summerside (1993-1995), where ♂ and ♀ denote male and female, repectively.

Variable		Summerside 1993	Summerside 1994	Summerside 1995	
Length(cm)		17.56±0.6	18.00±0.24	18.44±0.46	ns, ns
Weight(g)		85.15±8.6	79.02±3.48	82.25±6.80	ns, ns
K-Factor		1.48±0.03	1.28±0.01	1.25±0.01	ns, ns
Gonado-SI	(♂)	6.05±0.62	7.56±0.77	6.73±1.02	ns, ns
	(♀)	3.83±0.19	7.68±0.50	8.98±0.69	ns, ns
Hepato-SI	(♂)	1.69±0.11	1.79±0.82	1.95±0.15	ns, ns
	(♀)	2.93±0.37	3.35±0.11	3.51±0.20	ns, ns
Visceral-SI		2.98±0.18	3.48±0.10	7.08±0.69	ns, m
Spleno-SI		0.16±0.19	0.21±0.01	0.13±0.01	ns,n

m Summerside 1994 significantly different from Summerside 1995, $p<0.001$
n Summerside 1994 significantly different from Summerside 1995, $0.001<p<0.05$

Table A.5. Yearly differences for body parameters and somatic indices of *Tautogolabrus adspersus* collected at Gillams (1993-1995), where ♂ and ♀ denote male and female, repectively.

Variable		Gillams 1994	Gillams 1995	
Length(cm)	(♂)	14.63±0.43	19.50±1.50	p
	(♀)	16.09±0.35	18.59±0.49	o
Weight(g)	(♂)	43.37±4.48	103.79±25.27	p
	(♀)	58.00±3.59	90.07±7.04	o
K-Factor		1.26±0.01	1.36±0.03	ns
Gonado-SI	(♂)	3.28±0.40	5.58±2.19	p
	(♀)	4.93±0.48	12.43±0.75	
Hepato-SI		2.23±0.10	3.48±0.17	ns[1]
Visceral-SI		3.50±0.13	4.68±0.30	ns
Spleno-SI	(♂)	0.18±0.01	0.51±0.01	ns
	(♀)	0.19±0.02	0.18±0.05	ns

o Gillams 1994 significantly different from Gillams 1995, $p<0.001$

p Gillams 1994 significantly different from Gillams 1995, $0.001<p<0.05$

(x)[1] significant interaction, where (x)=(o, or p)

Table A. 6. Yearly growth rate differences of *Tautogolabrus adspersus* collected at Birchy Cove (1993-1995), where ♂ and ♀ denote male and female, respectively. Regression equation : Dressed weight (Y) = Length(X) - Intercept.

Birchy Cove 1993	Birchy Cove 1994	Birchy Cove 1995	
♂ Y=19.2Length-252.4	♂ Y=15.5Length-193.7	♀ Y=17.7Length-230.3	ns
♀ Y=17.8Length-213.4	♀ Y=12.2Length-138.1	♀ Y=18.1Length-243.2	i,k

i Birchy Cove 1993 significantly different from Birchy Cove 1994, $p<0.001$
k Birchy Cove 1994 significantly different from Birchy Cove 1995, $p<0.001$

Table A. 7. Yearly growth rate differences of *Tautogolabrus adspersus* collected at Summerside (1993-1995), where ♂ and ♀ denote male and female, respectively. Regression equation : Dressed weight (Y) = Length(X) - Intercept.

Summerside 1993	Summerside 1994	Summerside 1995
Y=15.3Length-182.0	Y=13.59Length-165.7	Y=14.4Length-183.4 n

n Summerside 1994 significantly different from Summerside 1995, $0.001<p<0.05$

Table A. 8. Yearly growth rate differences of *Tautogolabrus adspersus* collected at Gillams (1994-1995), where ♂ and ♀ denote male and female, respectively. Regression equation : Dressed weight (Y) = Length(X) - Intercept.

Gillams 1994	Gillams 1995
Y=9.98Length-102.7	Y=13.98Length-169.9 o

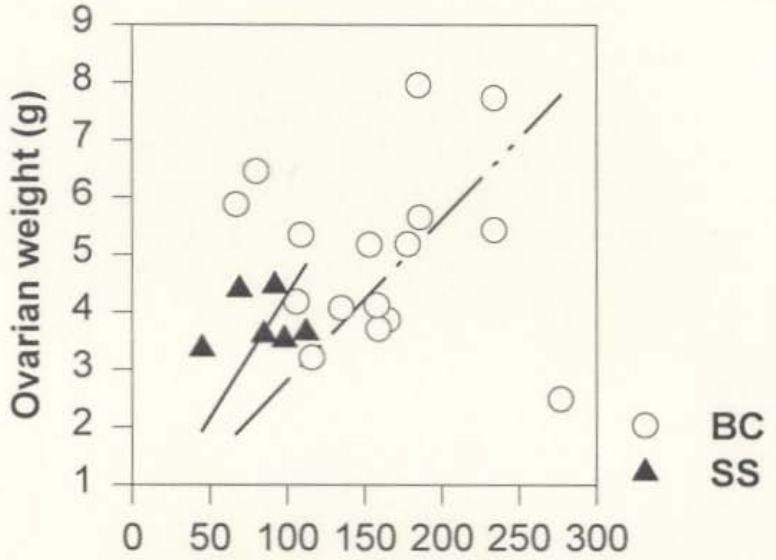
o Gillams 1994 significantly different from Gillams 1995, $p<0.001$

Table A.9. Site sexual differences between male and female *Tautogolabrus adspersus* for body parameters and somatic indices (SI) collected at Birchy Cove, Summerside and Gillams (1995), where ♂ and ♀ denote male and female, respectively.

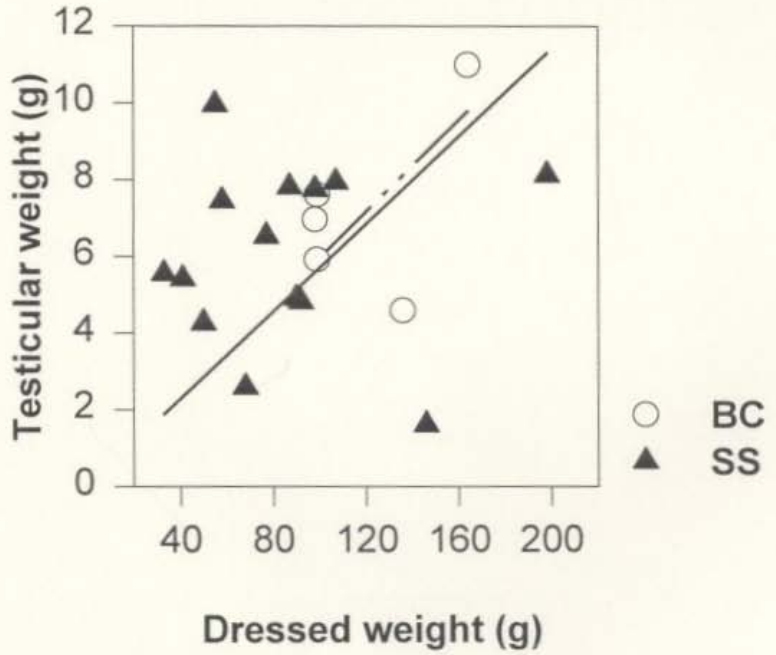
Variable		Birchy Cove n=6(♂), 19(♀)	Summerside n=7(♂), 18(♀)	Gillams n=2(♂), 21(♀)	
Splenosomatic Index	(♂)			0.51±0.40	
	(♀)			0.18±0.05	h^1

h Male and female significantly different, $0.001<p<0.05$

$(x)^1$ significant interaction, where (x)=(h)

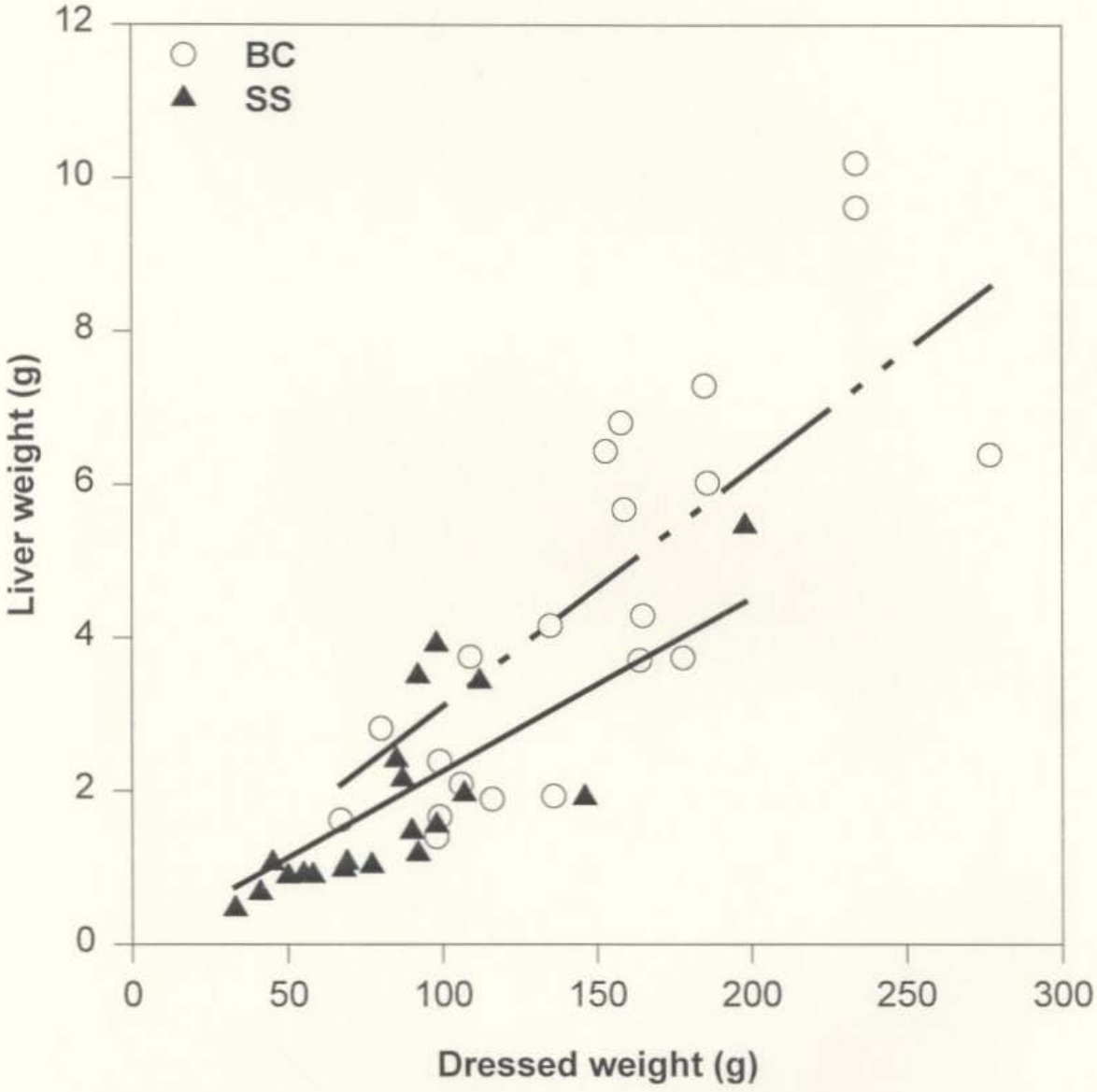


BC:Ovarian weight=0.05 dressed weight - 0.54, p=0.009, r=0.65
SS:Ovarian weight=0.04 dressed weight + 0.11, p=0.009, r=0.92
Regresion over groups:p=0.73



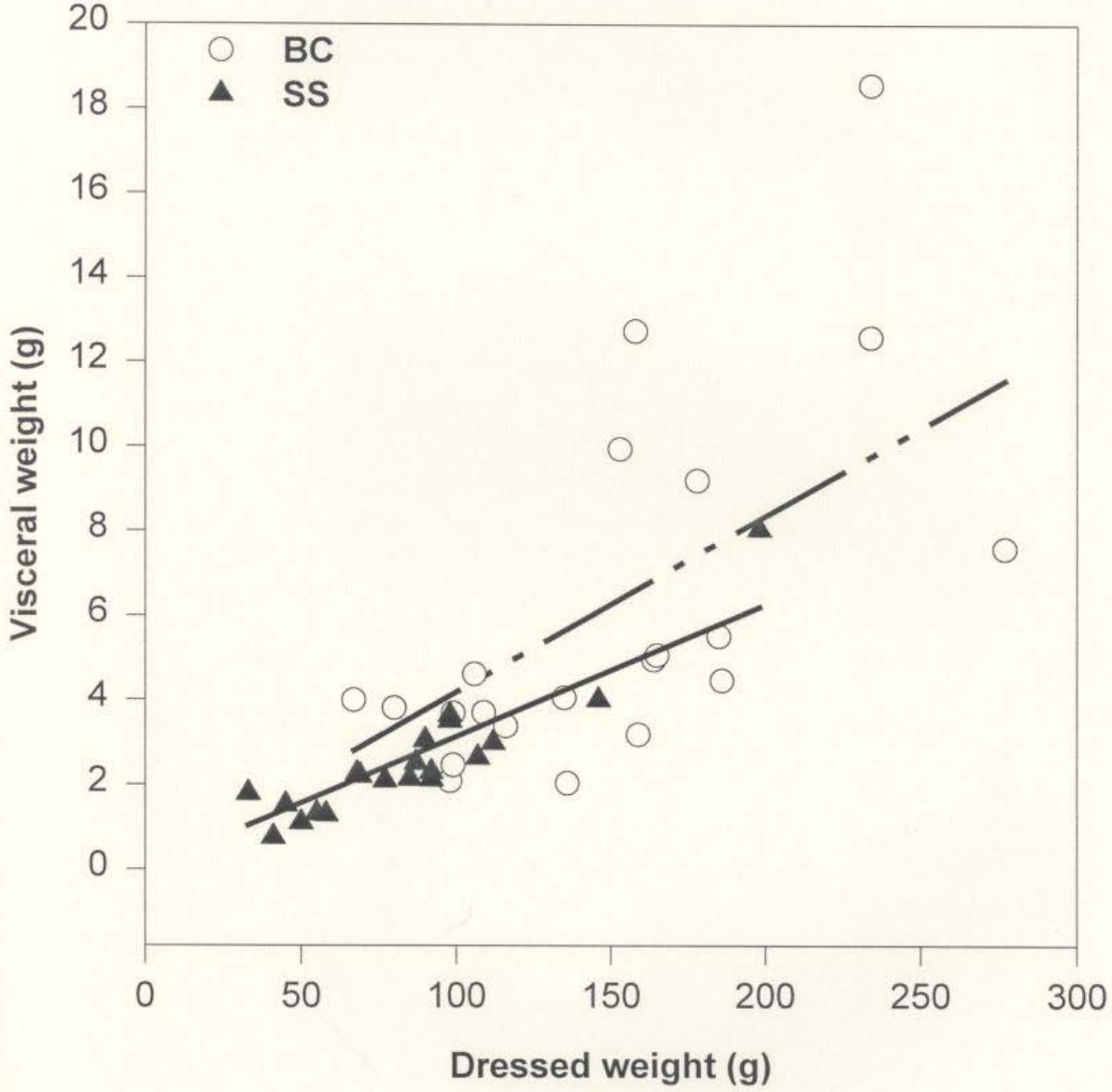
BC:Testicular weight=0.14 dressed weight - 7.70, p=0.10, r=0.81
SS:Testicular weight=0.07 dressed weight - 0.41, p=0.002, r=0.75
Regression over groups:p=0.28

Figure A. 2. Dressed vs ovarian/testicular weights regression analyses for Birchy Cove (BC) and Summerside (SS), 1993.



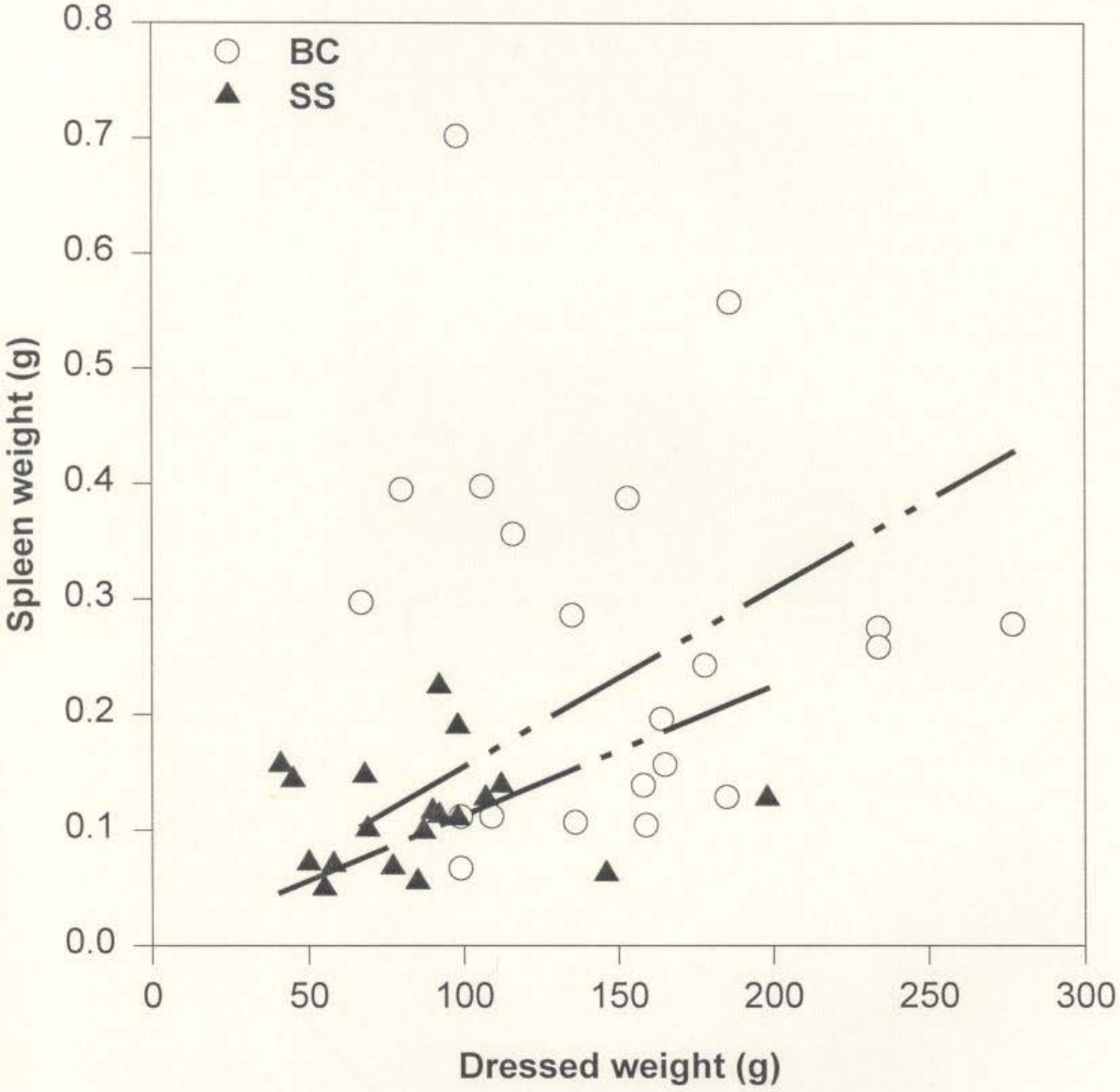
BC:Liver weight=0.04 dressed weight - 0.85, p=0.0001, r=0.79
SS:Liver weight=0.03 dressed weight - 0.49, p < 0.00005, r=0.80
Regression over groups:p=0.44

Figure A. 3. Dressed vs liver weights regression analyses for Birchy Cove (BC) and Summerside (SS), 1993.



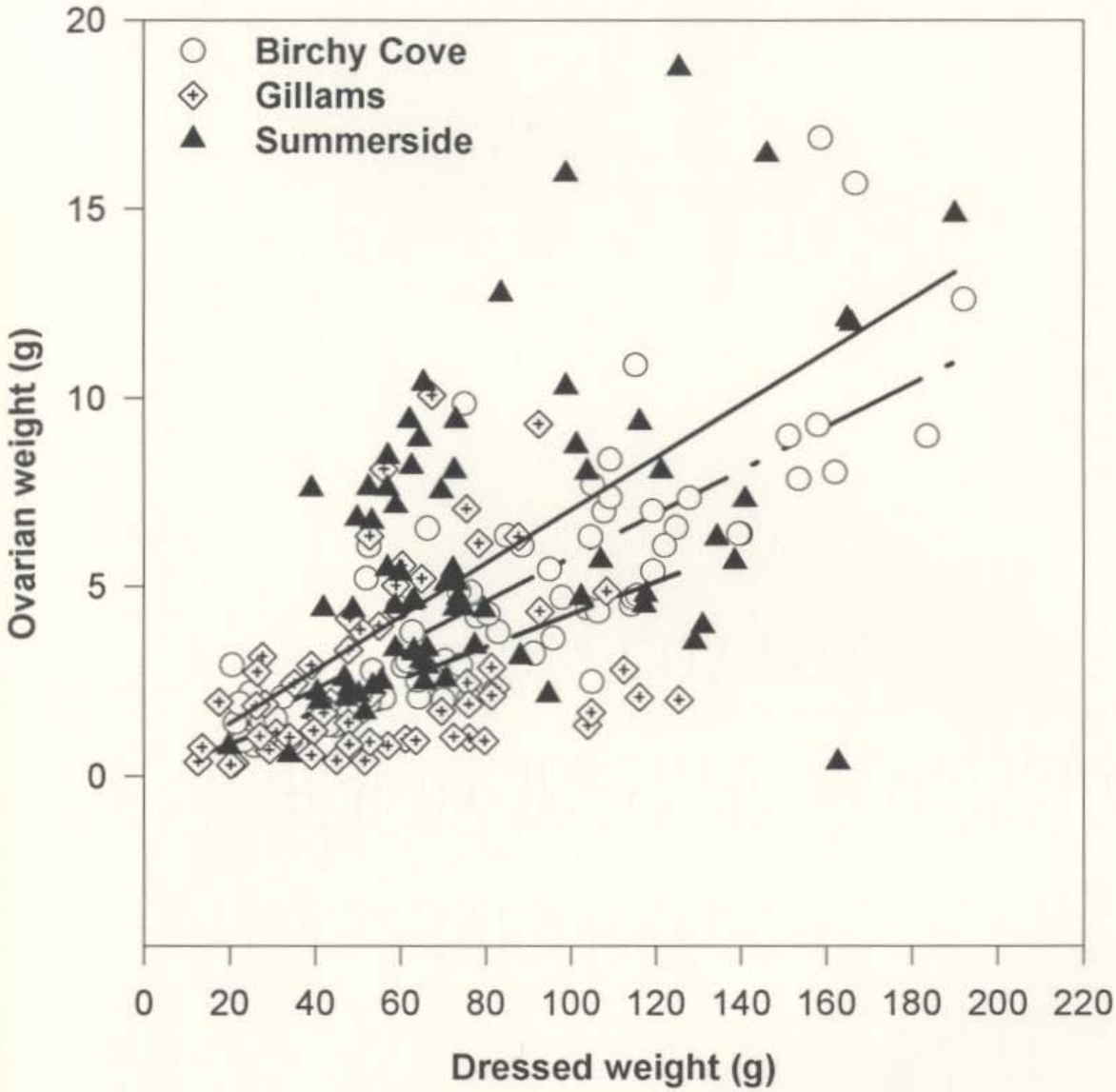
BC:Visceral weight=0.05 dressed weight - 1.18, p=0.01, r=0.60
SS:Visceral weight=0.04 dressed weight - 0.61, p < 0.00005, r=0.92
Regression over groups:p=0.75

Figure A. 4. Dressed vs visceral weights regression analyses for Birchy Cove (BC) and Summerside (SS), 1993.



BC:Spleen weight=0.19*10^{2-} dressed weight - 0.025, p=0.01, r = 0.60
SS:Spleen weight=0.15*10^{2-} dressed weight + 0.003, p < 0.00005, r = 0.79
Regression over groups:p=0.76

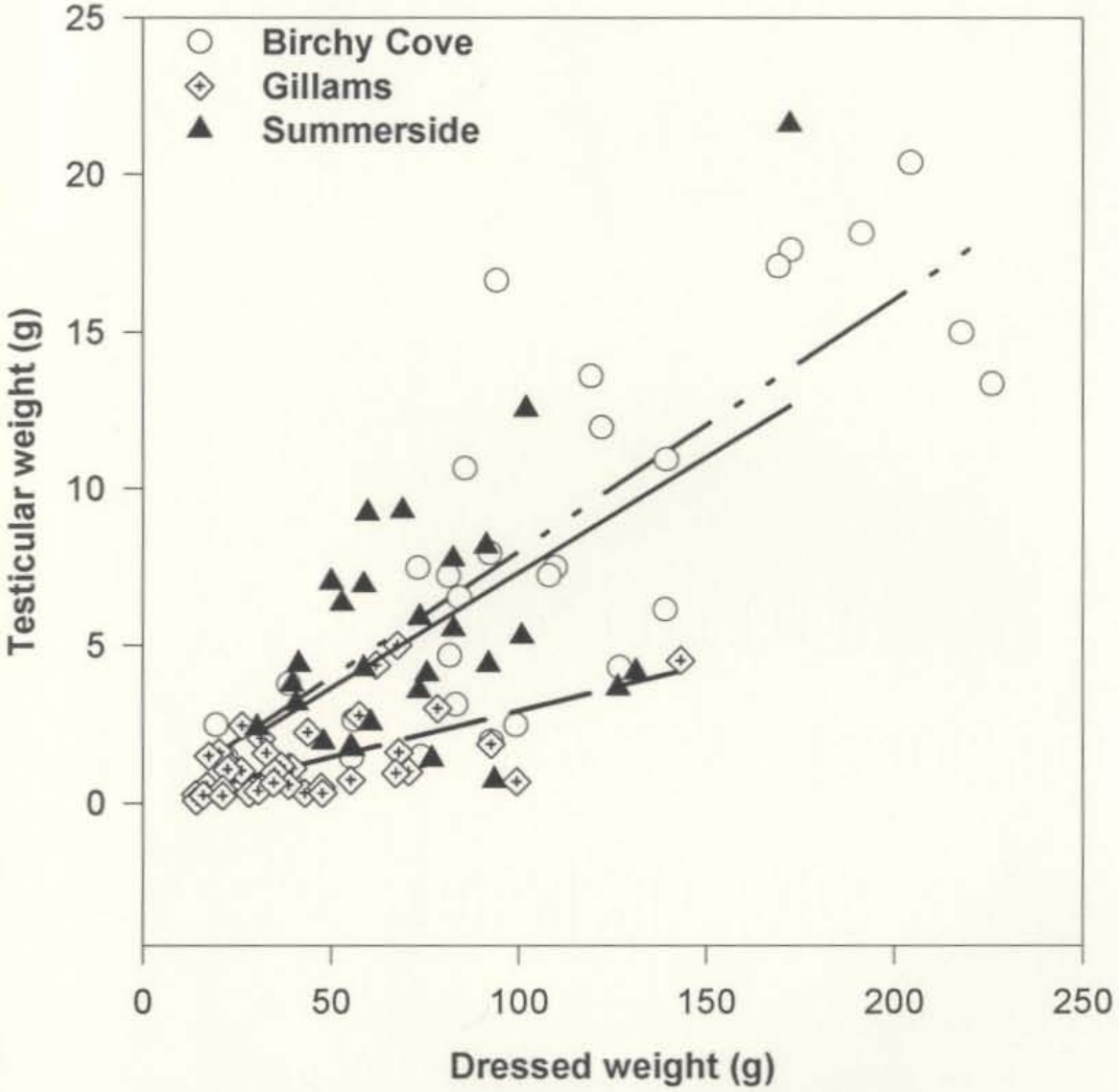
Figure A. 5. Dressed vs spleen weights regression analyses for Birchy Cove (BC) and Summerside (SS), 1993.



BC:Ovarian weight=0.06 dressed weight - 0.50, p < 0.00005, r=0.82
SS:Ovarian weight=0.05 dressed weight + 1.79, p < 0.00005, r=0.59
GM:Ovarian weight=0.03 dressed weight + 1.12, p=0.01, r = 0.32

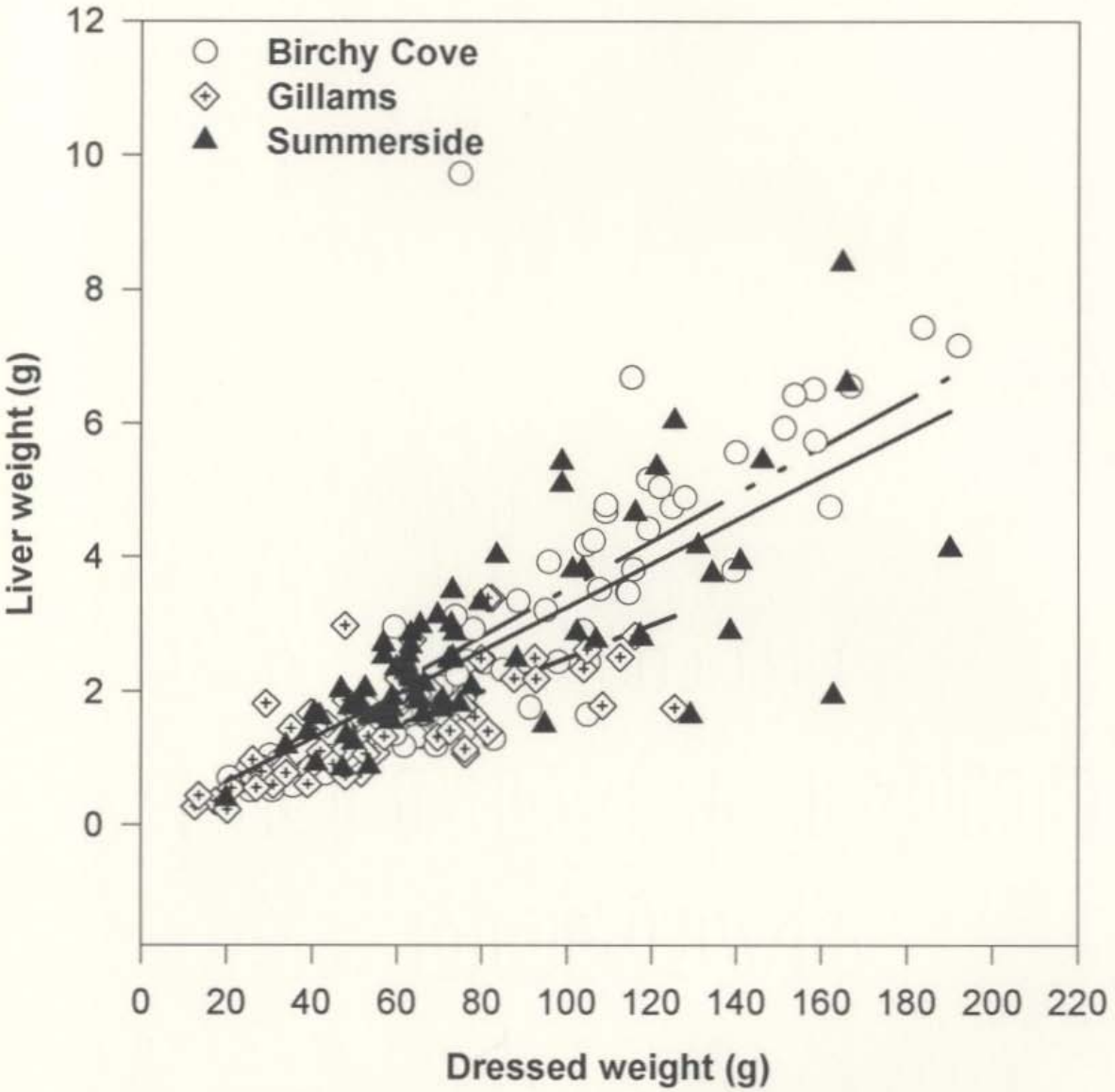
Regression over groups:BC vs GM, p=0.002
Regression over groups:SS vs GM, p=0.0001

Figure A. 6 a. Dressed vs ovarian weights regression analyses for Birchy Cove (BC), Summerside (SS) and Gillams (GM), 1994.



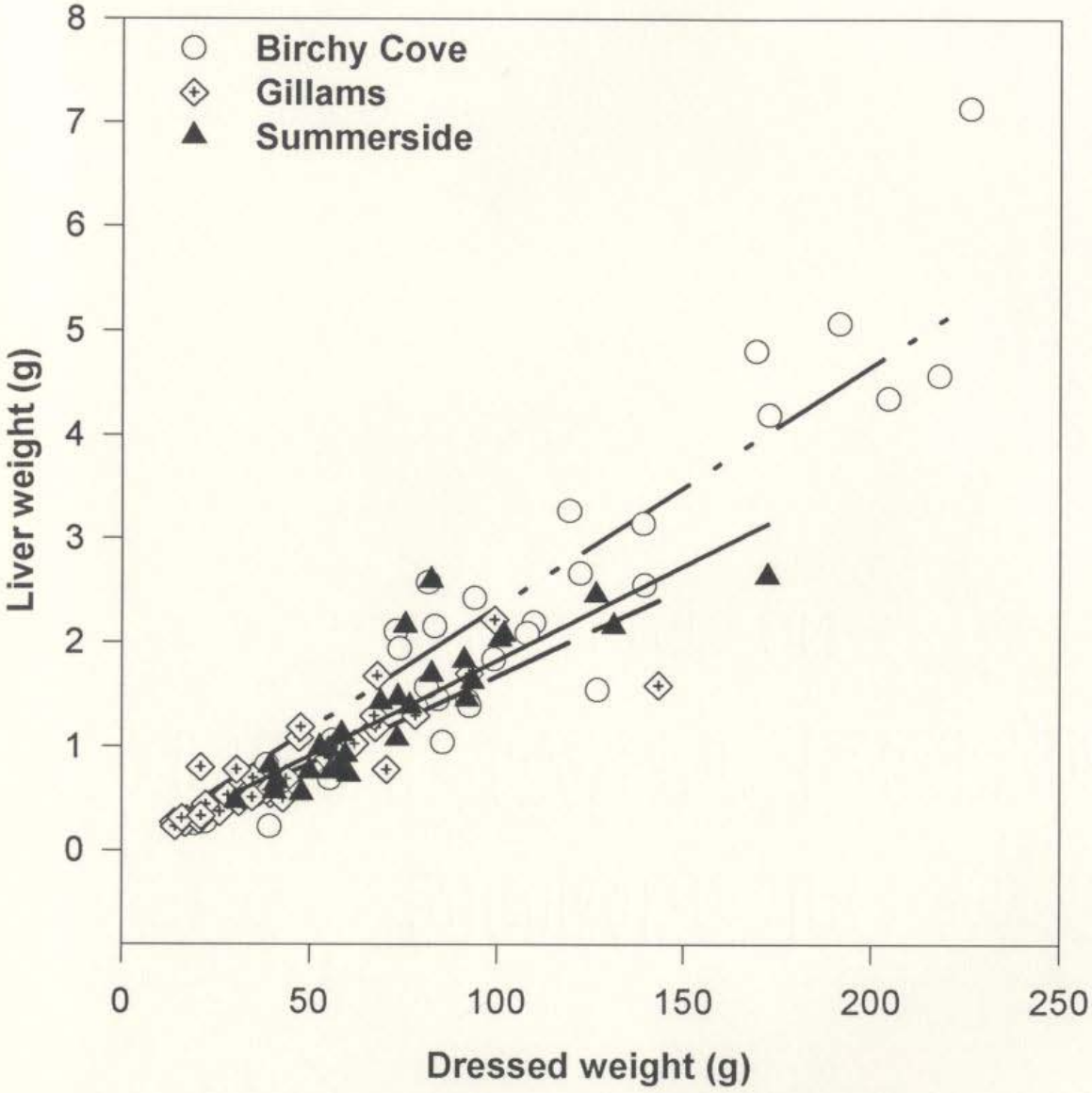
BC:Testicular weight=0.09 dressed weight - 1.04, p < 0.00005, r=0.81
SS:Testicular weight=0.07 dressed weight - 0.04, p=0.002, r=0.56
GM:Testicular weight=0.03 dressed weight + 0.25, p=0.0002, r=0.57,
Regression over groups:BC vs GM, p=0.0001
Regression over groups:SS vs GM, p=0.0002

Figure A. 6 b. Dressed vs testicular weights regression analyses for Birchy Cove (BC), Summerside (SS) and Gillams (GM), 1994.



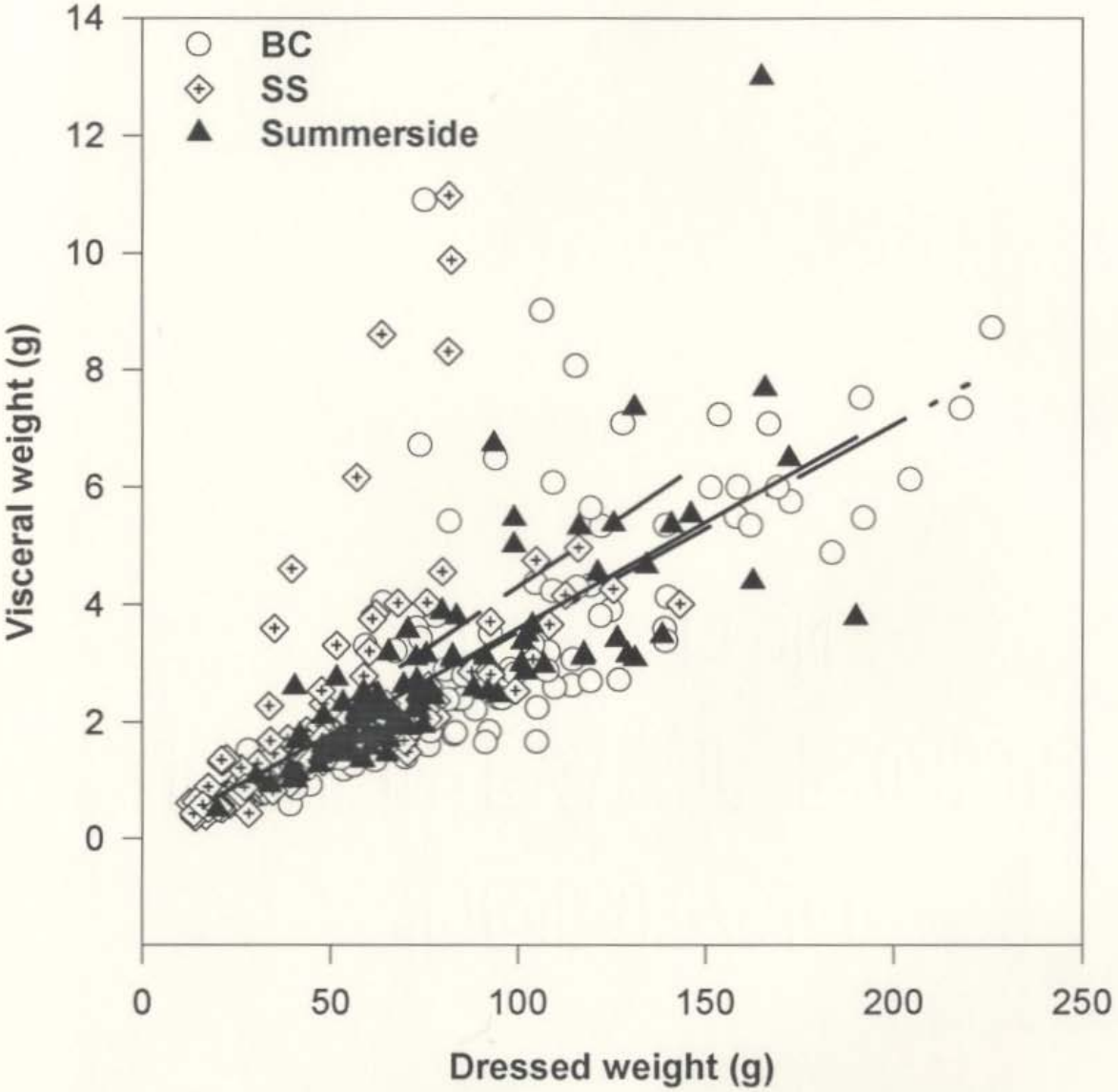
BC:Liver weight=0.04 dressed weight - 0.57, p < 0.00005, r=0.85
SS:Liver weight=0.03 dressed weight + 0.32, p < 0.00005, r=0.73
GM:Liver weight=0.02 dressed weight + 0.32, P < 0.00005, r=0.72
Regression over groups:BC vs GM, p=0.00001
Regression over groups:SS vs GM, p=0.0002

Figure A. 7 a. Dressed vs female liver weights regression analyses for Birchy Cove (BC), Summerside (SS) and Gillams (GM), 1994.



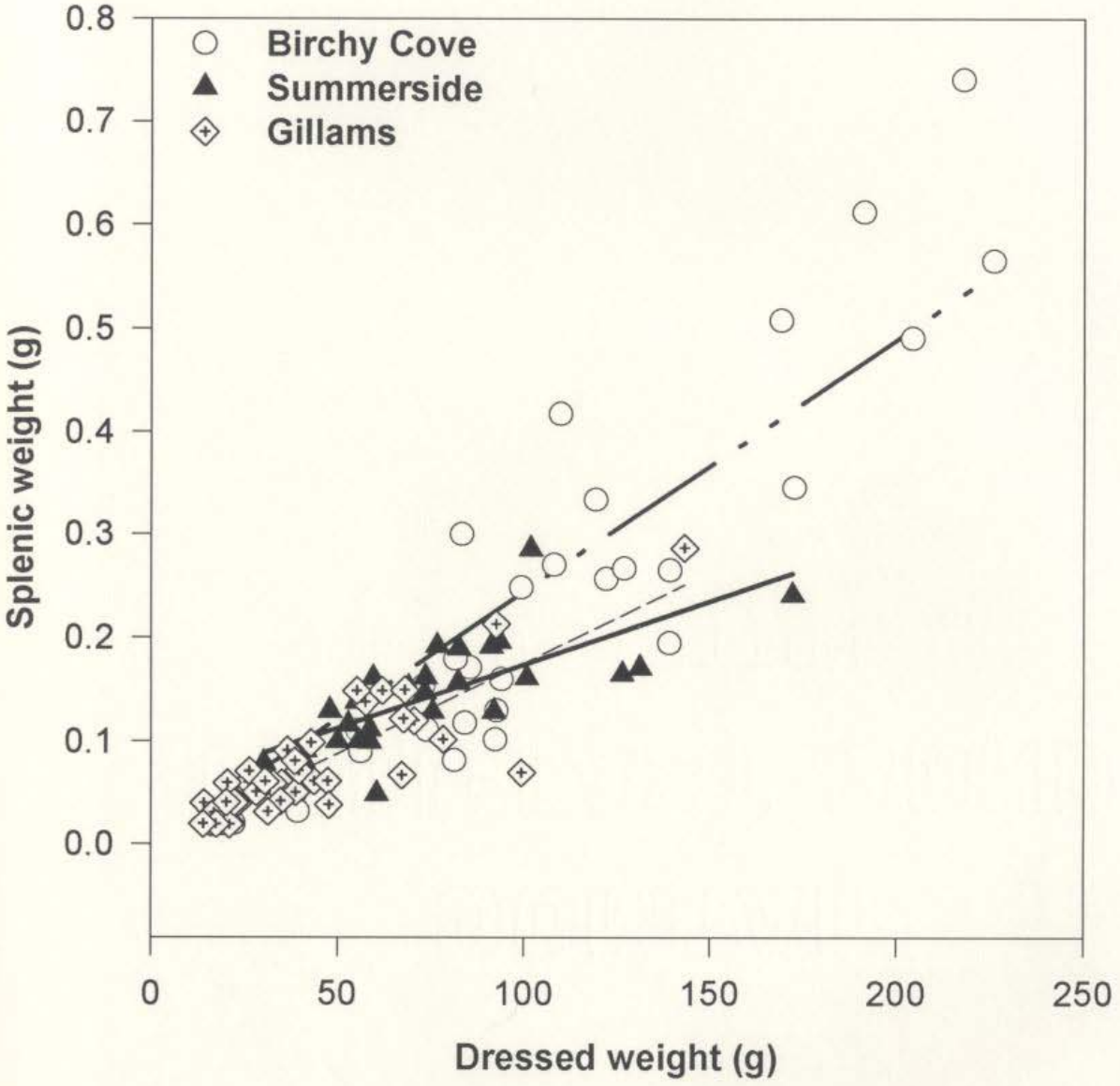
BC:Liver weight=0.03 dressed weight - 0.52, p < 0.00005, r=0.93
SS:Liver weight=0.02 dressed weight + 0.001, P < 0.00005, r=0.87
GM:Liver weight=0.02 dressed weight - 0.1, p < 0.00005, r=0.87
Regression over groups:BCvs GM, p=0.001
Regression over groups:SS vs GM, p=0.25

Figure A. 7 b. Dressed vs male liver weights regression analyses for Birchy Cove (BC), Summerside (SS) and Gillams (GM), 1994.



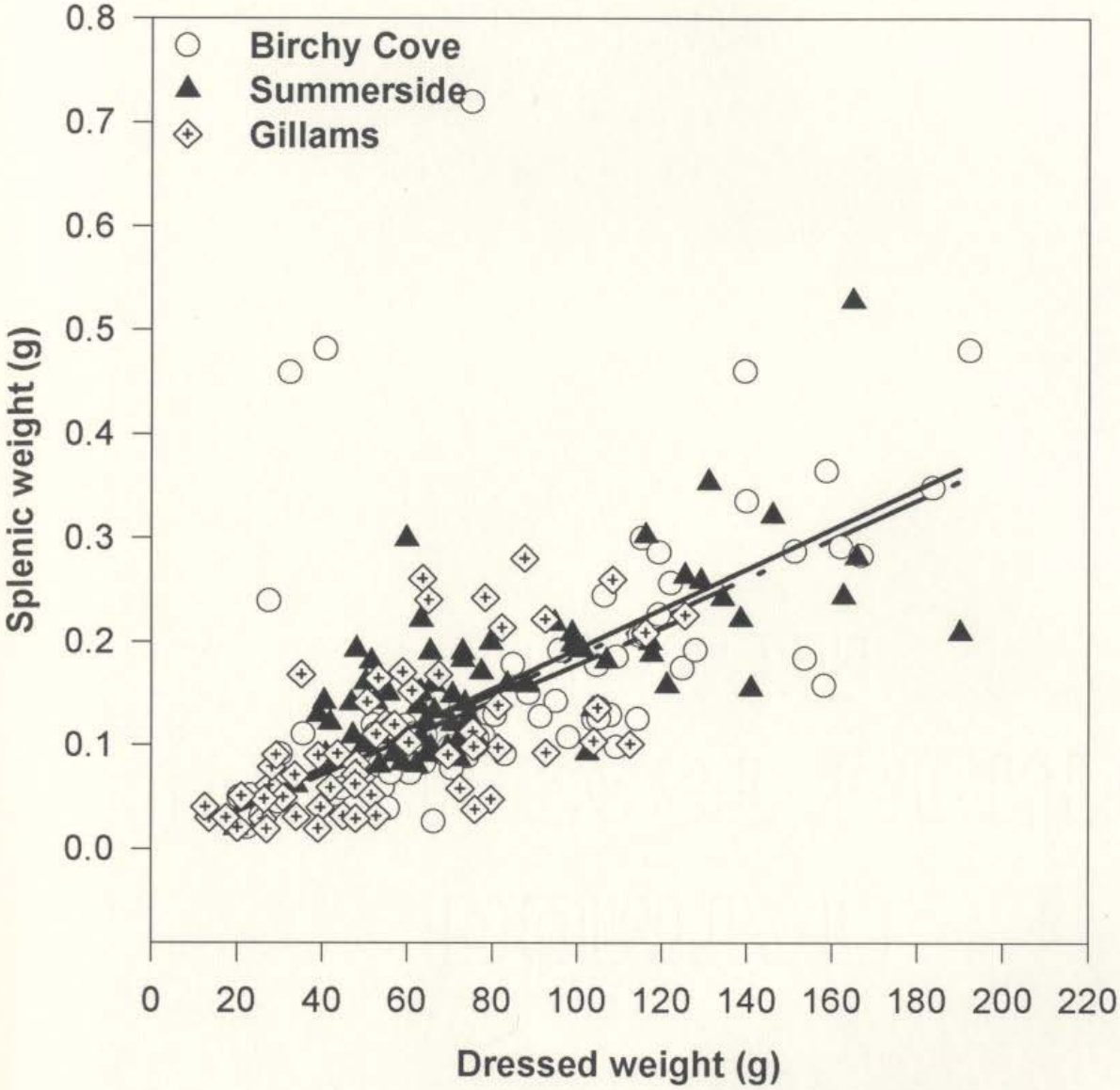
BC:Visceral weight=0.04 dressed weight - 0.06, p < 0.00005, r=0.76
SS:Viseceral weight=0.04 dressed weight - 0.28, p < 0.00005, r=0.78
GM:Visceral weight=0.04 dressed weight - 0.05, p < 0.00005, r=0.61
Regression over groups:BC vs GM, p=0.05
Regression over groups:SS vs GM, p=0.03

Figure A. 8 Dressed vs visceral weights regression analyses for Birchy Cove (BC), Summerside (SS) and Gillams (GM), 1994.



BC:Spleen weight=0.23*10^{-2} dressed weight - 0.02, p < 0.00005, r=0.71
SS:Spleen weight=0.15*10^{-2} dressed weight + 0.04, p < 0.00005, r=0.71
GM:Spleen weight=0.17*10^{-2} dressed weight + 0.01, p < 0.00005, r = 0.71
Regression over groups:BC vs GM, p=0.19
Regression over groups:SS vs GM, p=0.02

Figure A. 9 a. Dressed vs male splenic weights regression analyses for Birchy Cove (BC), Summerside (SS) and Gillams, 1994.



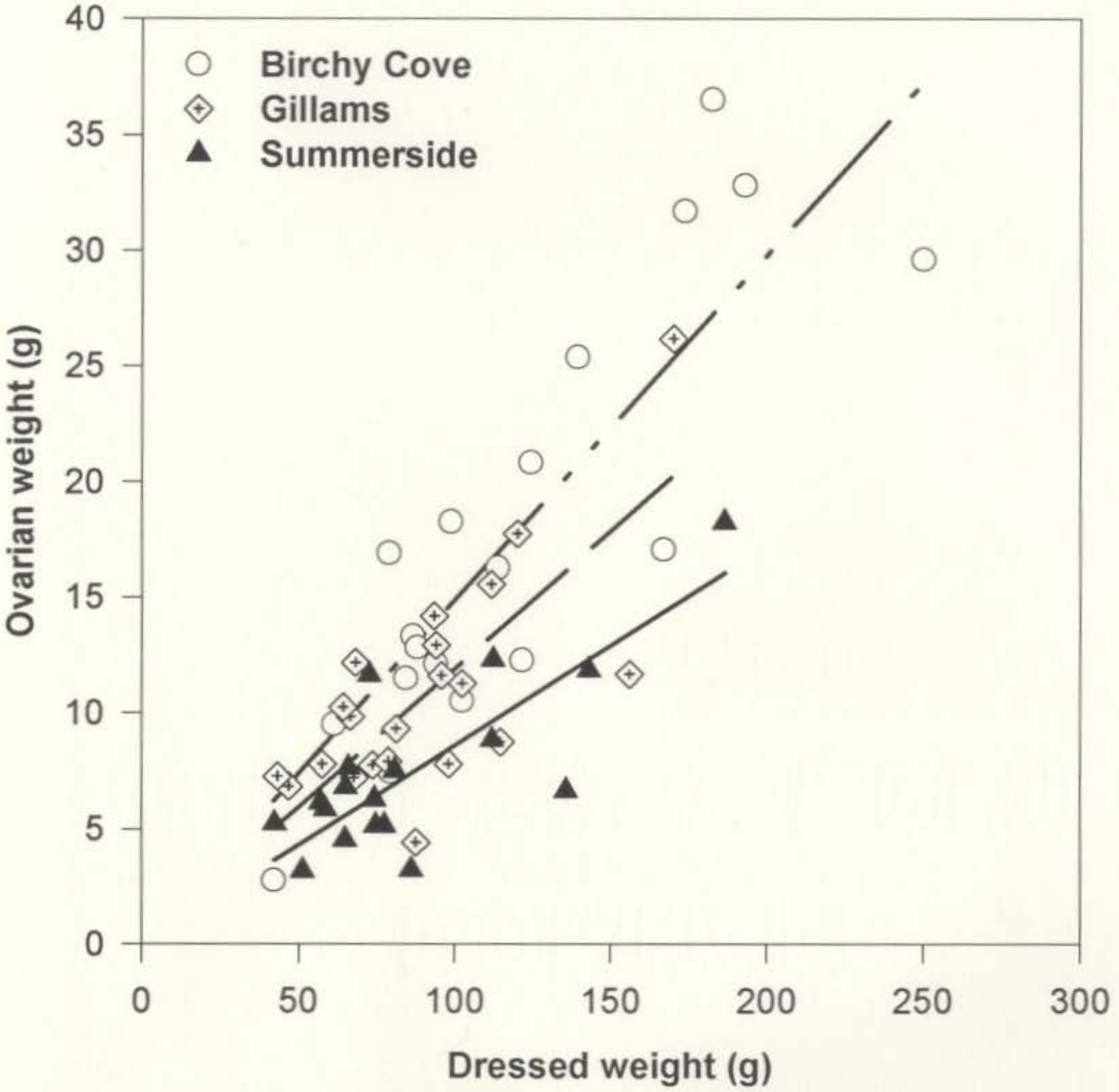
BC:Spleen weight=$0.23*10^{-2}$ dressed weight - 0.02, $p < 0.00005$, r=0.71

SS:Spleen weight=$0.15*10^{-2}$ dressed weight + 0.04, $p < 0.00005$, r=0.71

GM:Spleen weight=$0.17*10^{-2}$ dressed weight + 0.01, $p < 0.00005$, r = 0.71

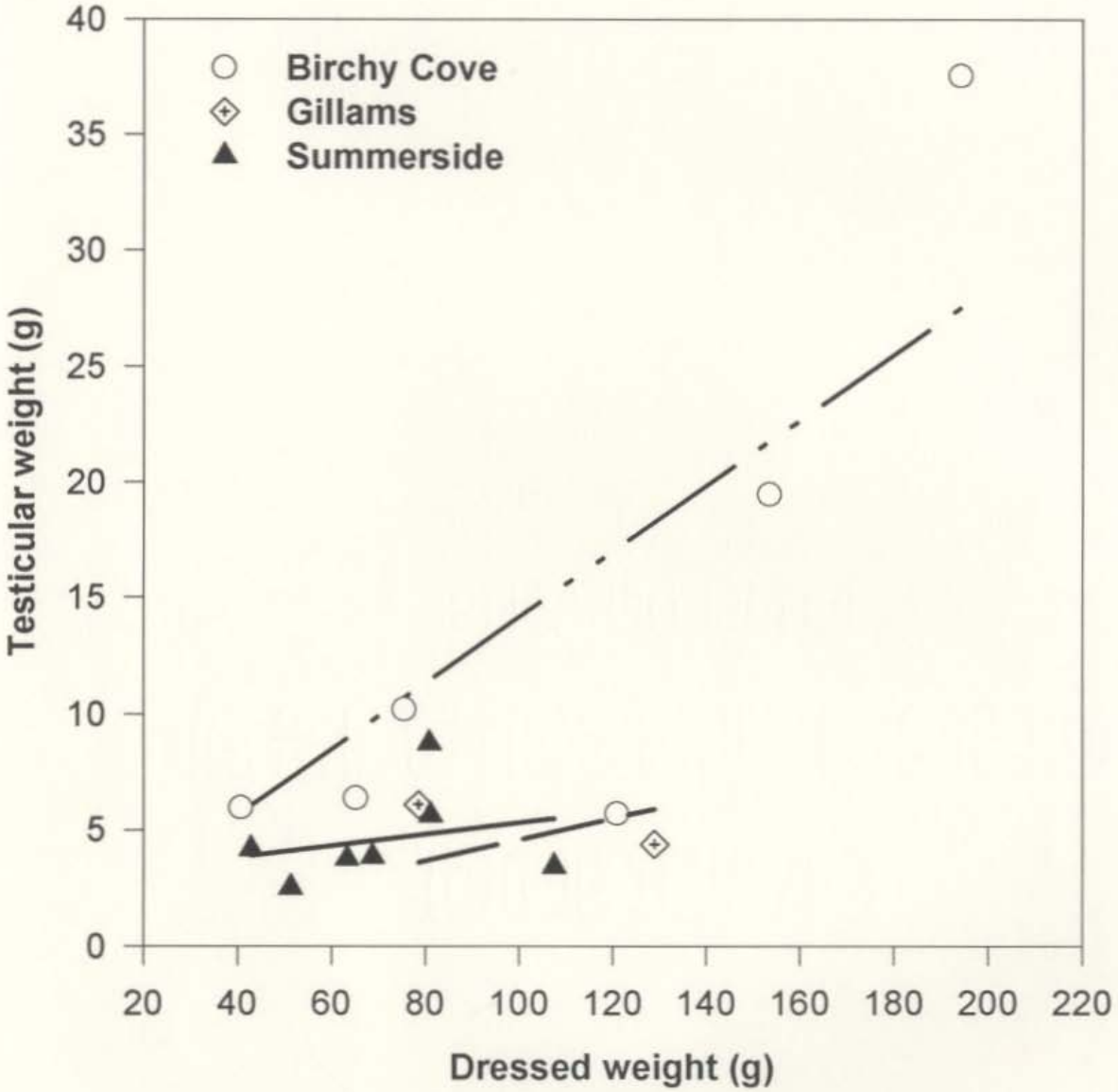
Regression over groups:BC vs GM, p=0.19
Regression over groups:SS vs GM, p=0.02

Figure A. 9 b. Dressed vs female splenic weights regression analyses for Birchy Cove (BC), Summerside (SS) and Gillams, 1994.



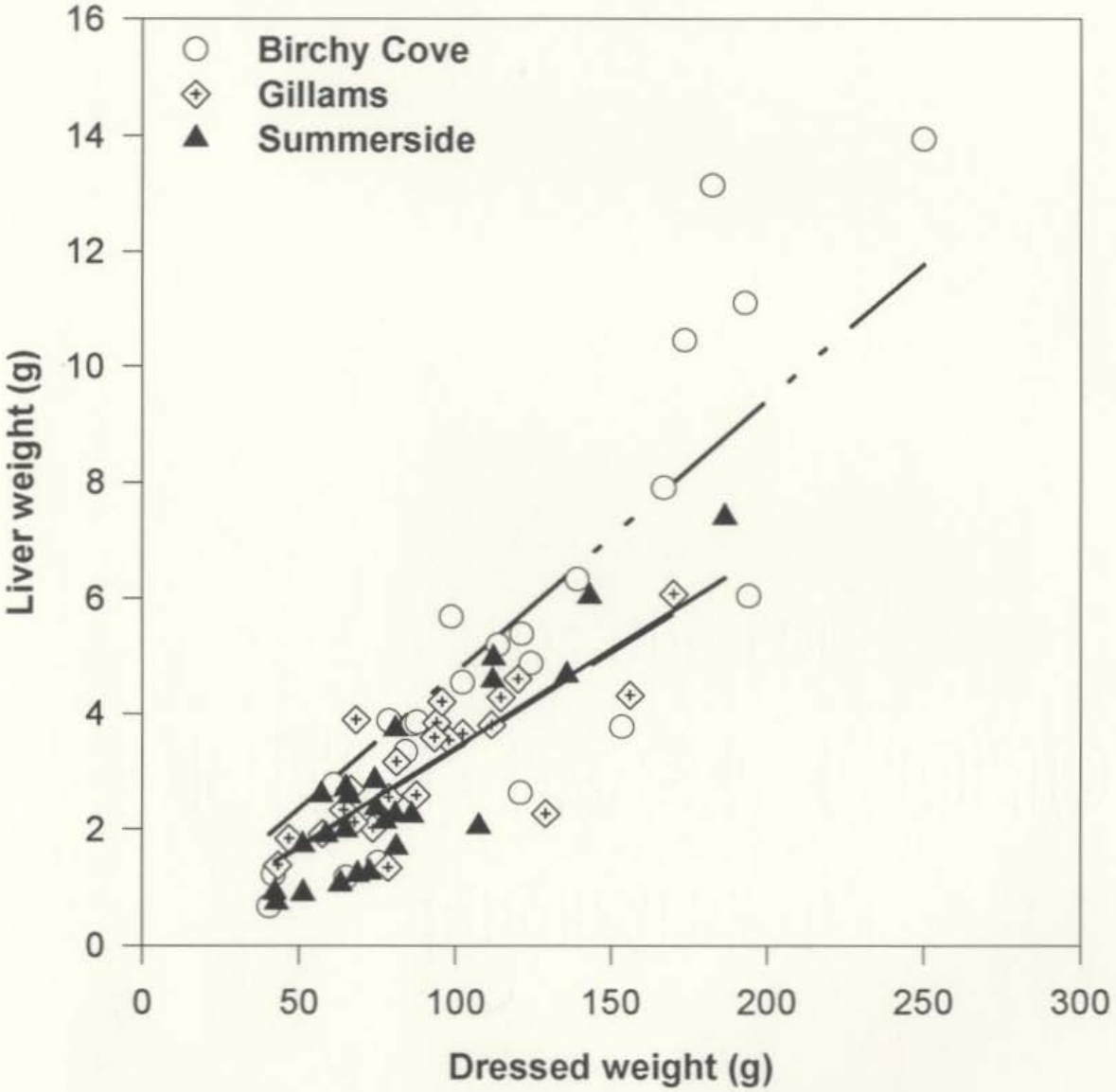
BC:Ovarian weight=0.15 dressed weight - 0.66, p < 0.00005, r=0.86
SS:Ovarian weight=0.08 dressed weight + 0.79, p=0.00002, r=0.79
GM:Ovarian weight=0.11 dressed weight + 1.44, p=0.0003, r=0.71
Regression over groups:BC vs GM, p=0.07
Regression over groups:SS vs GM, p < 0.00005

Figure A. 10 a. Dressed vs ovarian weights regression analyses for Birchy Cove (BC), Summerside (SS) and Gillams (GM), 1995.



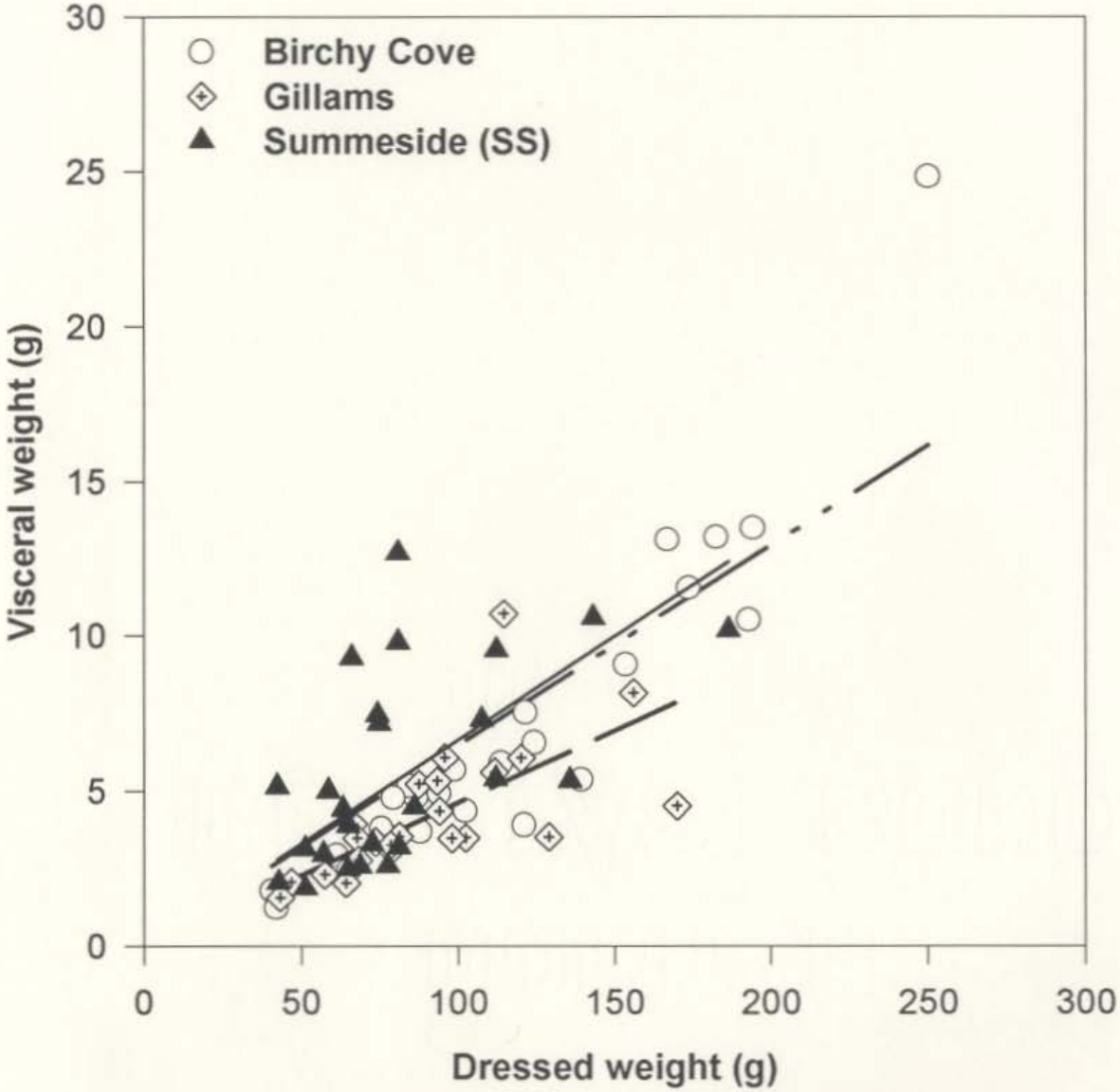
BC:Testicular weight=0.19 dressed weight - 5.82, p=0.03, r=0.86
SS:Testicular weight=0.02 dressed weight + 2.82, p=0.57, r=0.26
GM: Not enough cases to execute regression analysis, n=2

Figure A. 10 b. Dressed vs testicular weights regression analyses for Birchy Cove (BC), Summerside (SS) and Gillams (GM), 1995.



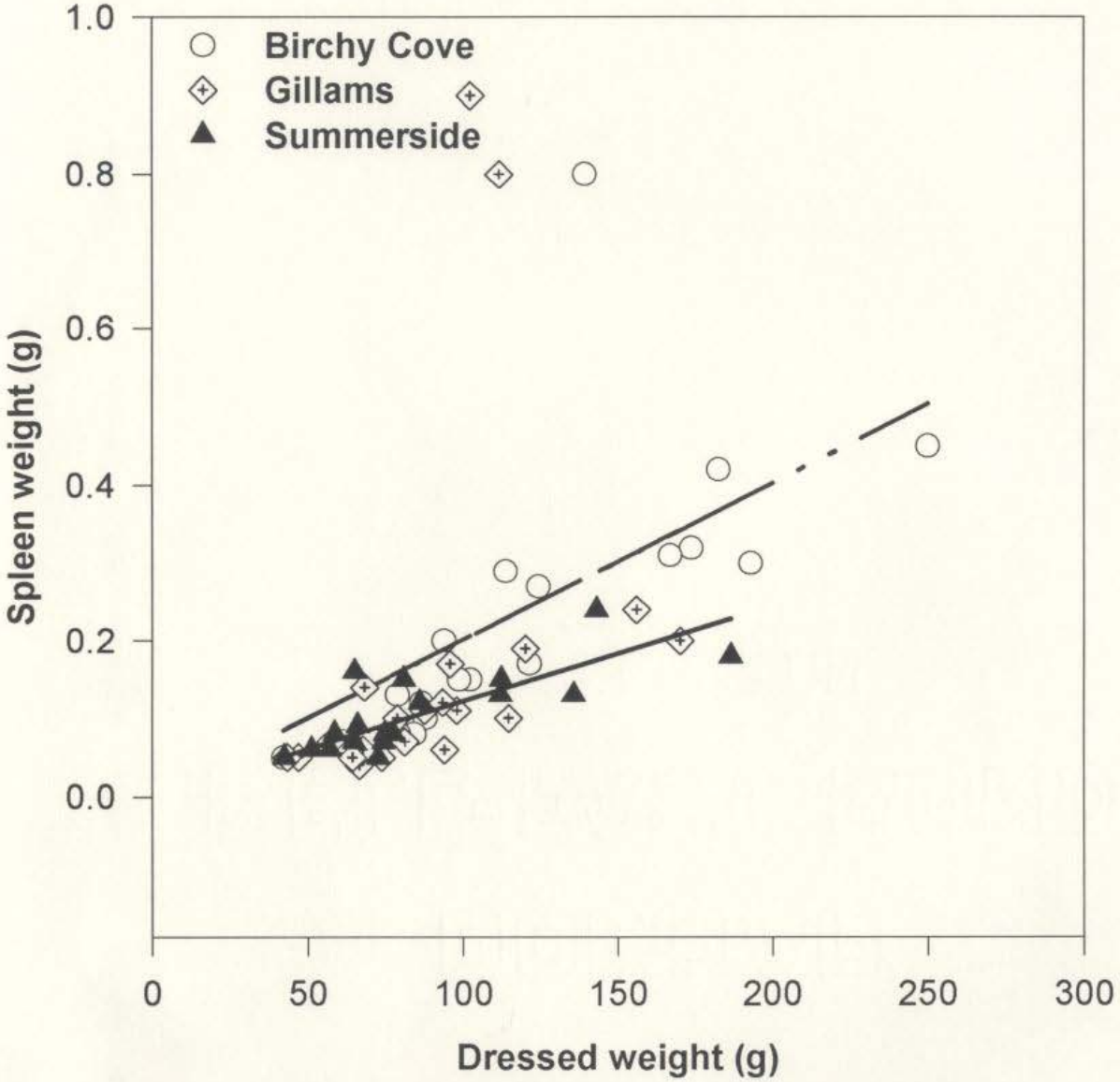
BC:Liver weight=0.06 dressed weight - 1.78, p < 0.00005, r=0.89
SS:Liver weight=0.04 dressed weight - 1.03, p < 0.00005, r=0.90
GM:Liver weight=0.03 dressed weight + 0.49, p < 0.00005, r=0.78
Regression over groups:BC vs GM, p=0.002
Regression over groups:SS vs GM, p=0.06

Figure A. 11. Dressed vs liver weights regression analyses for Birchy Cove (BC), Summerside (SS) and Gillams (GM), 1995.



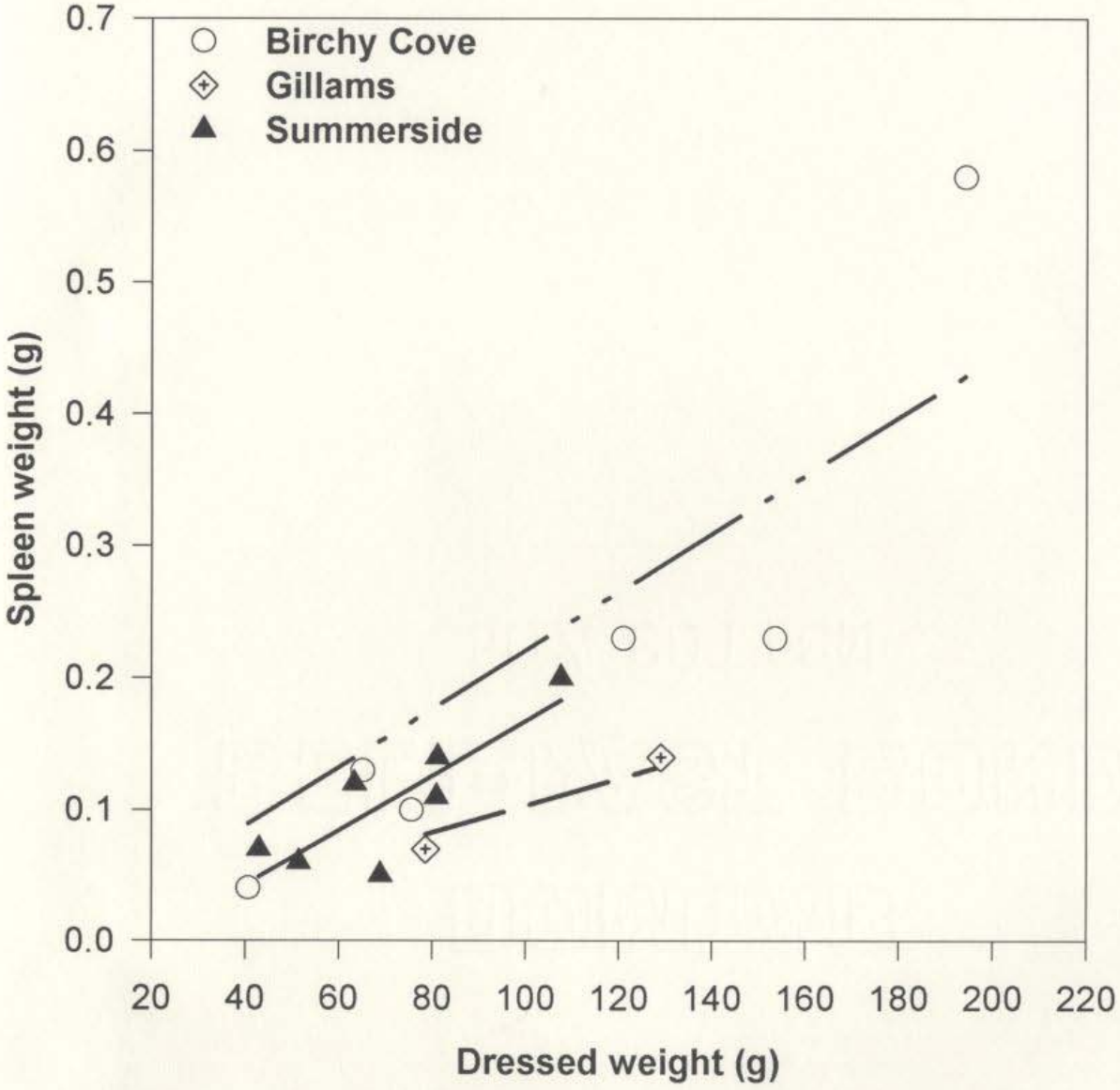
BC:Visceral weight=0.09 dressed weight - 3.69, p< 0.00005, r=0.93
SS:Visceral weight=0.05 dressed weight + 1.46, p=0.004, r=0.56
GM:Visceral weight=0.04 dressed weight + 0.50, p=0.001, r = 0.63
Regression over groups:BC vs GM, p=0.002
Regression over groups:SS vs GM, p=0.02

Figure A. 12. Dressed vs visceral weights regression analyses for Birchy Cove (BC), Summerside (SS) and Gillams (GM), 1995.



BC:Spleen weight=0.23*10^{-2} dressed weight - 0.05, p=0.002, r=0.68
SS:Splenic weight=0.11*10^{-2} dressed weight + 0.02, p=0.0002, r=0.77
GM:Spleen weight=0.002 dressed weight - 0.06, p =0.10, r=0.36
Regression over groups:BC vs GM, p=0.96
Regression over groups:SS vs GM, p=0.33

Figure A. 13 a. Dressed vs female splenic weights regression analyses for Birchy Cove (BC), Summerside (SS), Gillams (GM), 1995.



BC:Spleen weight=0.30*10^{-2} dressed weight - 0.11, p=0.01, r=0.92
SS:Splenic weight=0.21*10^{-2} dressed weight - 0.04, p=0.03, r=0.84
GM:Not enough cases to execute regression, n=2

Figure A. 13 b. Dressed vs male splenic weights regression analyses for Birchy Cove (BC), Summerside (SS) and Gillams (GM), 1995.