EVIDENCE OF CHRONIC STRESS IN WINTER FLOUNDER, PLEURONECTES (= PSEUDOPLEURONECTES) AMERICANUS LIVING ADJACENT TO A PULP AND PAPER MILL IN ST. GEORGE'S BAY, WESTERN NEWFOUNDLAND

DUANE EDWARD BARKER

Evidence of chronic stress in winter flounder, *Pleuronectes* (= *Pseudopleuronectes*) *americanus* living adjacent to a pulp and paper mill in St. George's Bay, Western Newfoundland.

by

Duane Edward Barker

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Department of Biology

Memorial University of Newfoundland

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Abstract

Winter flounder, *Pleuronectes (= Pseudopleuronectes) americanus*, living adjacent to a pulp and paper mill at Port Harmon, Stephenville, Newfoundland, were compared with flounder from a reference site, St. George's, 12 km from the mill, for signs of chronic stress. Several bioindicators of stress in fish were employed, including condition factor, organ somatic indices, haematological values, external lesions, and parasitofauna. Winter flounder from Port Harmon, (effluent site) showed significantly lower condition factors (K-factors) and hepatosomatic indices (HSI) indicative of depleted energy reserves, and physiological impairment, than those from St. George's. Delayed spawning was evident in both male and female winter flounder from Port Harmon compared with those from St. George's. Blood haemoglobin, haematocrit, and lymphocyte levels were significantly lower at Port Harmon, than in samples from St. George's. Fin necrosis of the caudal, dorsal and anal fins, was greater (in terms of prevalence and intensity) in flounder from Port Harmon. The prevalence and intensity of intestinal nematodes was significantly higher at Port Harmon, than St. George's; possibly the result of differences in diet. Conversely, the prevalence and intensity of intestinal acanthocephalans was significantly lower at Port Harmon, and was possibly related to effluent discharge. No differences were

found in a comparison of prevalence and intensity of intestinal digeneans. The prevalence and severity of infection of a parasitic microsporan protozoan, *Glugea stephani*, and the encysted metacercaria of the digenean, *Cryptocotyle lingua*, were, however, significantly higher among flounder from Port Harmon. Anaemia, low lymphocyte levels, a high prevalence of fin necrosis and parasitemias (*Glugea stephani*, *Cryptocotyle lingua*) are all suggestive of immunosuppression. Since the above differences were not attributed to differences in basic water parameters (temperature, salinity, conductivity, pH, and dissolved oxygen) at the two sites, the chronic stress evident in winter flounder from Port Harmon is most likely attributed to pulp mill effluent discharged at Port Harmon.

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Dedication

This thesis is dedicated to the memory of both my grandfathers, Mr. T. Barker and Mr. P. Taylor, who sadly passed away during the completion of this project . God bless them.

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List of abbreviations and symbols used

GSI = gonadosomatic index

HSI = hepatosomatic index

SSI = splenosomatic index

VSI = visceralsomatic index

HCRT = haematocrit

wgt = weight

TCDD = tetrachlorodibenzo-*para*-dioxin

K-factor = condition factor

(♀) = female

(♂) = male

HB = haemoglobin

LYM = lymphocyte number

Introduction

Anthropogenic pollutants are continually being added to the marine ecosystem and considerable interest has focused on the chronic effects of such pollutants on marine communities. Several studies recently have reported the effects of pulp and paper effluent on fish assemblages and these results have demonstrated weight loss, impaired liver function, skin lesions, fin necrosis, cellular hypertrophy, disruption of the immune system, presence of tumours and changes in parasitofauna (Adams et al.,1992; Andersson et al.,1988; Couillard et al., 1988; Khan et al.,1992; Hodson et al.,1992; Lehtinen, 1990; Lehtinen et al.,1984; Lindesjoo and Thulin, 1990; Lindstrom - Seppa and Oikari, 1990; McMaster et al.,1991; Munkittrick et al.,1992). Many toxic chemicals have been identified in effluents from pulp mills using chlorine (Cl_2) for bleaching, including chlorophenols, resin acids, furans, and dioxins (Bettis, 1991; Waldichuck, 1990). One of the most toxic chemical compounds in pulp effluents is the dioxin : 2,3,7,8 - tetrachlorodibenzo-*para*-dioxin (TCDD), which is produced as a by-product when chlorine is used as a pulp bleaching agent (Bettis, 1991; Waldichuck, 1990). High levels of carcingenic dioxins found in the invertebrate tissues and sediment, located close to pulp mills, caused several shellfish fisheries in 1988 in British Columbia to be closed (Waldichuck, 1990) .

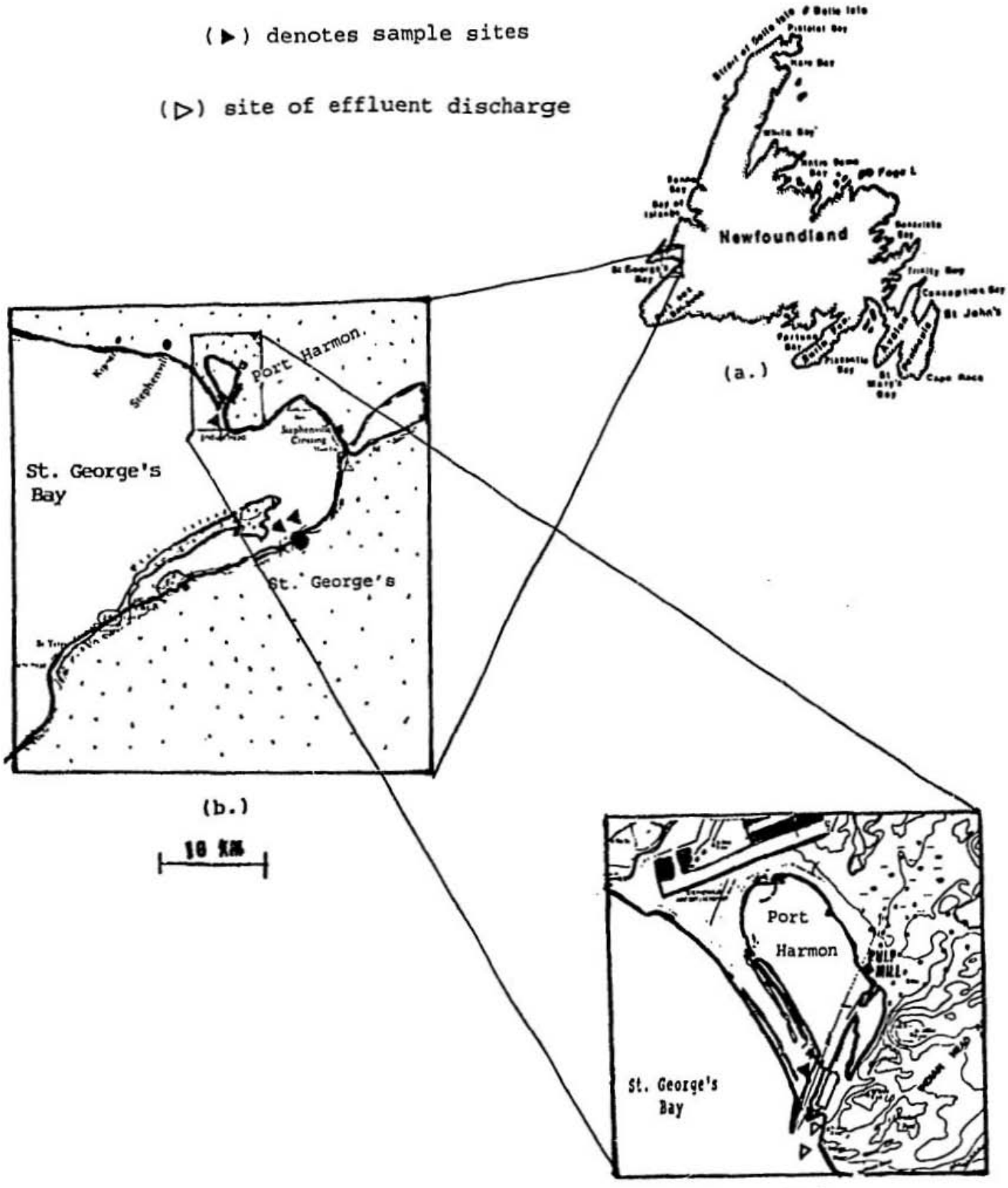
Newfoundland currently has three pulp and paper mills in operation: one in central Newfoundland (Grand Falls - Windsor) which discharges its effluent into the Exploits River, and two on the west coast of Newfoundland, Corner Brook (Bay of Islands) and Stephenville (St. George's Bay), which discharge their effluent into the marine environment.

Stephenville first opened the mill at Port Harmon in 1974 producing linerboard, which ran until 1977 (Anon., 1981a). In 1981, the mill was reopened by Abitibi-Price Inc. as a newsprint mill which uses thermomechanical (TMP) pulp, mixed with a maximum of 10% pre-bleached imported kraft pulp (Anon., 1981a; 1981b). A confirmed report from the public relations division of Abitibi-Price in Stephenville stated that chlorine was used for pulp bleaching during linerboard production, but currently, sodium hydrosulphite is used as the bleaching chemical on its stock pulp. The type of bleach used on the imported kraft pulp (15,000 tonnes annually - Anon., 1981a) was not disclosed. The effluent discharged from the Stephenville mill is temporarily stored in a lagoon for a period of 24 to 48 hours, receives primary treatment, and is pumped directly into the Port Harmon - St. George's Bay area at an average rate of 18,000 cubic meters per day (Abitibi-Price Public relations, pers. comm.) (Figure 1c.). The effluent discharge pipe has a mean diameter of 1.5 meters, and is located at a depth of approximately 5.5 meters. Values of absobable organic halogens

Figure 1: Sample site depicting location of St. George's Bay (a.), sample sites of St. George's Bay (b.), and Port Harmon effluent site (c.)

(▶) denotes sample sites

(▷) site of effluent discharge



(AOX), biological oxygen demand (BOD), acute toxicity, and effluent temperature were not disclosed.

Port Harmon supports a year round population of winter flounder ("black-back"), *Pleuronectes* (formerly *Pseudopleuronectes*) *americanus*. Winter flounder are a relatively important commercial species belonging to the family Pleuronectidae, a group of flatfishes characterized by having both eyes on the right side of the head (Scott and Scott, 1988). Winter flounder seldom exceed 46 cm in length, and are common inhabitants of inshore areas, where they are caught and marketed as fillets of "sole" or "flounder" (Pitt, 1984; Scott and Scott, 1988). In Newfoundland waters during fall (October, November), adult winter flounder migrate into shallow waters, cease feeding and submerge in the substrate, where they remain until emerging to spawn in spring (April,May) (VanGuelpen and Davis, 1979). Winter flounder tend to prefer temperatures less than 14°C in north temperate regions, and larger adults (> 26 cm) will often migrate to deeper, cooler inshore waters during summer.

Winter flounder are "sight-feeders" and diurnally consume a variety of benthic prey including : polychaetes, bivalves, amphipods, isopods, decapods, coelenterates, as well as the eggs of herring (*Clupea harengus*), capelin (*Mallotus villosus*) sculpin (*Myoxocephalus spp.*), and winter flounder (*P. americanus*) (Keats, 1990; Kennedy, 1964; MacDonald and Waiwood,

1987). MacDonald and Waiwood (1987) calculated a daily feeding ration for winter flounder to be 1.77% body weight/day during the summer months when feeding rates are highest.

The winter flounder, *P. americanus*, is a host to many parasites including : (i) Acanthocephala (*Echinorhynchus, Corysoma spp.*), (ii) Nematoda (*Contracecum, Pseudoterranova spp.*), (iii) Digenea (*Derogenes, Fellodistomum, Lecithaster, Podocotyle, Cryptocotyle spp.*), (iv) Cestoidea (*Bothriocephalus, Bothriomonus spp.*) and (v) Protozoa (*Glugea stephani*) (Margolis and Arthur, 1979; Ronald, 1963; Scott, 1982; Takvorian and Cali, 1984).

Stress may be defined as "...the sum of all physiological responses that occur when animals attempt to establish or maintain homeostasis, the stressor being an environmental alteration, and stress - the organism's response..." (Adams, 1990). Chronic stress or sublethal stress is common among wild populations; its effects are manifested over time and may alter the entire life cycle of an animal, whereas, acute stress is often lethal, and its short term effects may act on one component only of an animal's life cycle but could be extremely harmful (Adams, 1990). Adams (1990) discussed the validity and usefulness of several techniques that can be used as bioindicators of stress in fish populations; these techniques can be summarized as: (i) cellular genetics, (ii) enzyme activity (P450, MFO), (iii) histopathological studies,

(iv) organismic indices (condition factors, somatic indices), (v) blood parameters (haemoglobin, haematocrit, lymphocyte numbers), and (vi) species diversity. A review by Khan and Thulin (1991) examines the uses of parasitic infestations in fish as bioindicators of pollution.

The winter flounder is an ideal specimen to use as an ecological indicator of pollution based on the aforementioned benthic lifestyle and associated parasitofauna. Thus, the present study was undertaken to compare winter flounder, *P. americanus*, populations living adjacent to the Stephenville - Port Harmon pulp mill, and those from a reference site, St. George's, using several techniques which are bioindicators of chronic stress.

Materials and Methods

Field Sampling

This study was conducted on the west coast of Newfoundland, St. George's Bay (Figure 1). Sampling occurred during the spring, summer and fall of 1991 and 1992. No winter or early spring samples were obtained as the areas were frozen during this period. Samples were collected from Port Harmon (48° 31'N,58° 33'W) (approx. 1 km from the effluent discharge site) and from a reference site, St. George's (48° 26'N,58° 30'W) (approx. 12 km from the effluent site and free of effluent discharge). The bottom substrate of the Port Harmon sample site consisted of soft, muddy sediment mixed with wood fibres. The depth at the wharf site varied from 3 - 4 meters, which then increased at a 45° angle until a maximum depth of 13 meters was attained about 40 meters from the shoreline. The Port Harmon site is a high marine traffic area, used by local fishermen, the Coast Guard, and as a conduit to export mill products. During the summer of 1992, considerable bottom dredging occurred in the Port Harmon basin. Benthic flora common to the area included: Phaeophytes (*Laminaria, Fucus, Chorda, Desmarestia, Punctaria spp.*), Rhodophytes (*Chondrus, Palmaria, Ceramium, Ptilota, Polysiphonia, Phyllophora spp.*) Chlorophytes (*Ulva, Enteromorpha, Cladophora spp.*), and sea grass, Angiospermae (*Zostea sp.*). The local fauna included

molluscs (*Mytilus, Hiatella, Mya, Littorina, Acmacea, Tonicella spp.*), annelids (*Nereis, Myicola spp.*), arthropods (Amphipoda, Isopoda, Decapoda), and fish (*Pleuronectes americanus, Myoxocephalus scorpius, M. octodecimspinosus, Tautogolabrus adspersus, Microgadus tomcod*).

The bottom substrate at the St. George's site consisted of fine sand interspersed with occasional rock projections. The St. George's site was slightly shallower, being 2.5 - 3.0 meters near the wharf, with a gentle slope of about 10° to a depth of 5 - 6 meters approximately 300 meters from the shoreline. Both the flora and fauna were similar to those at Port Harmon, with the exception that echinoderms (*Asterias, Ophiopholus spp.*) and skates (*Raja sp.*) were found only in the St. George's area.

Samples were collected from depths of 2 - 10 meters by SCUBA divers using a dipnet (8 cm mesh size) or by hook and line from public wharves at each site. These wharves were primarily used by local fishermen for unloading and processing their catch. As a consequence of fishing activity, both sites were areas of fish offal disposal, providing ample foraging opportunities for the local fish assemblages. Water samples were collected in plastic 125 ml bottles and water temperatures were recorded at the surface (0 m.) and bottom (6 m.) from each sampling site during each sampling session of 1992.

The sampling in 1991 was a preliminary study in which

only length, weight, fin necrosis, and number and frequency of intestinal parasites were recorded from captured winter flounder. During 1992, fish were sacrificed via spinal severance following which a blood sample was taken using a 3ml heparinized syringe and a no.23 guage needle. A thin blood smear was made from each blood sample which was kept on ice until further analysis at the laboratory. Total length (nearest 0.1 cm) and eviscerated weight (nearest 0.1g) of each fish was recorded, along with the weight (nearest 0.001g) of the following organs: liver, spleen, gonad, and stomach (+ intestine). Samples of gill, liver, spleen, and gonad were placed in a 10 % formalin: 90 % phosphate buffer fixative for a histopathological study by Khan et al. (1993). Stomachs were individually tagged and frozen (1991) or placed in 10 % formalin (1992) for analysis.

The extent of fin necrosis on each fish was recorded at this time by measuring the amount of caudal fin eroded plus the length of each caudal fin. Any other sites of fin necrosis were also noted (i.e. dorsal fin).

The encysted metacercaria of *Cryptocotyle lingua* were enumerated from the following sites on each fish : (i) right pectoral fin, (ii) right pelvic fin, (iii) first 10 posterior fin rays of the dorsal and anal fins, and (iv) first 5 dorsal fin rays of the caudal fin (see Appendix A).

Salinity and conductivity were measured from each water

sample using a standard portable YSI meter, while dissolved oxygen levels and pH were measured using a Hach™ kit (which includes methods).

Laboratory Analysis

Blood haemoglobin levels (g./100 ml) were measured using a standard, portable American Optical™ haemoglobinometer, and blood haematocrit levels (% blood cells / total blood) were recorded following the procedures outlined in Blaxhall (1972). Blood smears were stained with 5ml Giemsa : 95ml phosphate buffer (pH 7.6) for 45 minutes and examined with a compound microscope at 1000x (oil immersion) to record the number of lymphocytes per 1000 erythrocytes for each fish.

Oocytes from preserved ovaries of mature female winter flounder were measured using a compound microscope eyepiece micrometer at 40x. The number of oocytes from each mature female was estimated by calculating the mean number of oocytes per three 2.0 gram samples of ovary and multiplying this mean number by the total ovarian weight (minus weight of ovarian wall and ducts).

Stomachs were dissected into a sieve tray (0.025mm mesh size) and the frequency of prey items and the prevalence and number of the following helminth taxonomic groups were recorded : (i) Acanthocephala, (ii) Nematoda, (iii) Digenea, and (iv) Cestoidea. Parasites were individually stained using

a trichome / hematoxylin stain as outlined in Schmidt (1988). In addition, the presence or absence of a parasitic microsporidan, *Glugea stephani*, on or in the viscera of each fish was made by microscopic examination.

Data Analysis

Somatic indices were calculated in the following manner :

(i) Condition Factor **KFactor** = 100x (eviserated weight/total length');

(ii) Hepatosomatic Index **HSI** = 100x (liver weight / eviscerated wgt);

(iii) Splenosomatic Index **SSI** = 100x(spleen weight / eviscerated wgt)

(iv) Visceralsomatic Index **VSI** = 100x(stomach weight/eviscerated wgt) (stomach weight includes intestine + food weight);

(v) Gonadosomatic Index **GSI** =100x (gonad wgt /gonad +eviscerated wgt);

In addition, **percent fin eroded (%)** was defined as 100 x (necrotic length / caudal length).

Statistical analysis of the data was performed using two computer software packages, SPSSX™ and MINITAB™. Values between both groups of fish were compared using a oneway ANOVA, with a Duncan's multiple groups test. Prevalence between both groups was compared using a G-test as outlined in Sokal and Rohlf (1987). Finally, a simple linear regression was used to test for correlation between: (i) weight and length, and (ii) caudal fin length and total length.

Figures and tables were provided using SPSS-GRAPHICS™ and Word Perfect™.

Results

Sample Statistics

A total of 400 winter flounder were collected from St. George's Bay during 1991 and 1992, 257 specimens from the study site at Port Harmon and 143 specimens from St. George's. Specimens were obtained in June, July, and September of 1991, and during May, June, July, August, and November of 1992. No winter flounder were found during the November 1991 sampling, and only 8 fish were collected (5 from Port Harmon, 3 from St. George's) during the November 1992 sampling . Due to the small sample size, and hence, large statistical variance, samples collected in November were omitted from monthly statistical comparisons.

Total length ranged from 11.0 - 45.7 cm, with most winter flounder in the range 22 - 28 cm (Figures 2,3). The mean length of the Port Harmon (1991-92) sample was 26.8 cm and it was significantly greater (p = 0.0013) than the St. George's (1991-92) sample (25.4 cm).

A simple linear regression of length-weight relationship of winter flounder from each pooled collection produced the following equations : (i) Port Harmon $Y_{(wgt)} = 24.55X_{(len)} - 442.39$ ($r^2 = .913$, s.e. = 46.44, $p < .001$), (ii) St. George's $Y_{(wgt)} = 21.97X_{(len)} - 382.86$ ($r^2 = .914$, s.e. = 34.01, $p < .001$). No statistical significance was observed between the two equat-

Figure 2: Length frequency histogram for P. americanus collected from Port Harmon during 1991 and 1992.

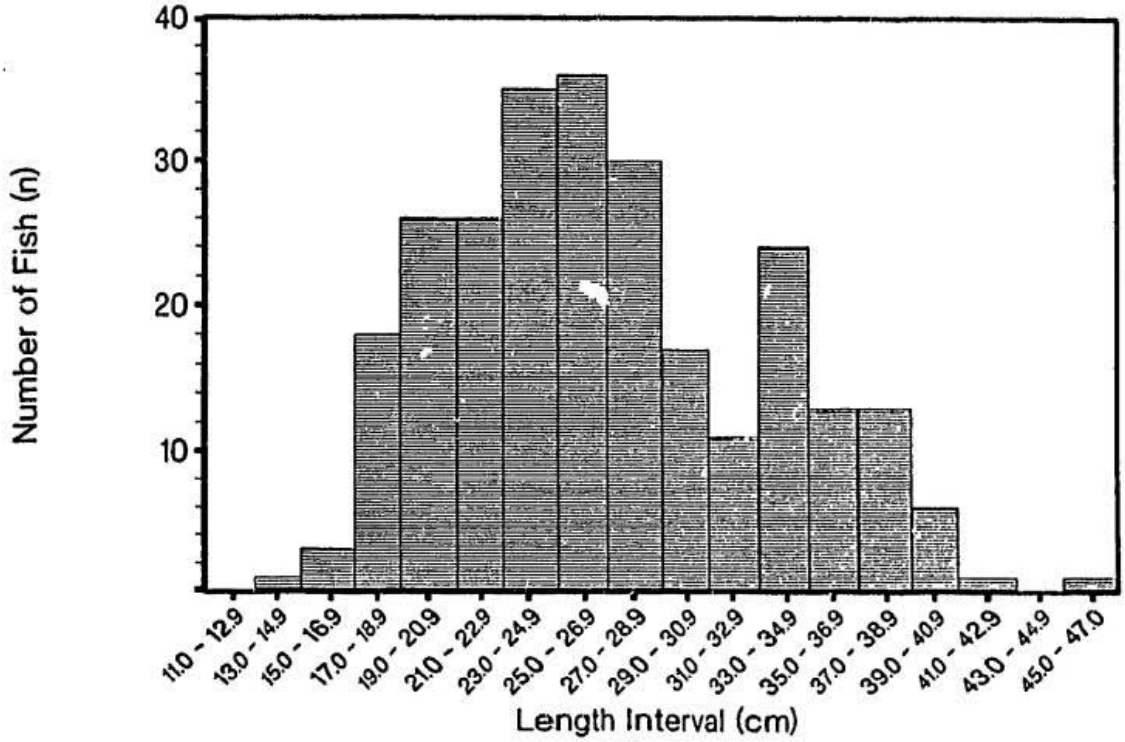
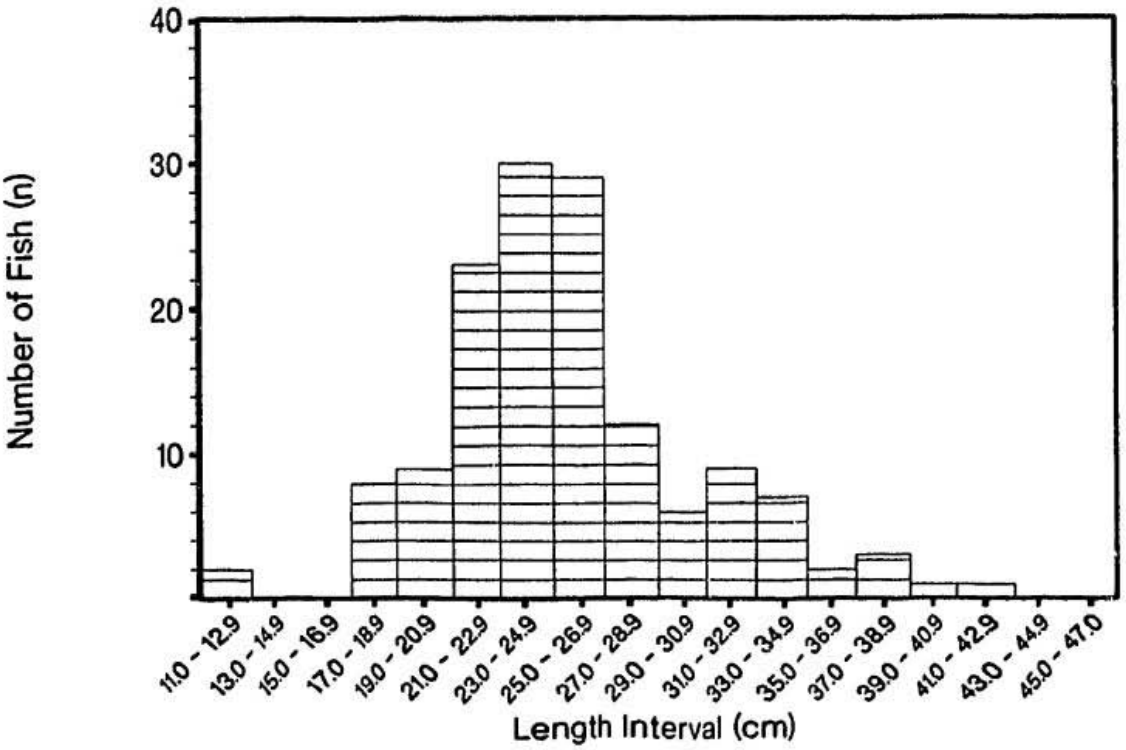


Figure 3: Length frequency histogram for P. americanus collected from St. George's during 1991 and 1992.



ions (p = 0.971). A comparison of length and weight using a one-way ANOVA for each month revealed no significant differences between any two pairs except the May and August 1992 samples, in which Port Harmon winter flounder were significantly greater in length ($p < 0.0001$) and dressed weight ($p < 0.0001$) than winter flounder collected from the reference site, St. George's (Table 1a). The pooled collections from 1991 showed no significant differences in length and weight, but the pooled summer (June,July, August) collections from 1992 showed significantly greater ($p < 0.0001$) lengths and weights in Port Harmon's sample than in the St. George's sample (Table 1b).

In both total collections, females slightly outnumbered the males. In the Port Harmon sample, 55% (141) were females while 45% (116) were males. In the St. George's sample, 59% (84) were females, while 41% (59) were males. These percentages were not significant when compared using a G-test.

Body and Organismic Indices

A comparison of monthly condition factors (K-factors) of winter flounder from both sites, using a one-way ANOVA, revealed significantly greater K-factor values ($p < 0.0001$) in all winter flounder collected from the reference site, St. George's, except during July and September 1991, and May 1992 (Table 1a). An analysis of summer pooled K-factors revealed

Table 1a: Length, weight, and condition factor (K-factors) of samples of winter flounder, *Pleuronectes americanus*, collected from Port Harmon and St. George's, during 1991 and 1992.

Month		Port Harmon	St. George's
June '91	Length (cm)	23.4 ± 1.2	20.2 ± 2.9
	Weight (g)	156.9 ± 35.2	121.8 ± 39.7
	K-factor (w/l^3)	0.91 ± 0.04	1.14 ± 0.04 **
	n	27	6
July '91	Length (cm)	24.3 ± 1.2	24.5 ± 1.8
	Weight (g)	164.8 ± 25.1	188.5 ± 40.4
	K-factor (w/l^3)	1.19 ± 0.04	1.24 ± 0.03
	n	4	4
Sept.'91	Length (cm)	21.9 ± 1.1	24.1 ± 1.8
	Weight (g)	120.3 ± 19.8	167.5 ± 39.4
	K-factor (w/l^3)	1.06 ± 0.05	1.12 ± 0.04
	n	9	8
May '92	Length (cm)	29.7 ± 0.6	26.6 ± 0.5 **
	Weight (g)	274.3 ± 15.3	194.8 ± 12.3 **
	K-factor (w/l^3)	0.92 ± 0.01	0.93 ± 0.01
	n	114	99
June '92	Length (cm)	23.3 ± 0.9	21.8 ± 1.1
	Weight (g)	124.3 ± 15.4	121.4 ± 17.2
	K-factor (w/l^3)	0.90 ± 0.03	1.09 ± 0.03 **
	n	19	11
July '92	Length (cm)	26.2 ± 0.8	24.1 ± 1.1
	Weight (g)	222.4 ± 20.1	175.6 ± 27.1
	K-factor (w/l^3)	1.05 ± 0.02	1.17 ± 0.04 **
	n	63	8
Aug. '92	Length (cm)	24.4 ± 0.7	20.4 ± 1.4 **
	Weight (g)	158.0 ± 13.6	98.3 ± 14.9 **
	K-factor (w/l^3)	1.03 ± 0.02	1.13 ± 0.04 **
	n	21	7

Note : length expressed as total length, **weight** expressed as eviscerated weight. Values expressed as $\bar{X} \pm$ s.e.
Both males and females used together because no difference
"**" $p < 0.05$

Table 1b: Pooled length, weight, and condition factor (K-factors) of samples of winter flounder, *Pleuronectes americanus*, collected from St. George's Bay during summer 1991 and 1992.

Year		Port Harmon	St. George's
Summer '91	Length (cm)	23.2 ± 0.91	22.8 ± 1.22
	Weight (g)	149.5 ± 24.2	156.9 ± 9.0
	K-factor (w/l^3)	0.97 ± 0.03	1.16 ± 0.02 **
	n	40	18
Summer '92	Length (cm)	25.3 ± 0.51	22.1 ± 0.66 **
	Weight (g)	191.2 ± 13.4	131.8 ± 12.8 **
	K-factor (w/l^3)	1.01 ± 0.01	1.17 ± 0.02 **
	n	103	26

Notes: "**" $p < 0.05$

Summer '91 = June, July, Sept. pooled
Summer '92 = June, July, Aug. pooled

length expressed as total length, **weight** expressed as eviscerated weight. Values expressed as $\bar{X}$ ± s.e. Both males and females used together because no difference

significantly higher ($p<0.0001$) K-factor values in St.George's compared with Port Harmon for both 1991 and 1992 (Table 1b). There was also a noticeable trend for K-factor values to increase during summer months, peaking in July, then decreasing during fall and winter for both sample sites (Figure 4). No differences between male and female K-factors were observed.

Monthly hepatosomatic indices (HSI) were significantly greater ($p <0.0001$) for winter flounder from Port Harmon during May and August (1992); but, were significantly lower ($p < 0.0001$ for winter flounder from Port Harmon during June and July (1992) when compared with winter flounder from St. George's (Table 2; Figure 5). Pooled hepatosomatic indices from summer of 1992 showed significantly higher ($p < 0.0001$) HSI values in St. George's winter flounder (Table 2).

No significant differences were observed between monthly splenosomatic (SSI) ($p = 0.677$) and visceralsomatic indices (VSI) ($p=0.109$) of Port Harmon and St. George's winter flounder (Table 2; Figures 6,7). Pooled summer VSI values were significantly greater ($p=0.028$) in St. George's winter flounder, but pooled summer SSI values were non-significant ($p = 0.242$) (Table 2).

Gonadosomatic indices (GSI) were significantly higher ($p = 0.0006$) for both males and females from Port Harmon during May and June (1992) and were not significant ($p=0.061$) for females from Port Harmon during July and August (1992) (Table 2; Figures 8,9). Scatterplots of GSI and length were const-

Figure 4: Mean (±s.e.) K-Factor values for *P. americanus* collected from St. George's Bay During 1991 and 1992.

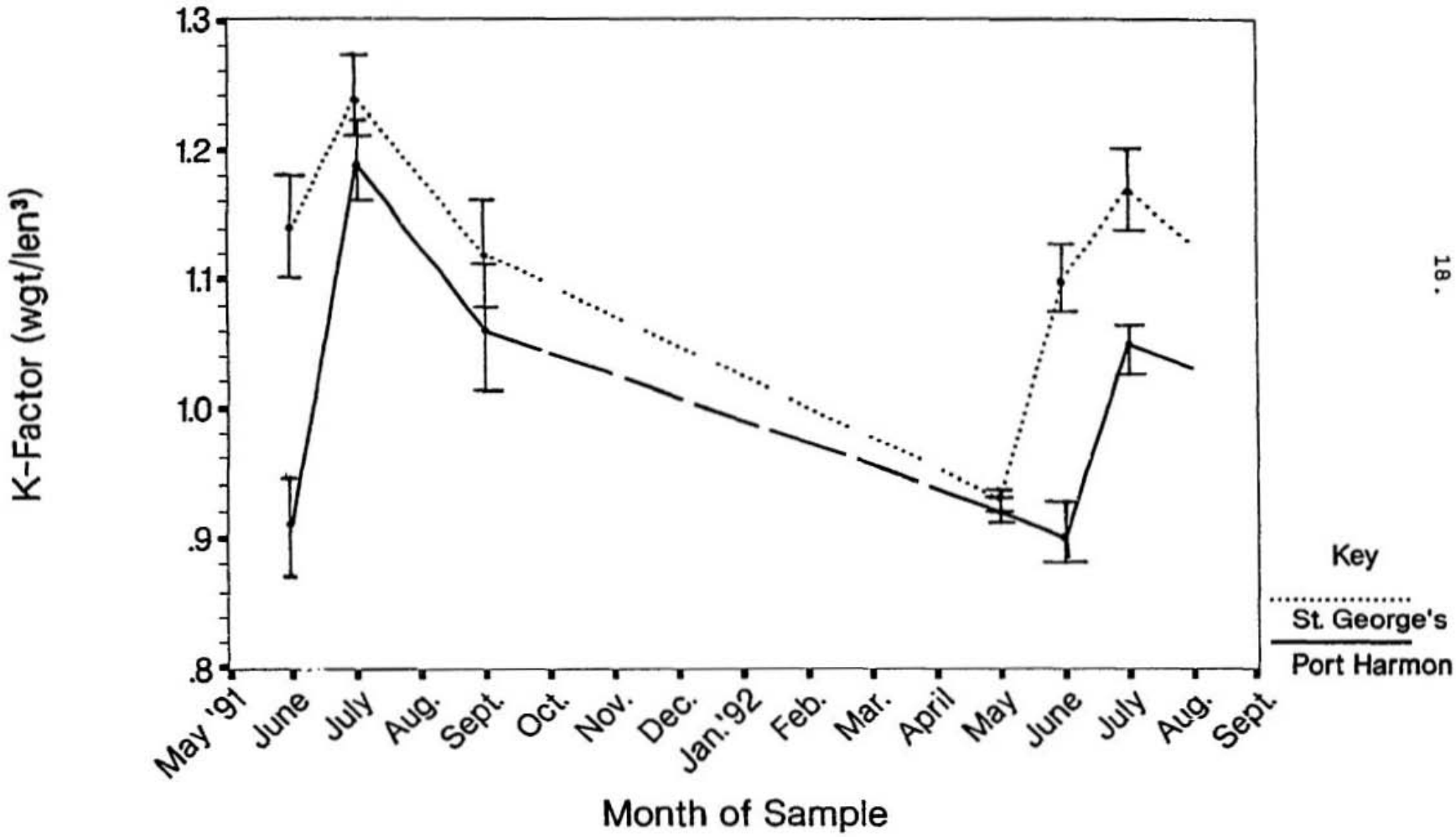


Table 2 : Mean (±s.e.) organ somatic indices of samples of *P.americanus* collected from Port Harmon and St. George's during 1992.

Month		Port Harmon	St. George's	
May '92	HSI	1.24 ± 0.04	0.99 ± 0.04	**
	SSI	0.13 ± 0.01	0.12 ± 0.01	
	VSI	7.70 ± 0.37	6.95 ± 0.30	
	GSI (♀)	9.93 ± 1.11	7.39 ± 1.00	**
	GSI (♂)	6.22 ± 0.62	4.98 ± 0.38	**
	n	108	96	
June '92	HSI	1.60 ± 0.17	2.01 ± 0.10	**
	SSI	0.11 ± 0.01	0.12 ± 0.01	
	VSI	8.36 ± 0.84	10.06 ± 0.60	
	GSI (♀)	3.51 ± 1.59	0.81 ± 0.08	**
	GSI (♂)	0.78 ± 0.04	0.54 ± 0.03	**
	n	19	11	
July '92	HSI	1.32 ± 0.06	1.79 ± 0.18	**
	SSI	0.12 ± 0.01	0.12 ± 0.01	
	VSI	7.92 ± 0.51	9.25 ± 0.97	
	GSI (♀)	1.95 ± 0.14	0.64 ± 0.10	*
	GSI (♂)	0.60 ± 0.04	0.56 ± 0.05	
	n	28	8	
Aug. '92	HSI	1.36 ± 0.07	0.94 ± 0.08	**
	SSI	0.12 ± 0.01	0.10 ± 0.01	
	VSI	6.09 ± 0.43	7.28 ± 0.77	
	GSI (♀)	2.02 ± 0.12	0.84 ± 0.47	*
	GSI (♂)	0.47 ± 0.02	0.62 ± 0.23	
	n	20	7	
Pooled Summer '92	HSI	1.41 ± 0.05	1.65 ± 0.11	**
	SSI	0.11 ± 0.01	0.11 ± 0.01	
	VSI	7.48 ± 0.35	9.06 ± 0.48	**
	n	67	26	

Notes : (i)**HSI** - Hepatosomatic Index, **SSI** - Splenosomatic Index
(ii)**VSI** - Visceralsomatic Index **GSI** - Gonadosomatic Index
(iii) **Summer '92** - June, July, August, 1992.

"**" $p < 0.05$ "*" $0.05 < p < 0.10$

Figure 5: Mean (±s.e.) hepatosomatic indices for *P. americanus* collected from St. George's Bay during 1992.

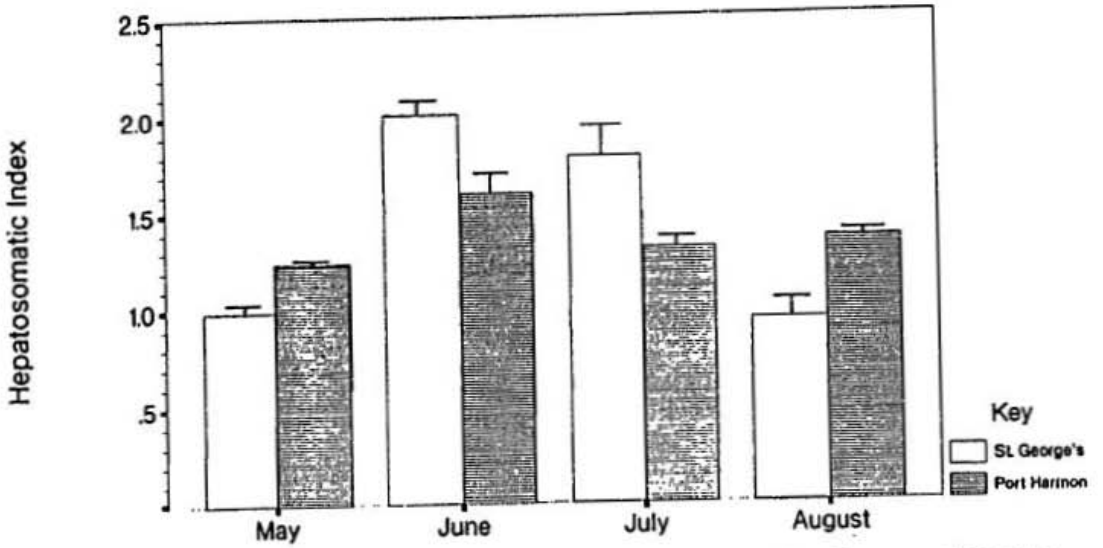


Figure 6: Mean (±s.e.) splenosomatic indices for *P. americanus* collected from St. George's Bay during 1992.

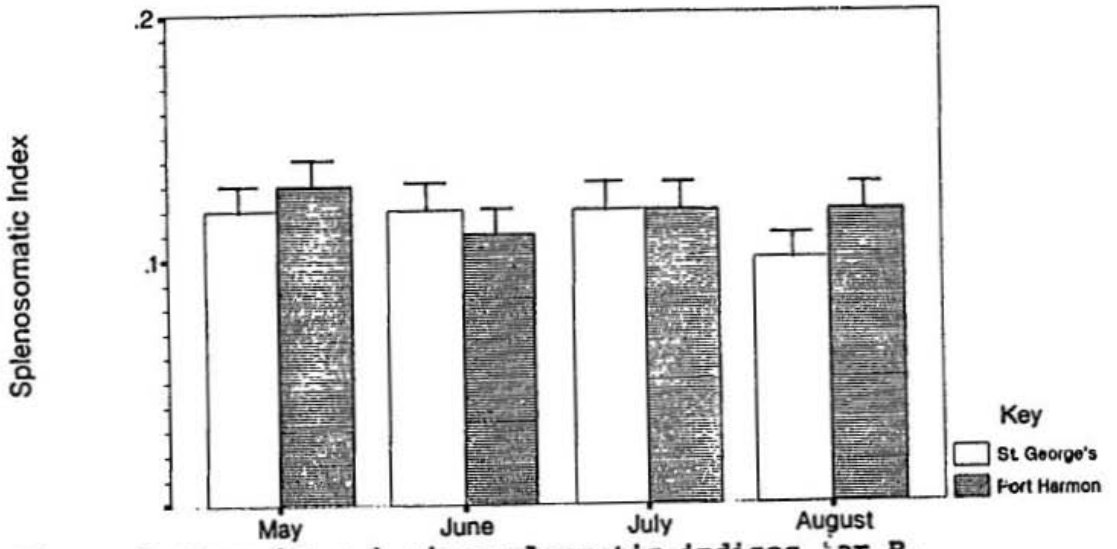


Figure 7: Mean (±s.e.) visceralsomatic indices for *P. americanus* collected from St. George's Bay during 1992.

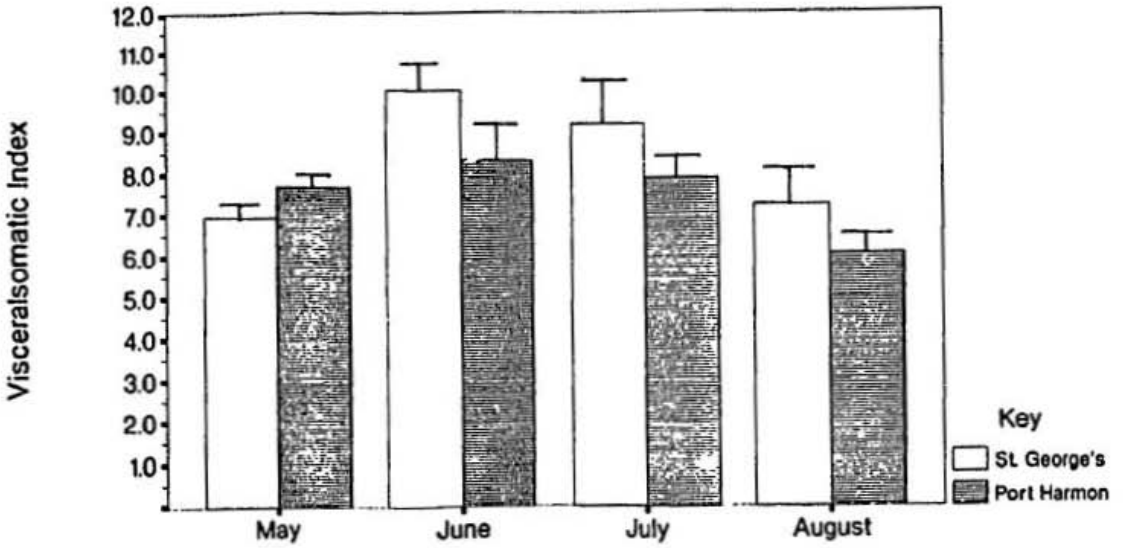


Figure 8: Mean (±s.e.) gonadosomatic indices for female *P. americanus* collected from St. George's Bay during 1992.

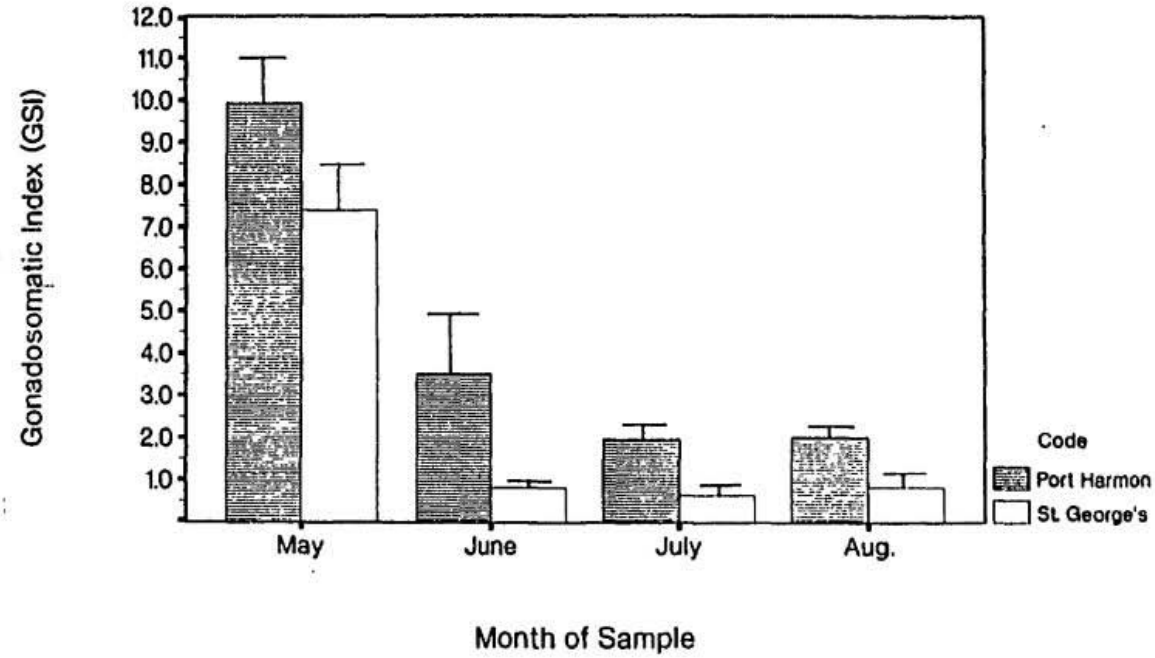
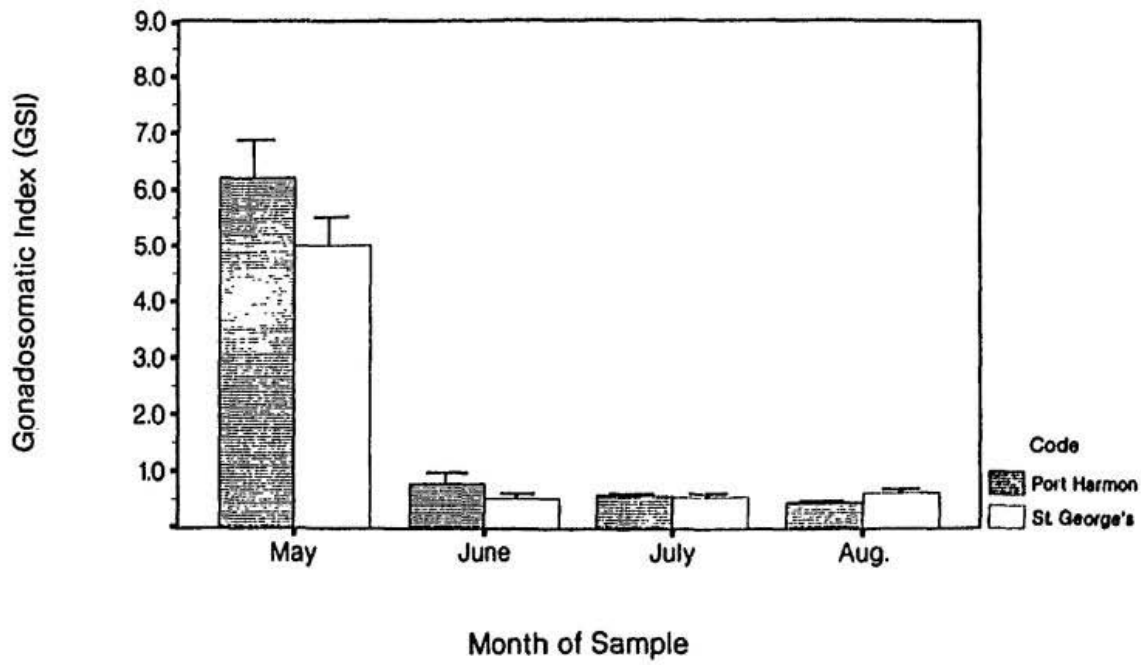


Figure 9: Mean (±s.e.) gonadosomatic indices for male *P. americanus* collected from St. George's Bay during 1992.



ructed for both groups of winter flounder of each sex, from the two study sites, to present a relative distribution of mature fish to non-mature (post spawned or immature). Based on Figure 10, it appears that most of the highest GSI values (reflecting mature fish) pertain to fish from Port Harmon. A similar scatterplot of GSI and length for females from both sites collected during summer 1992 (June, July, August), shows this same trend (Figure 11). Scatterplots of GSI and length for males collected during May 1992 (Figure 12) and summer 1992 (Figure 13), again show this trend - highest GSI values in Port Harmon fish.

An examination of the frequency of mature and non-mature (which includes immature and post spawned) male and female winter flounder from both sites showed significantly higher ($0.025 < p < 0.05$) frequencies of mature fish from Port Harmon during May 1992, and during summer 1992 (Figures 14, 15). During summer, there were no mature adult males or females at the St. George's site, but about 10% of the males and females from Port Harmon were mature and had not spawned.

Oocyte diameters were not significantly different ($p = 0.32$) between mature females collected from Port Harmon and St. George's during May ($\overline{X} = .402 \pm .014$ mm, $\overline{X} = .395 \pm 0.011$ mm respectively). There was also no significant difference ($p = 0.71$) in the mean number of oocytes produced by mature females from Port Harmon and St. George's during May($\overline{X} = [4.34 \pm 1.05] \times 10^5$, $\overline{X} = [3.07 \pm 0.65] \times 10^5$ respectively). The mature females collected during June from Port Harmon had significantly larger ($p = 0.0013$) oocytes ($X = .651 \pm .012$ mm) than

Figure 10: Scatterplot of GSI and length for female P. americanus collected from St. George's Bay during May 1992.

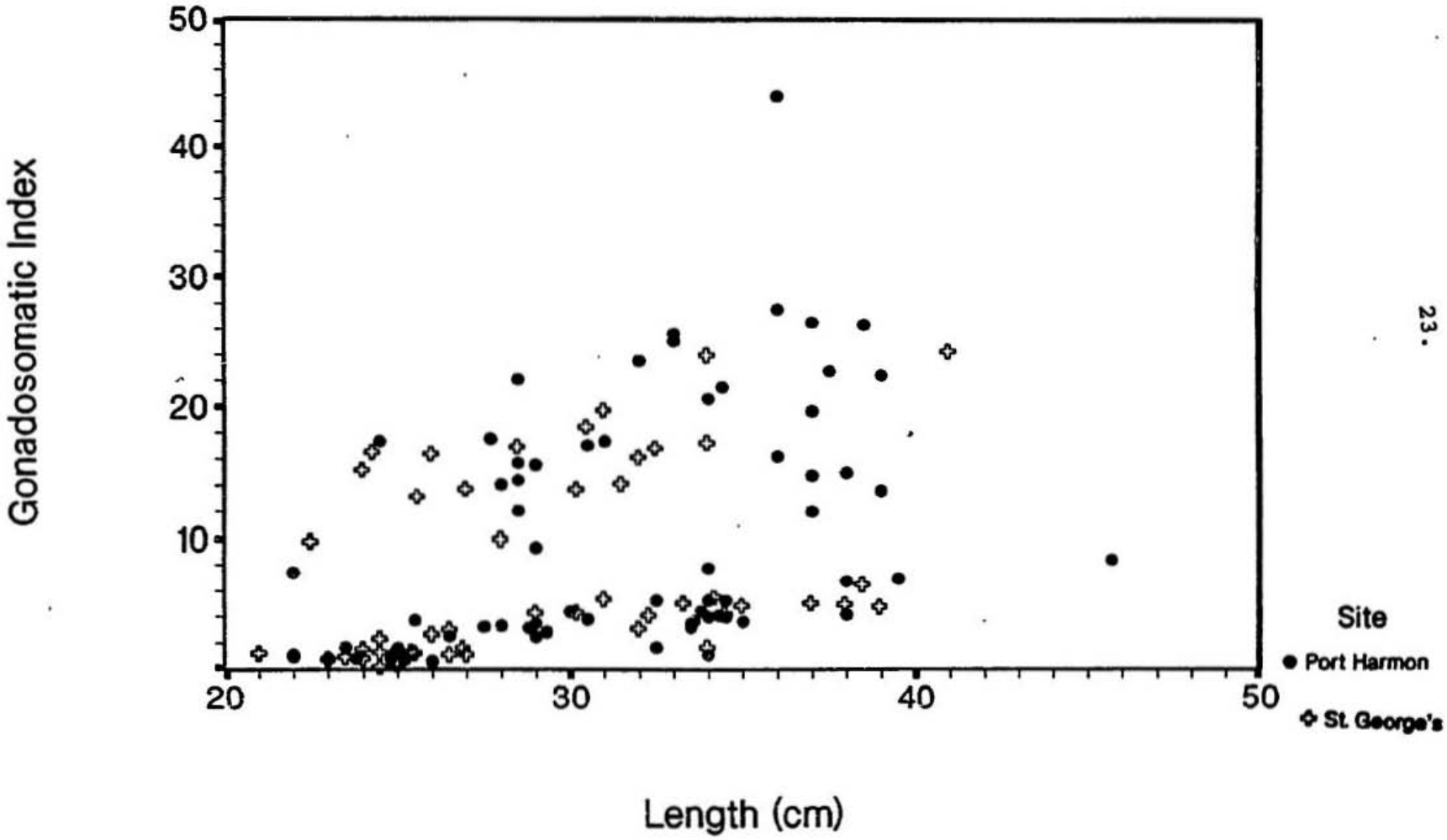
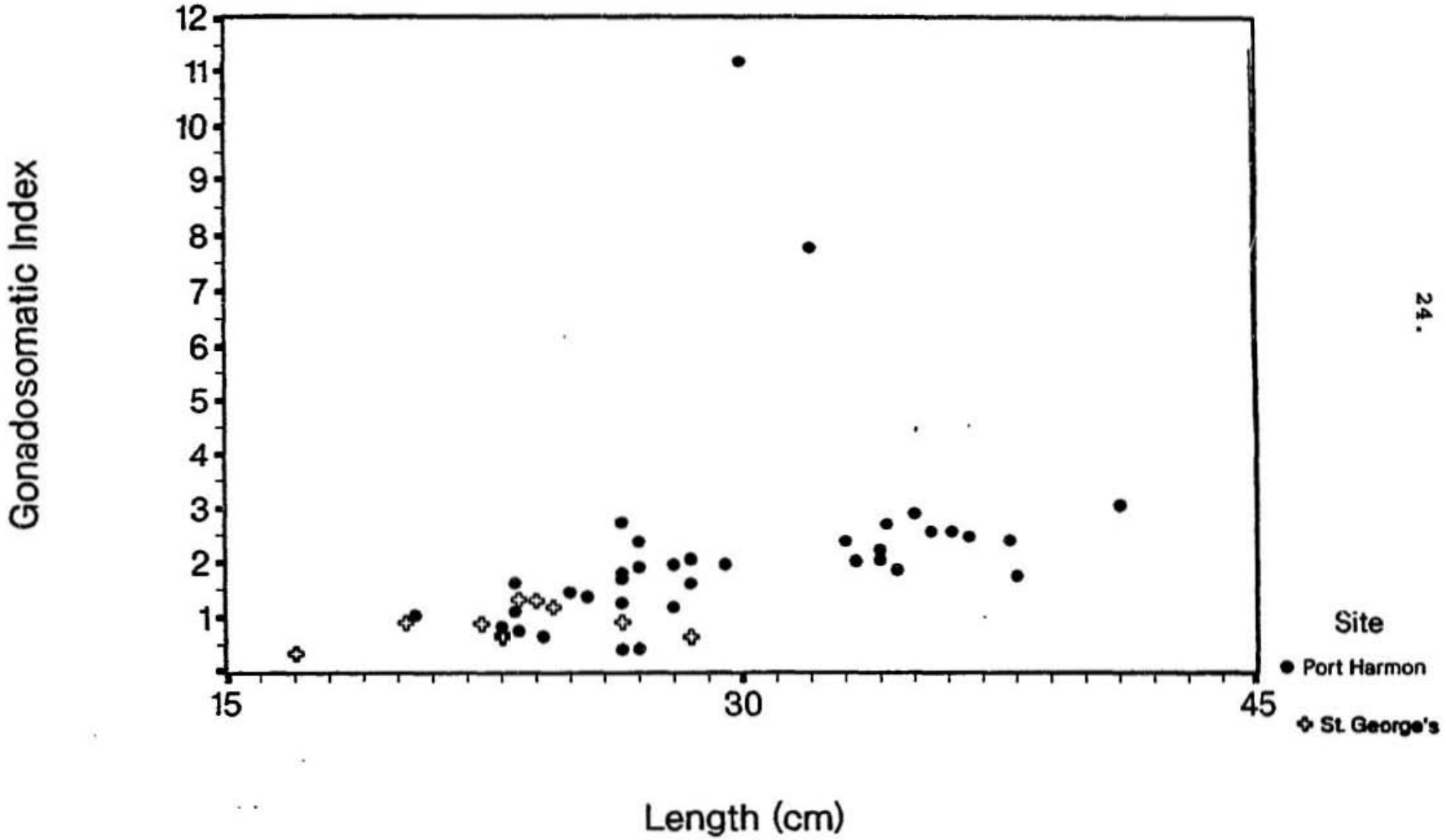


Figure 11: Scatterplot of GSI and length for female P. americanus collected from St. George's Bay during summer 1992.



Note: 'Summer' includes June, July, and August.

Figure 12: Scatterplot of GSI and length for male P. americanus collected from St. George's Bay during May 1992.

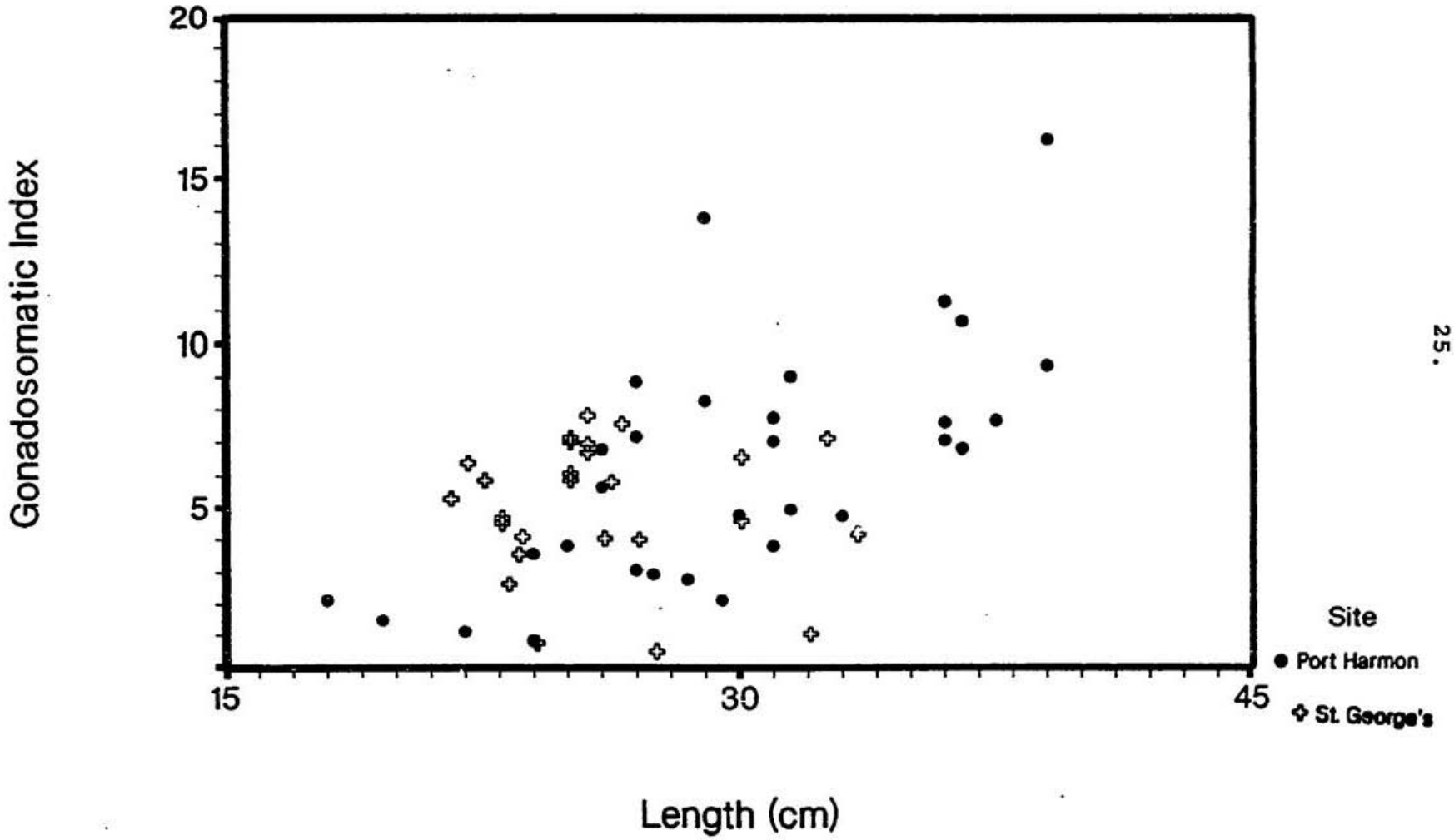
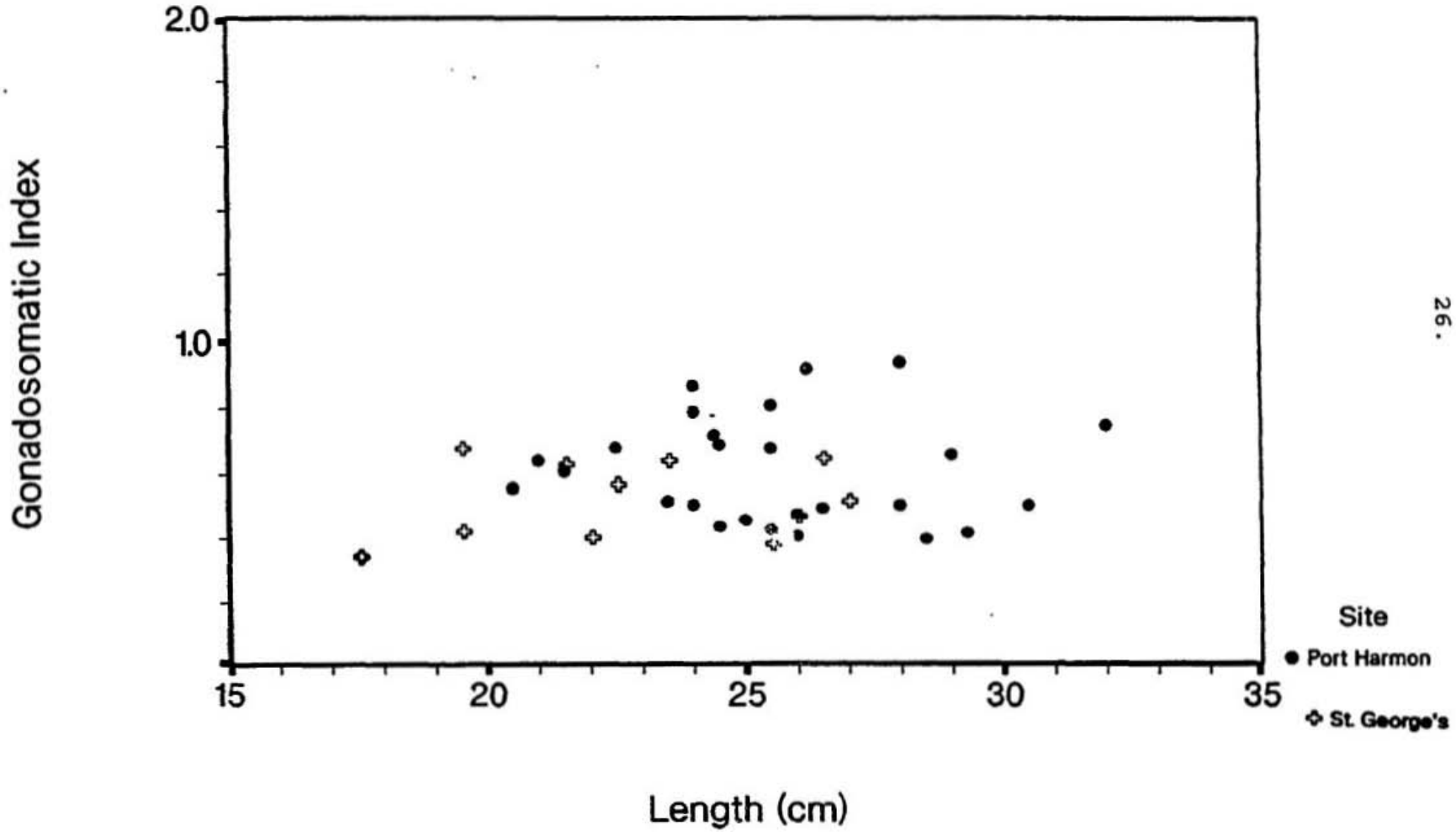
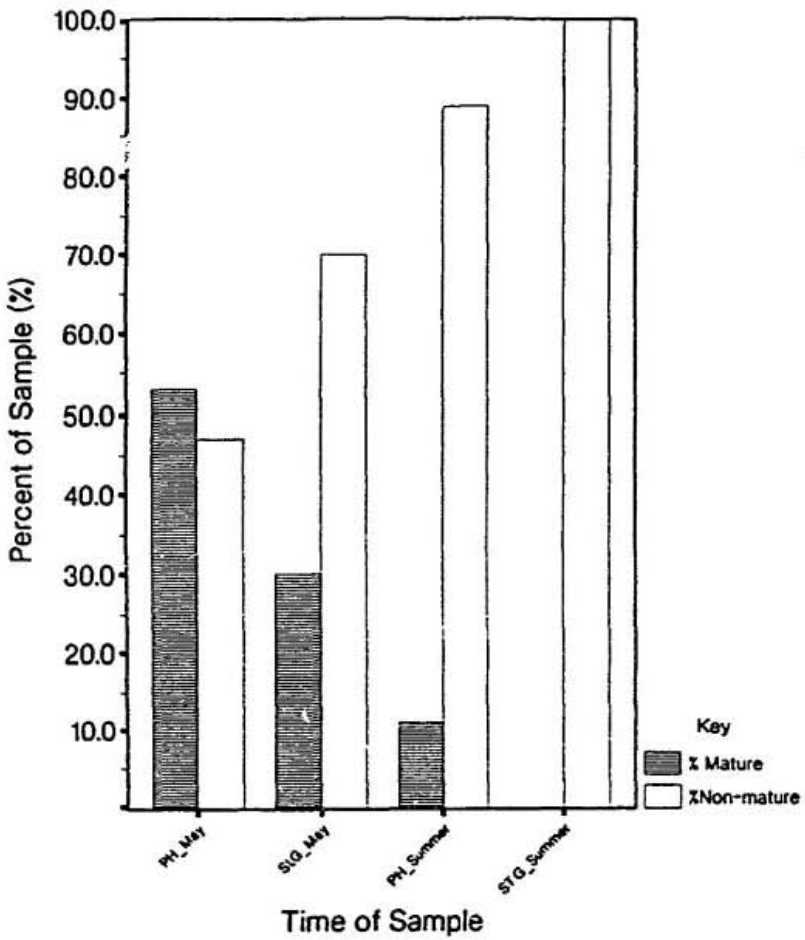


Figure 13: Scatterplot of GSI and length of male P. americanus collected from St. George's Bay during summer 1992.



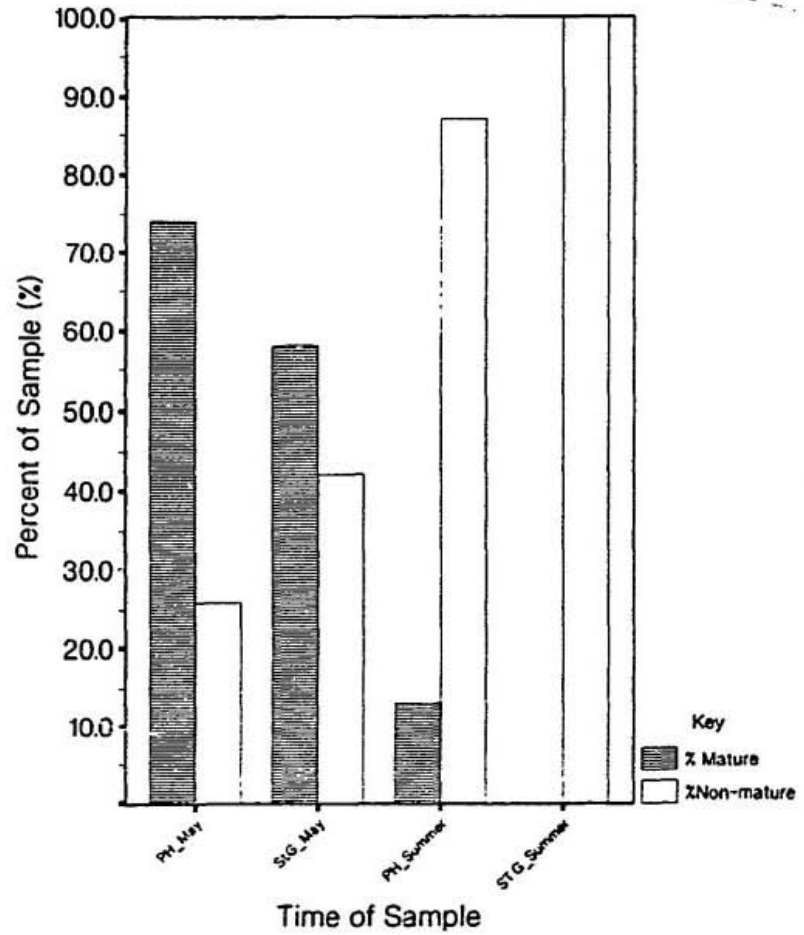
Note: 'Summer' includes June, July, and August

Figure 14: Frequency of mature / non-mature (post spawned and immature) female *P. americanus* collected from St. George's (St.G) and Port Harmon (PH) during 1992.



Note : "summer" includes the months of June,July, and August

Figure 15: Frequency of mature / non-mature (post spawned and immature) male *P. americanus* collected from St. George's (St.G) and Port Harmon (PH) during 1992.



Note : "summer" includes the months of June,July, and August

the fish collected in May 1992.

Haematological Values

An analysis of mean monthly haemoglobin (%g) levels showed significantly higher ($p < 0.0001$) levels of haemoglobin in the blood of flounder from St. George's than Port Harmon during all months except June 1992 (Table 3, Figure 16).

Comparison of monthly mean haematocrit (%) levels revealed significantly higher ($p < 0.0001$) haematocrit levels in St. George's fish during each month (Table 3, Figure 17).

Monthly mean numbers of lymphocytes (per 1000 erythrocytes) were significantly greater ($p < 0.0001$) consistently in St. George's flounder; in some samples this increase was nearly two-fold : 42.58 vs. 88.13 in July, 43.52 vs. 86.43 in August (Table 3, Figure 18).

Finally, pooled summer blood samples showed significantly greater ($p = 0.0004$) values of haemoglobin, haematocrit, and lymphocyte number at St. George's than at Port Harmon.

Fin necrosis

There was evidence of fin necrosis in winter flounder captured at both sites. The most common sites of erosion were the caudal, dorsal and anal fins. The monthly frequency of caudal fin necrosis (% sample with fin necrosis) was signif-

Table 3: Haematological values of *P. americanus* collected from St. George's Bay during 1992.

Month		Port Harmon	St. George's
May '92	Haemoglobin (%g)	4.17 ± 0.17	4.75 ± 0.19**
	Haematocrit (%)	16.0 ± 0.90	21.1 ± 1.10**
	Lymphocyte No.	13.77 ± 1.98	50.63 ± 9.78**
	n	62	55
June '92	Haemoglobin (%g)	3.05 ± 0.23	3.45 ± 0.24
	Haematocrit (%)	---	---
	Lymphocyte No.	43.73 ± 3.89	69.00 ± 9.71**
	n	19	11
July '92	Haemoglobin (%g)	3.41 ± 0.18	5.43 ± 0.26**
	Haematocrit (%)	22.5 ± 2.00	40.6 ± 3.00**
	Lymphocyte No.	42.58 ± 3.57	88.13 ± 9.71**
	n	20	8
Aug. '92	Haemoglobin (%g)	4.82 ± 0.49	6.33 ± 0.27**
	Haematocrit (%)	32.2 ± 3.10	39.5 ± 3.40**
	Lymphocyte No.	43.52 ± 3.31	86.43 ± 9.18**
	n	20	7
Pooled Summer'92			
	Haemoglobin (%g)	3.71 ± 0.21	4.83 ± 0.29**
	n	59	26
	Haematocrit (%)	27.2 ± 2.0	40.1 ± 2.2 **
	n	40	15
	Lymphocyte No.	42.8 ± 2.0	79.6 ± 5.7 **
	n	20	26

Note : values expressed as $\bar{X}$ ± s.e.
Summer = June, July, Aug.
Lymphocyte no. expressed per 1000 erythrocytes

"**" $p < 0.05$

Figure 16: Mean (±s.e.) haemoglobin (%g) levels in blood of *P. americanus* collected from St. George's Bay during 1992.

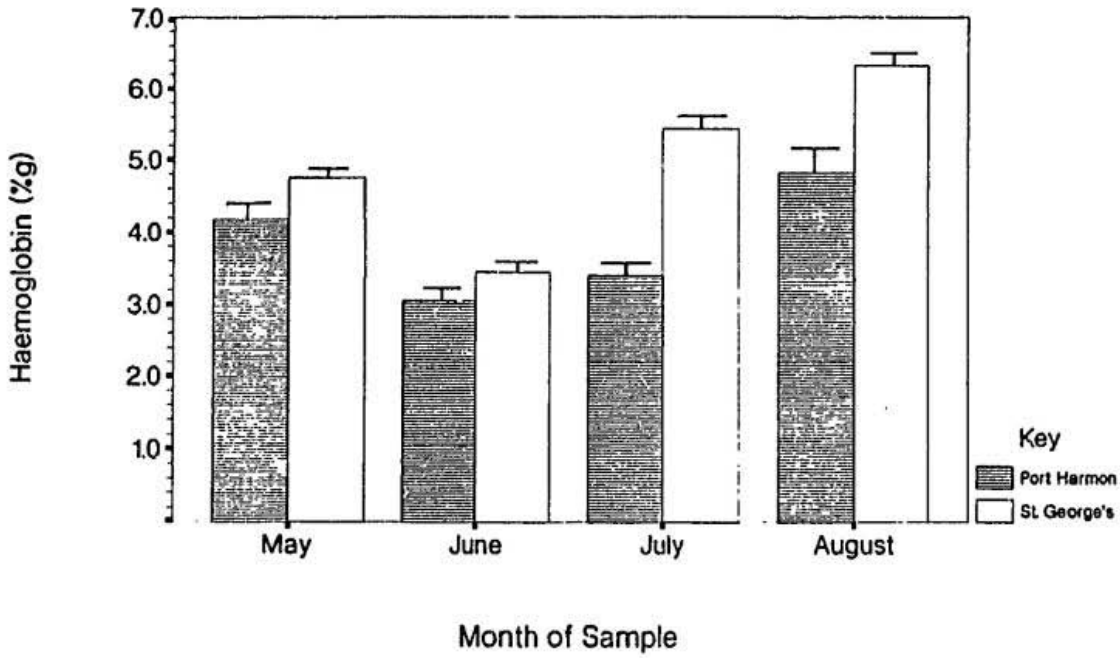


Figure 17: Mean (±s.e.) haematocrit (%) levels in blood of *P. americanus* collected from St. George's Bay during 1992.

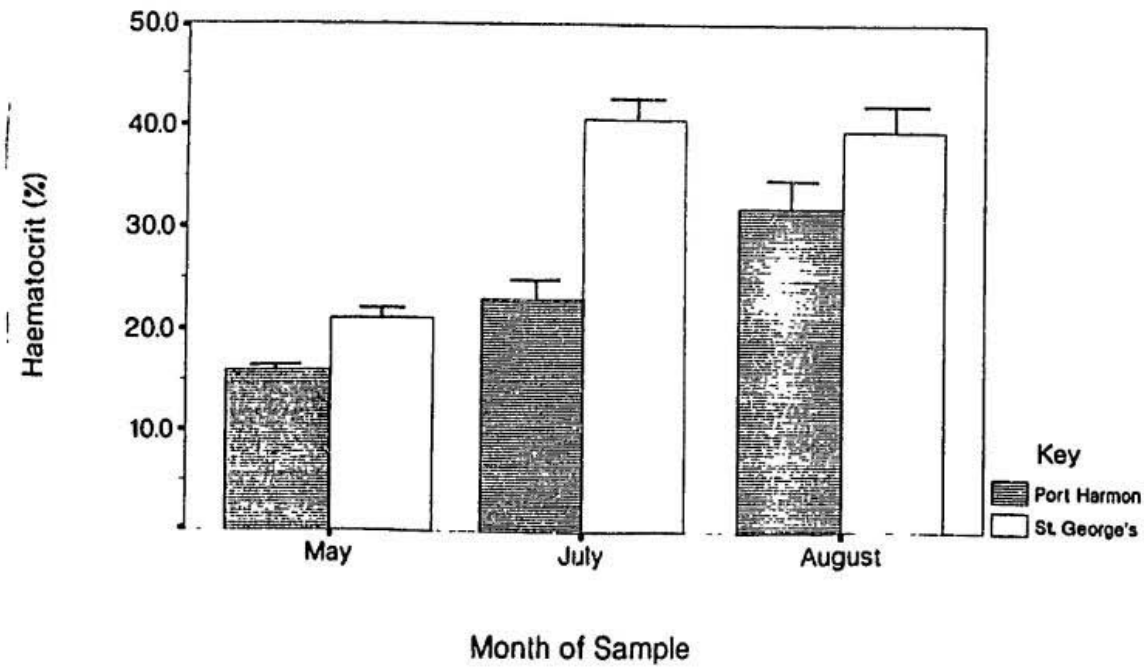
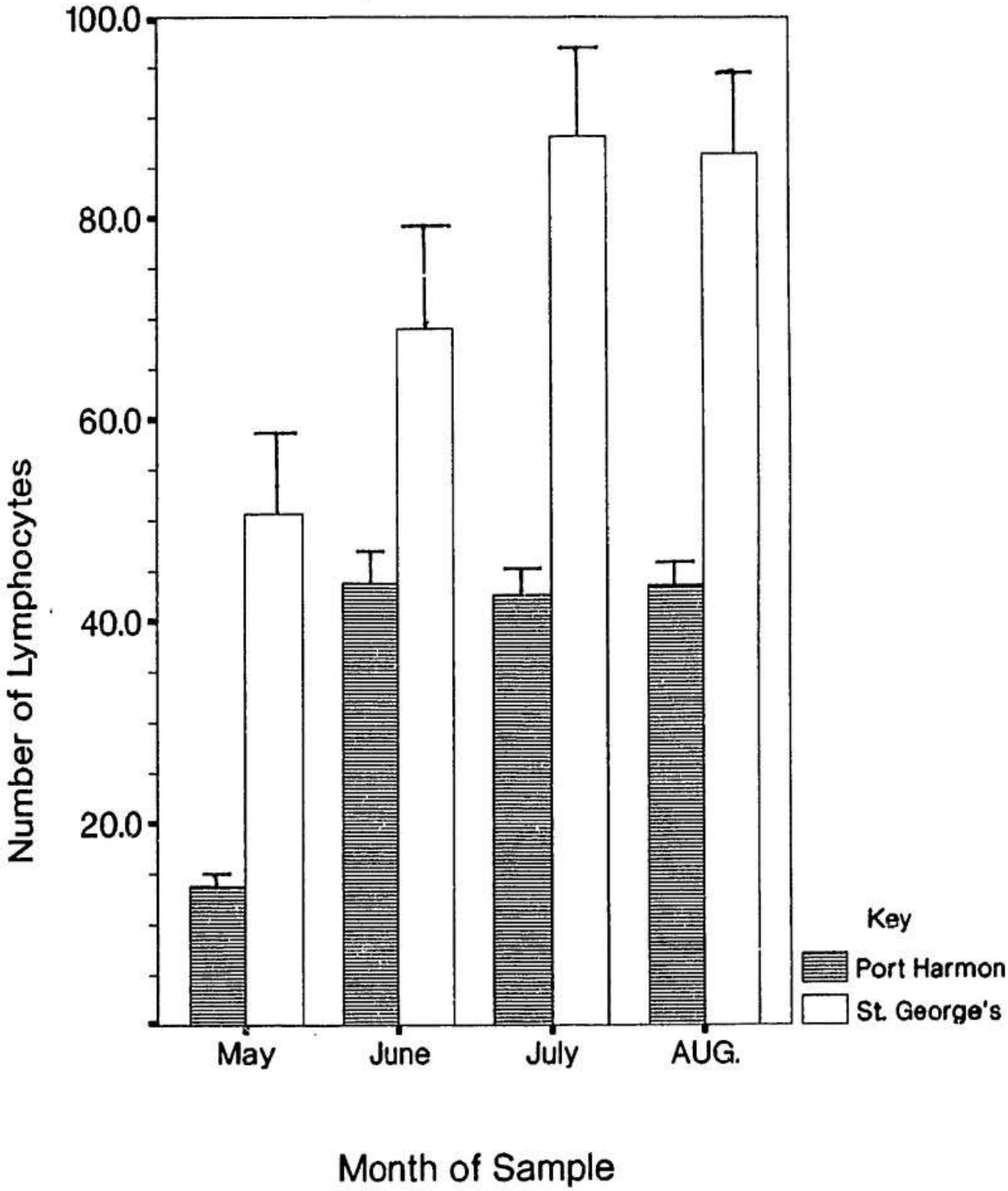


Figure 18: Mean (±s.e.) number of lymphocytes (per 1000 erythrocytes) in blood of *P. americanus* collected from St. George's Bay during 1992.



icantly higher ($0.025 < p < 0.05$) in fish from Port Harmon during each sample month of 1991 and 1992, with the exceptions of July and August 1992 than in samples from St. George's (Table 4a, Figure 19). There appeared to be an annual peak in fin necrosis during July,in flounder from both sites, and it decreased over fall and winter (Figure 19). The total frequency of fin necrosis in fish from St. George's increased from 11% in 1991 to 35% in 1992, but it was still significantly less ($0.025 < p < 0.05$) than the total fin necrosis frequency in fish from Port Harmon (Table 4b).

Each monthly prevalence of dorsal and anal fin necrosis was higher in flounder from Port Harmon, but was only significantly higher ($0.025 < p < 0.05$) during June 1992 (Table 4a).Prevalence of dorsal and anal fin rot in the entire pooled collections from 1992, were signifcantly higher ($0.01 < p < 0.025$) in flounder from Port Harmon than in flounder from the St. George's collection (Table 4b).

Comparing monthly frequencies of winter flounder with >1 site of caudal fin necrosis, samples from Port Harmon had a significantly higher ($0.025 < p < 0.05$) frequency during May 1992 (Table 4a, Figure 20). No fish with > 1 site of fin necrosis were collected from St. George's during June and July, when Port Harmon frequencies were 11% and 18% respectively (Table 4a). The frequency of the total sample of fish with > 1 site of fin necrosis was significantly greater ($p < 0.001$)

Table 4a: Summary statistics from analysis of fin necrosis of *P. americanus* collected from St. George's Bay during 1992.

Month	Variable	Port Harmon	St. George's
MAY	Caudal fin necrosis frequency (%)	45	33 **
	Prevalence of fish with >1 necrotic site (%)	21	6 **
	Mean no. necrotic sites ($\bar{X} \pm$ s.e.)	0.69 ± 0.09	0.39 ± 0.07 **
	% of caudal fin eroded ($\bar{X} \pm$ s.e.)	11.1 ± 1.6	7.3 ± 1.3 **
	Dorsal and anal fin necrosis (%)	18	12
	n	98	82
JUNE	Caudal fin necrosis frequency (%)	63	27 **
	Prevalence of fish with >1 necrotic site (%)	11	0
	Mean no. necrotic sites ($\bar{X} \pm$ s.e.)	0.74 ± 0.15	0.27 ± 0.14 **
	% of caudal fin eroded ($\bar{X} \pm$ s.e.)	18.0 ± 5.0	13.2 ± 7.4
	Dorsal and anal fin necrosis (%)	42	27 **
	n	19	11
JULY	Caudal fin necrosis frequency (%)	65	63
	Prevalence of fish with >1 necrotic site (%)	18	0 *
	Mean no. necrotic sites ($\bar{X} \pm$ s.e.)	0.91 ± 0.11	0.50 ± 0.19 **
	% of caudal fin eroded ($\bar{X} \pm$ s.e.)	23.7 ± 3.0	18.7 ± 9.5
	Dorsal and anal fin necrosis (%)	44	37
	n	63	8
AUG.	Caudal fin necrosis frequency (%)	46	40
	Prevalence of fish with >1 necrotic site (%)	27	20
	Mean no. necrotic sites ($\bar{X} \pm$ s.e.)	0.81 ± 0.20	0.60 ± 0.27
	% of caudal fin eroded ($\bar{X} \pm$ s.e.)	13.3 ± 3.5	21.0 ± 10.0
	Dorsal and anal fin necrosis (%)	33	43
	n	21	7

"****" $p < 0.001$ "**" $0.001 < p < 0.05$ "*" $0.05 < p < 0.10$

Figure 19: Annual frequency of fin necrosis in P. americanus collected from St. George's Bay during 1991 - 1992.

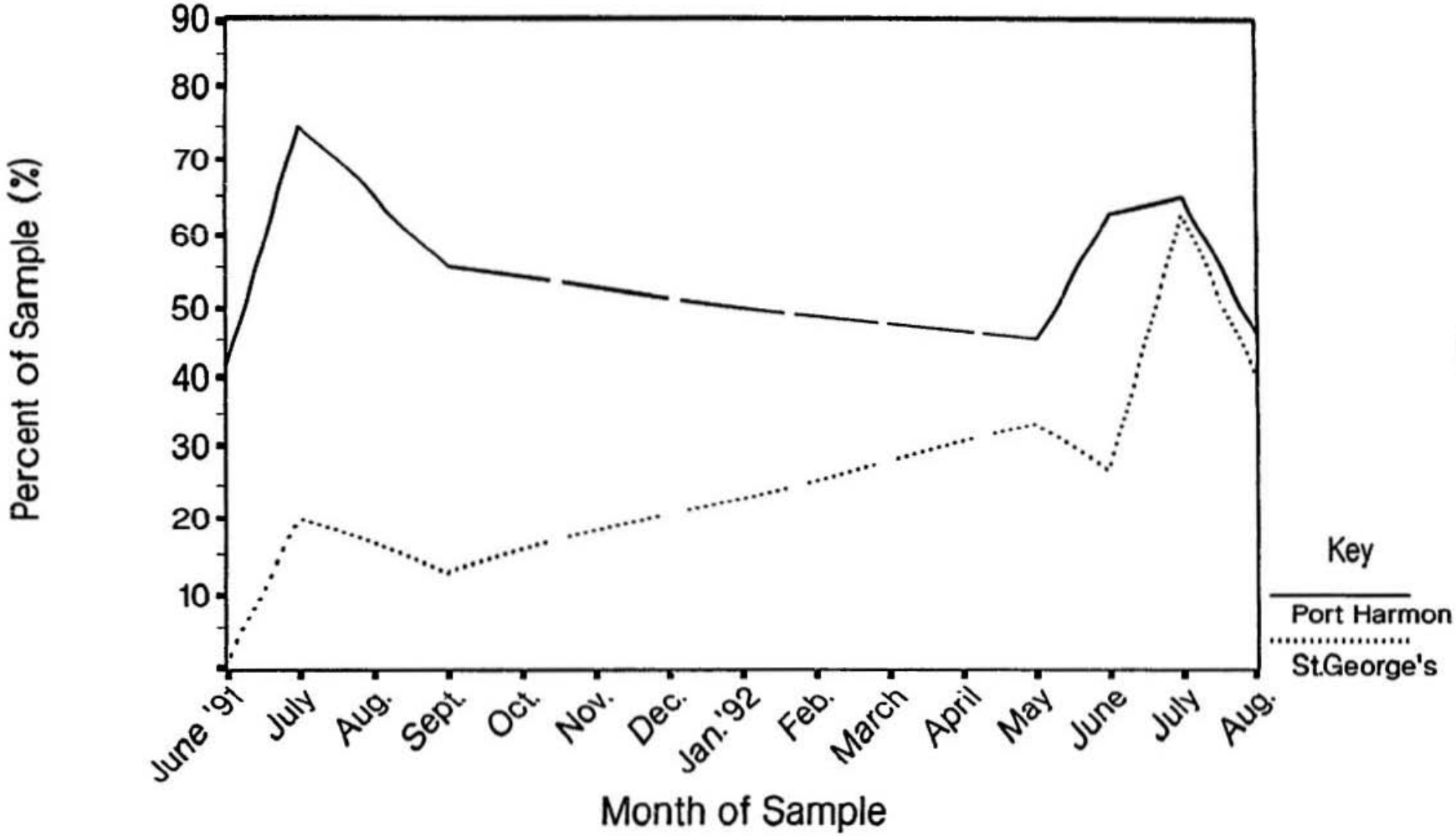
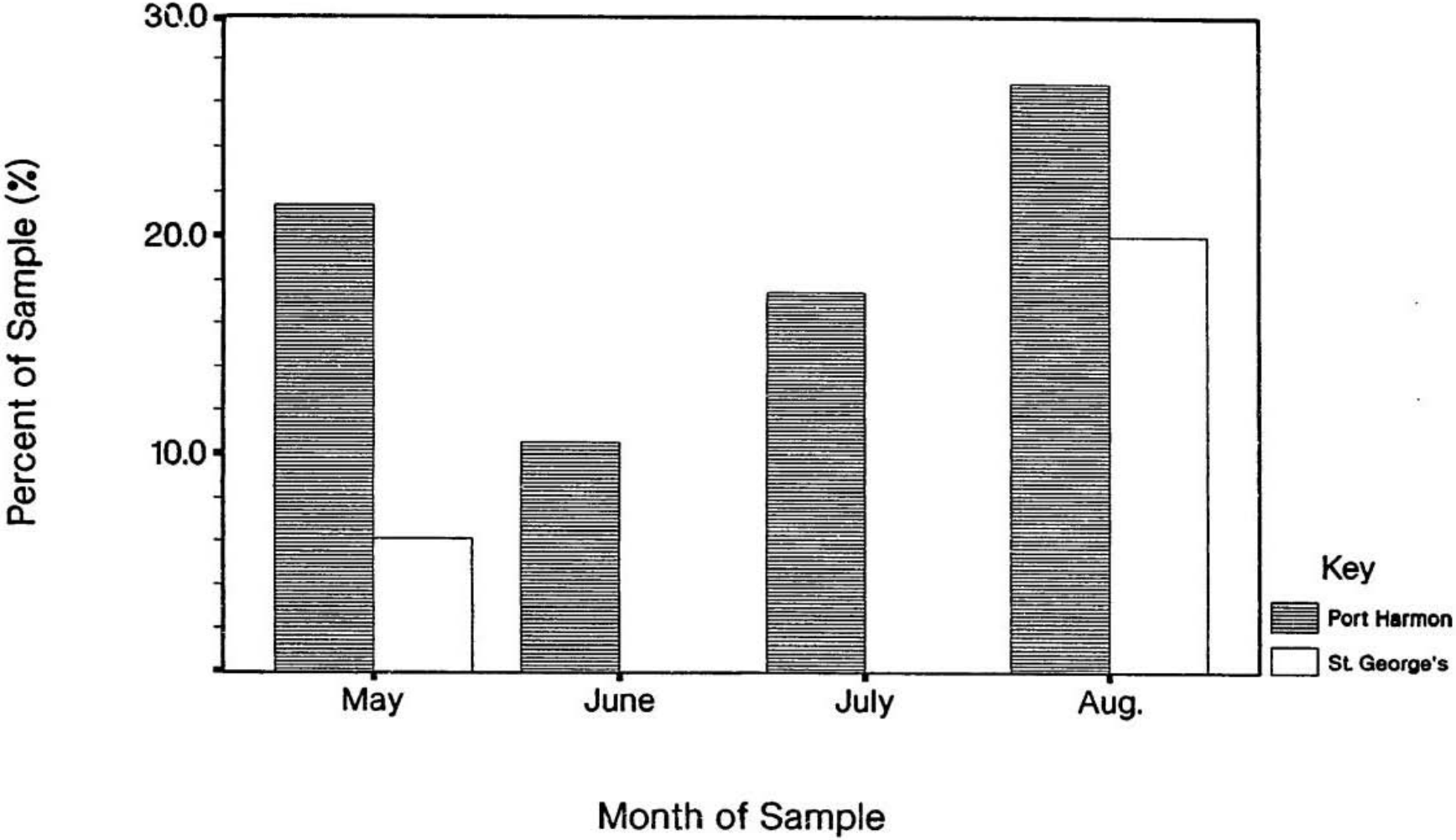


Table 4b: Pooled summary statistics from analysis of fin necrosis in *P. americanus* collected from St. George's Bay during 1992.

Variable	Port Harmon	St. George's
Caudal fin necrosis frequency (%) (1991%)	53 (57)	35 ** (11)***
Prevalence of fish >1 necrotic site (%)	20	6 ***
Mean no. of necrotic sites ($\overline{X} \pm$ s.e.)	0.78 ± 0.06	0.41 ± 0.06 ***
% of caudal fin eroded ($\overline{X} \pm$ s.e.)	15.9 ± 1.4	9.9 ± 1.7 **
Dorsal and anal fin necrosis (%)	30	19 **
Prevalence of skin ulcers (%)	11	5 **
n	201	108

"***" $p < 0.001$ "**" $0.001 < p < 0.05$ "*" $0.05 < p < 0.10$

Figure 20: Frequency of P. americanus with > 1 site of caudal erosion collected from St. George's Bay during 1992.



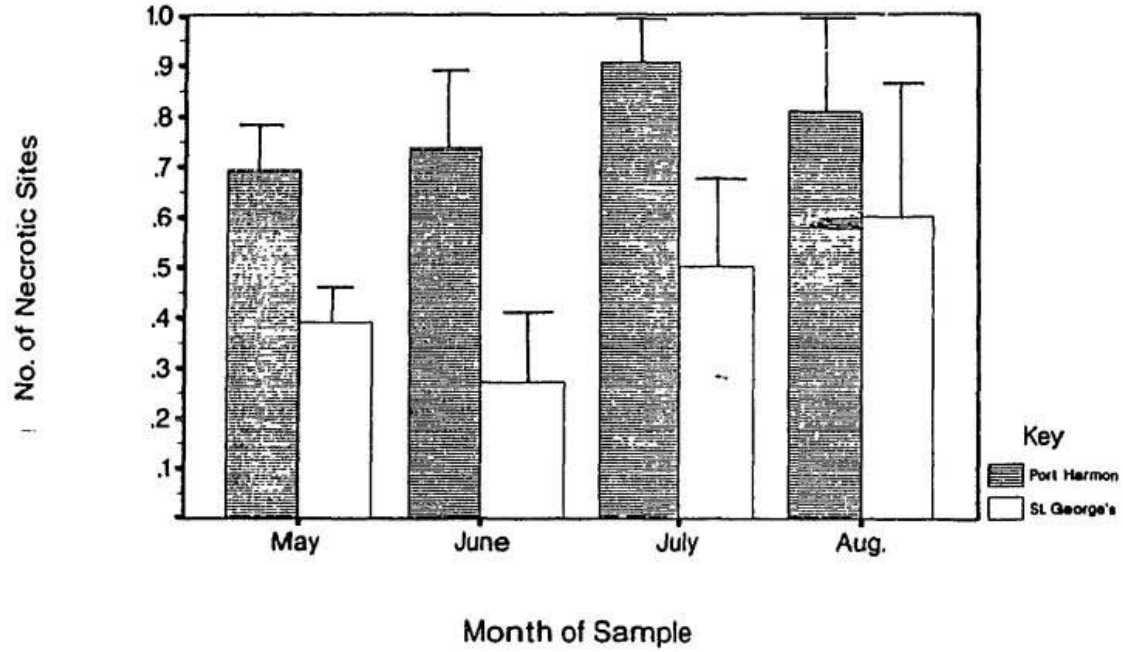
in Port Harmon fish compared with St. George's fish (20% and 6% respectively; Table 4b).

The mean number of necrotic sites on the caudal fin of winter flounder from Port Harmon was significantly greater (p = 0.011) than that of the St. George's sample during all months of 1992 except August (Table 4a, Figure 21). When all samples from 1992 were pooled, the mean number of necrotic sites in winter flounder from Port Harmon was significantly greater (p= 0.0001) than that of the samples from St. George's (Table 4b).

Mean percentage (%) of caudal fin eroded (length of necrotic site/length of caudal fin) showed considerable variation among groups. Only the May 1992 sample from Port Harmon was significantly greater ($p < 0.0001$) than that of St. George's (Table 4a, Figure 22). After all samples of 1992 were pooled, flounder from Port Harmon had a significantly greater ($p < 0.0001$) percentage of their caudal fin eroded than did flounder from St. George's (Table 4b).

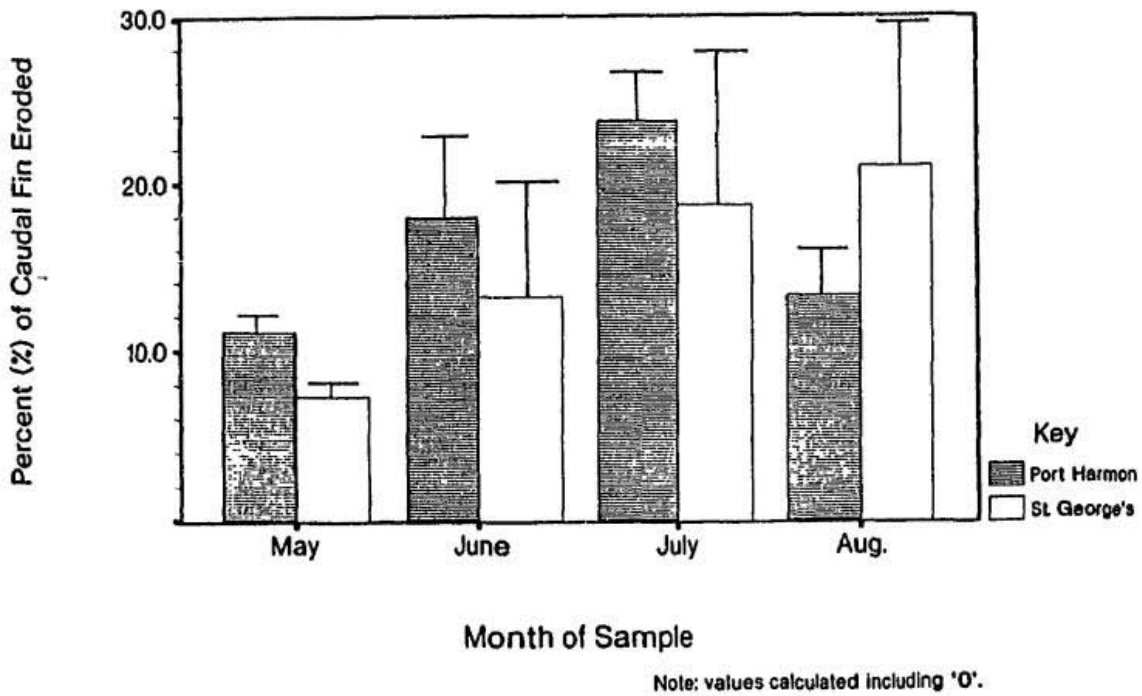
In an attempt to discern if there was any significant difference in caudal fin growth between the 2 groups of fish, a simple linear regression of caudal fin length and total length was calculated. The regression equation for Port Harmon fish was $Y_{(caud.len.)} = 0.184X_{(tot.len.)} + 0.001$ (r^2=.993, $p < 0.001$, s.e. = 0.451). The regression equation for St. George's fish was $Y_{(caud.len.)} = 0.188X_{(tot.len.)} + 0.002$ (r^2=.990, $p < 0.001$, s.e.= 0.497). There was no significant difference between both

Figure 21: Mean (±s.e.) number of necrotic sites on caudal fin of *P. americanus* collected from St. George's Bay during 1992



Note: values calculated including '0'.

Figure 22: Mean (±s.e.) percent (%) of caudal fin eroded in *P. americanus* collected from St. George's Bay during 1992.



Note: values calculated including '0'.

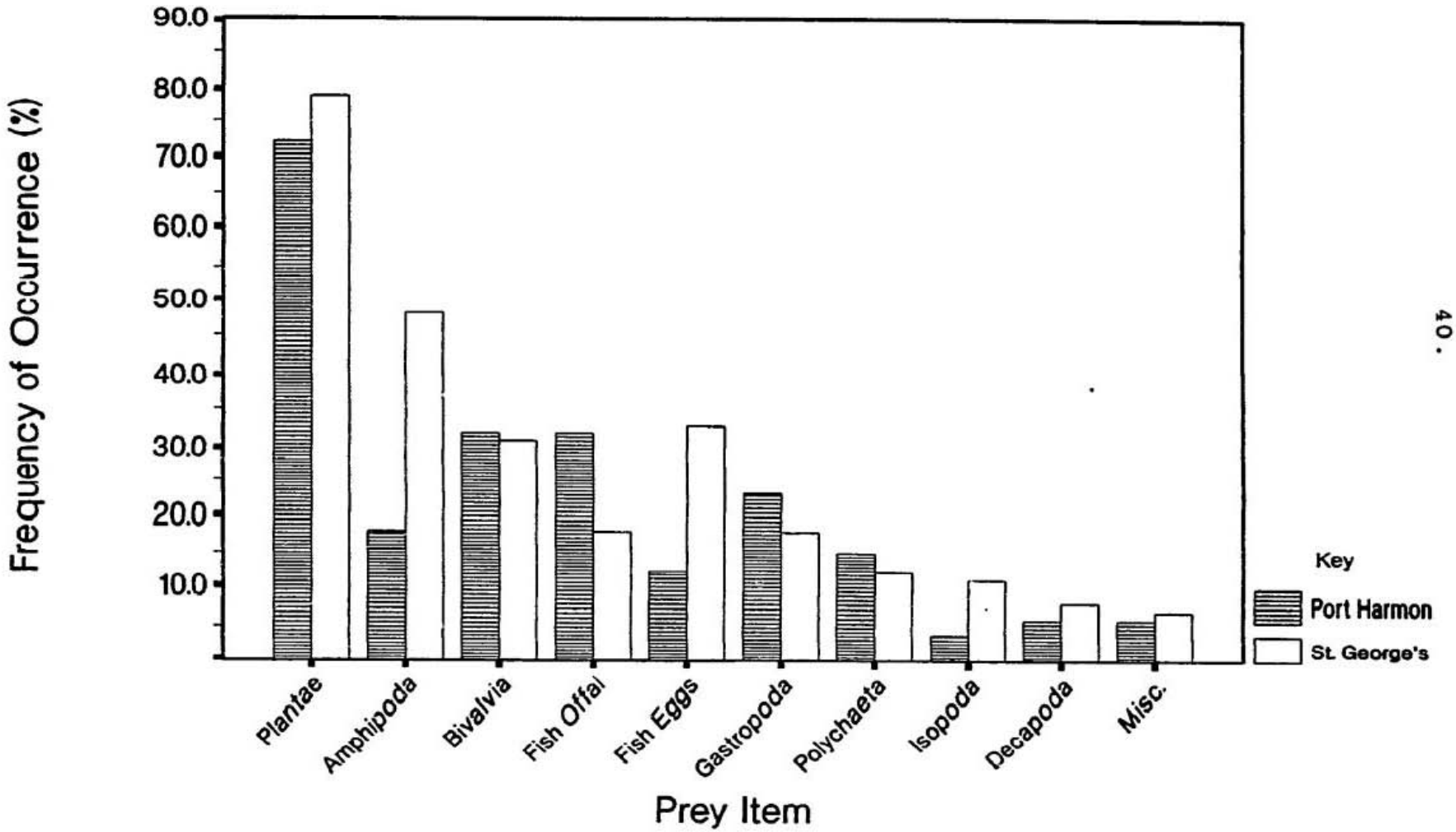
equations ($p = 0.671$), but the equations failed to demonstrate the occurrence of caudal fins that were previously eroded, but which had healed eventually. Among flounder from Port Harmon, there was evidence of fin ray fusion, and an overall malformation of the shape of the caudal fin. In addition, many fish from Port Harmon with fin necrosis showed hemorrhagic sites along the edges of the eroded area, which was also not demonstrated by the regression equation.

Finally, the total frequency of skin ulcers on winter flounder was significantly greater ($0.025 < p < 0.05$) in Port Harmon fish (11%) compared with St. George's sample (5%) (Table 4). Ulcers were commonly found on the left side (unpigmented, "blind side") but were occasionally found on the right side (pigmented, "eyed side") of winter flounder collected from Port Harmon.

Feeding and Parasitofauna

The diets of flounder from both sites was very similar. The dominant prey items (by frequency of occurrence) in the diet of winter flounder from Port Harmon and St. George's were: (i) Plantae (Clorophyta, Rhodophyta, Phaeophyta); (ii) Amphipoda; (iii) Bivalvia; (iv) fish offal; and (v) fish eggs (Figure 23). St. George's winter flounder consumed a significantly higher frequency of amphipods (48% vs. 18%, $p < 0.001$) and fish eggs (33% vs. 12%, $p < 0.001$) than Port Harmon winter flounder.

Figure 23: Frequency (%) of food items consumed by P. americanus collected from St. George's Bay during 1992.



Plantae = Div. Chlorphyta,Rhodophyta,Phaeophyta
Misc. = Echinodermata, Cnidaria, & 'non-food' items : rocks, wood chips

Port Harmon flounder consumed a higher frequency of fish offal than St. George's flounder (32% vs. 18%, $0.01 < p < 0.025$). No significant differences were observed between frequencies of any other common prey. All flounder from St. George's had stomachs varying in fullness from one-third to full, while 12 flounder (7%) from Port Harmon had empty stomachs. In addition some of the "non-food" items found in the stomachs of flounder from Port Harmon included rocks, wood chips, and paint.

The three most common intestinal helminth taxonomic groups recorded were Acanthocephala (*Echinorhynchus gadi*), Nematoda (larval *Anisakis sp.* + other anisakine type larvae), and Digenea (*Podocotyle atomon, Fellodistomum sp.*). Acanthocephalan prevalences (%) were significantly greater ($p < 0.05$, $p < 0.001$) in St. George's fish during each sample month of 1991 and 1992, except June 1991 and July 1992 (Table 5, 6a; Figure 24). Acanthocephalan prevalences peaked during summer at both sample sites and decreased over fall and winter (Figure 24). Mean monthly acanthocephalan intensities (number of helminths per fish) were significantly greater ($p < 0.0001$) in St. George's during each sample month of 1991 and 1992 except June 1991 (Table 5,6a; Figure 25). A comparison of pooled acanthocephalan prevalence and intensity for 1991 (Table 5) and separately for 1992 (Table 6b) shows a significantly higher prevalence ($p < 0.001$) and intensity ($p < 0.0001$) in St. George's during 1991 and 1992. Acanthocephalans were not found

Table 5: Mean (±s.e.) intensity and prevalence of intestinal helminths in *P. americanus* collected from Port Harmon and St. George's during 1991.

Month/ Parasite		Port Harmon	St. George's
June '91	*Acanthocephala*		
	Intensity	0.17 ± 0.11	0.33 ± 0.21
	Prevalence (%)	16	33
	Nematoda		
	Intensity	0.58 ± 0.41	0.0 **
	Prevalence (%)	25	0.0
July '91	*Acanthocephala*		
	Intensity	0.0	13.00 ± 6.67 **
	Prevalence (%)	0.0	75 ***
	Nematoda		
	Intensity	1.50 ± 0.87	0.75 ± 0.48
	Prevalence (%)	75	50
Sept. '91	*Acanthocephala*		
	Intensity	0.67 ± 0.29	10.25 ± 4.13 **
	Prevalence (%)	44	100 **
	Nematoda		
	Intensity	0.67 ± 0.24	0.0 **
	Prevalence (%)	56	0.0 **
Pooled			
	Acanthocephala		
	Intensity	1.33 ± 0.21	10.46 ± 3.18 **
	Prevalence (%)	20	72 ***
	n	40	18
	Nematoda		
	Intensity	1.73 ± 0.43	0.50 ± 0.25 **
	Prevalence (%)	65	13 ***
	n	40	18

Note : intensity defined as no. helminths / fish

"***" $p < 0.001$ "**" $0.001 < p < 0.05$ "*" $0.05 < p < 0.10$

Table 6a: Mean (±s.e.) intensity and prevalence of intestinal helminths in *P. americanus* collected from Port Harmon and St. George's during 1992.

Month/ Parasite		Port Harmon	St. George's
	Acanthocephala		
May '92	Intensity	0.0	0.06 ± 0.04 **
	Prevalence (%)	0.0	4 **
	Nematoda		
	Intensity	0.48 ± 0.13	0.01 ± 0.01 **
	Prevalence (%)	26	1 **
	Digenea		
	Intensity	1.67 ± 0.50	1.26 ± 0.29
	Prevalence (%)	35	33
	n	108	96
	Acanthocephala		
June '92	Intensity	0.16 ± 0.06	2.45 ± 0.65 **
	Prevalence (%)	5	73 ***
	Nematoda		
	Intensity	0.26 ± 0.13	0.45 ± 0.20
	Prevalence (%)	21	36
	Digenea		
	Intensity	2.84 ± 1.76	1.09 ± 0.61
	Prevalence (%)	47	36
	n	19	11
	Acanthocephala		
July '92	Intensity	0.44 ± 0.19	1.37 ± 0.73 **
	Prevalence (%)	22	50
	Nematoda		
	Intensity	1.48 ± 0.50	0.0 **
	Prevalence (%)	52	0.0 **
	Digenea		
	Intensity	0.33 ± 0.14	2.00 ± 1.45 *
	Prevalence (%)	22	50
	n	28	8
	Acanthocephala		
Aug. '92	Intensity	0.05 ± 0.03	5.71 ± 1.46 **
	Prevalence (%)	5	85 ***
	Nematoda		
	Intensity	1.67 ± 0.42	0.0 **
	Prevalence (%)	57	0.0 **
	Digenea		
	Intensity	0.0	0.0
	Prevalence (%)	0.0	0.0
	n	21	7

"***" $p < 0.001$ "**" $0.001 < p < 0.05$ "*" $0.05 < p < 0.10$

Figure 24: Annual acanthocephalan prevalence (%) in *P. americanus* collected from St. George's Bay during 1991-1992.

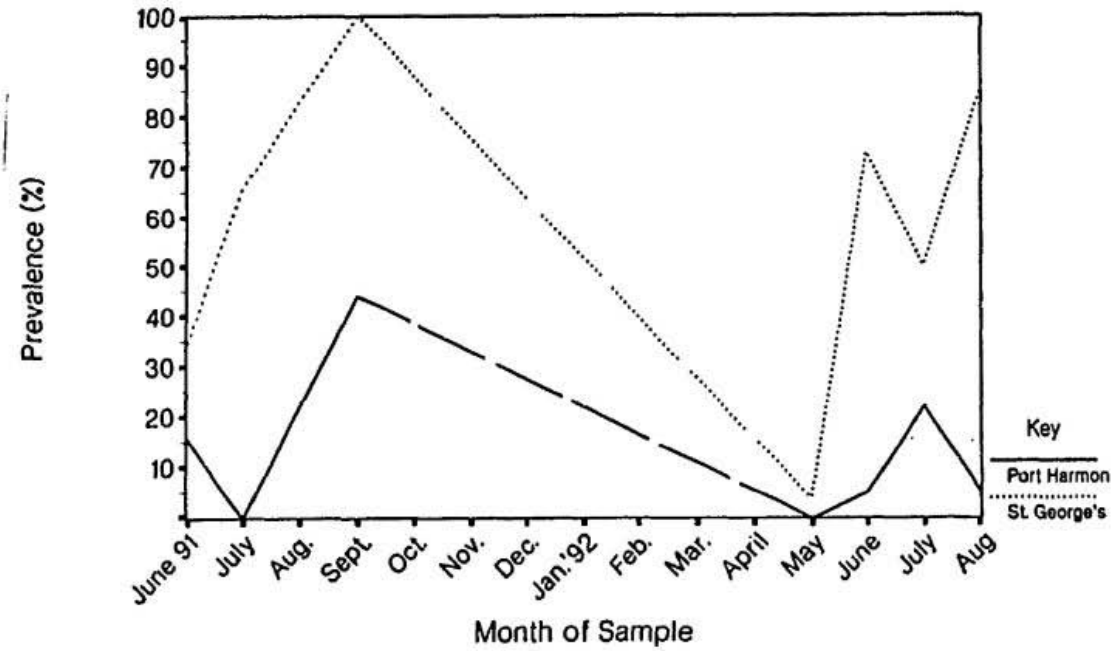


Figure 25: Mean (±s.e.) acanthocephalan intensity (#parasites/ fish) in *P.americanus* collected from St. George's Bay during 1991-1992.

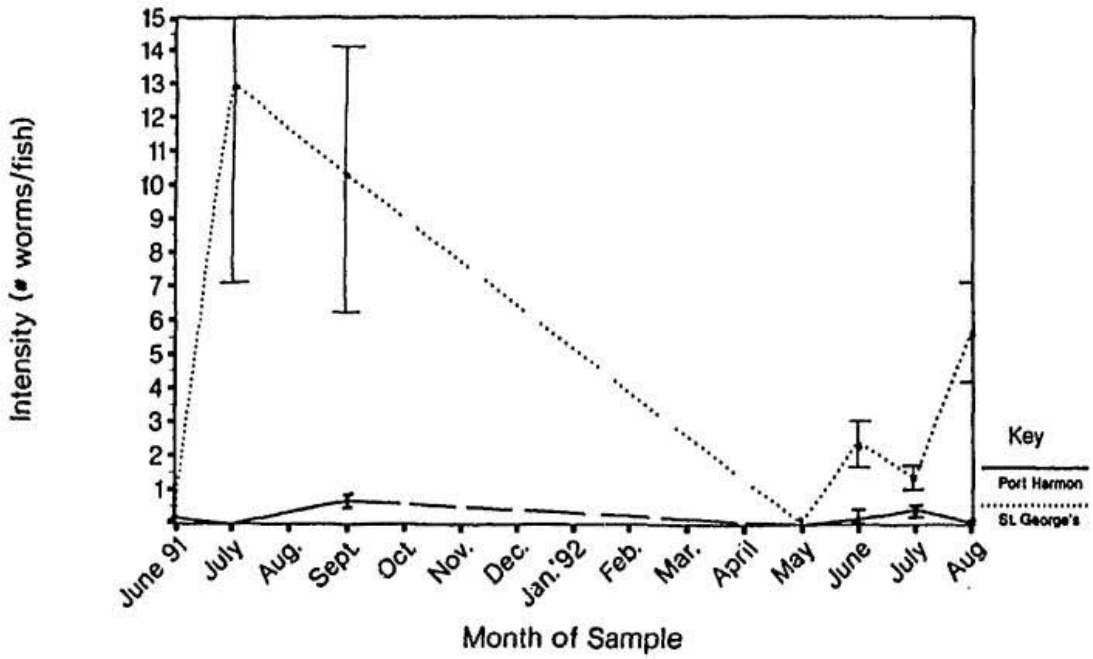


Table 6b: Pooled mean (±s.e.) intensity and prevalence of intestinal helminths in *P. americanus* collected from St. George's Bay during 1992.

Parasite	Port Harmon	St. George's
Acanthocephala		
Intensity	0.10 ± 0.04	0.79 ± 0.20 **
Prevalence (%)	8	53 ***
n	159	105
Nematoda		
Intensity	0.78 ± 0.13	0.06 ± 0.03 **
Prevalence (%)	39	9 ***
n	159	105
Digenea		
Intensity	1.36 ± 0.36	1.22 ± 0.25
Prevalence (%)	26	30
n	159	105

Note : intensity defined as no. helminths / fish

"***" $p < 0.001$ "**" $0.001 < p < 0.05$

in Port Harmon fish during July 1991 and May 1992.

All nematodes collected were either stage 3 or stage 4 anisakine larvae. Monthly nematode prevalences (%) were significantly higher in fish from Port Harmon ($0.025 < p < 0.05$) during each sample month except July 1991 and June 1992 (Table 5,6a; Figure 26). Nematode prevalence in Port Harmon's fish appear to be higher during 1991 than in 1992; while nematode prevalences in St. George's fish showed two distinct peaks (July 1991, June 1992) with very slight prevalences in the interim (Figure 26). Mean monthly nematode intensities were significantly greater in all samples from Port Harmon except during July 1991, and June 1992 (Table 5,6a; Figure 27). A comparison of pooled nematode prevalences and intensities from 1991 (Table 5) and 1992 (Table 6b) shows significantly higher prevalences ($p < 0.001$) and intensities ($p < 0.0001$) in Port Harmon fish during 1991 and 1992. Nematodes were not found in any of the St. George's fish during June and September 1991, and July and August 1992.

Digenea (Trematoda) were observed in winter flounder collected from both Port Harmon and St. George's in May, June, and July (1992), but were not found in 1991. No significant differences were observed in prevalences and intensity of digenes in winter flounder from both sites ($p = 0.258$). (Table 6a, Figures 28, 29). A comparison of pooled prevalences and intensities, again showed no significant differences ($p =$

Figure 26: Annual nematode prevalence (%) in *P. americanus* collected from St. George's Bay during 1991-1992.

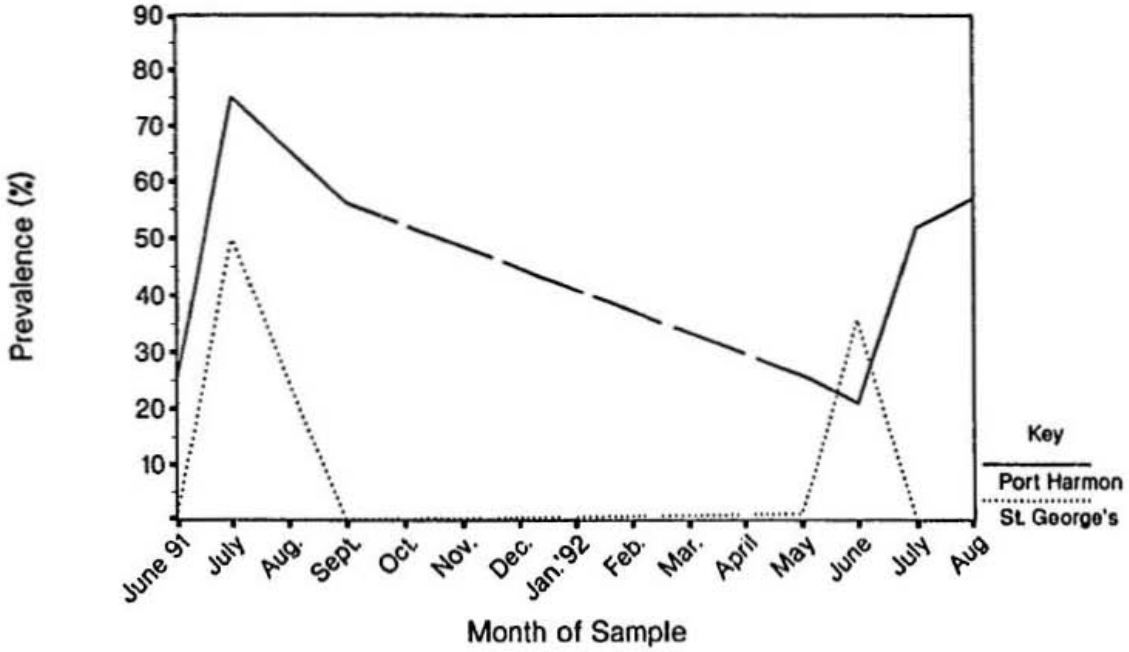
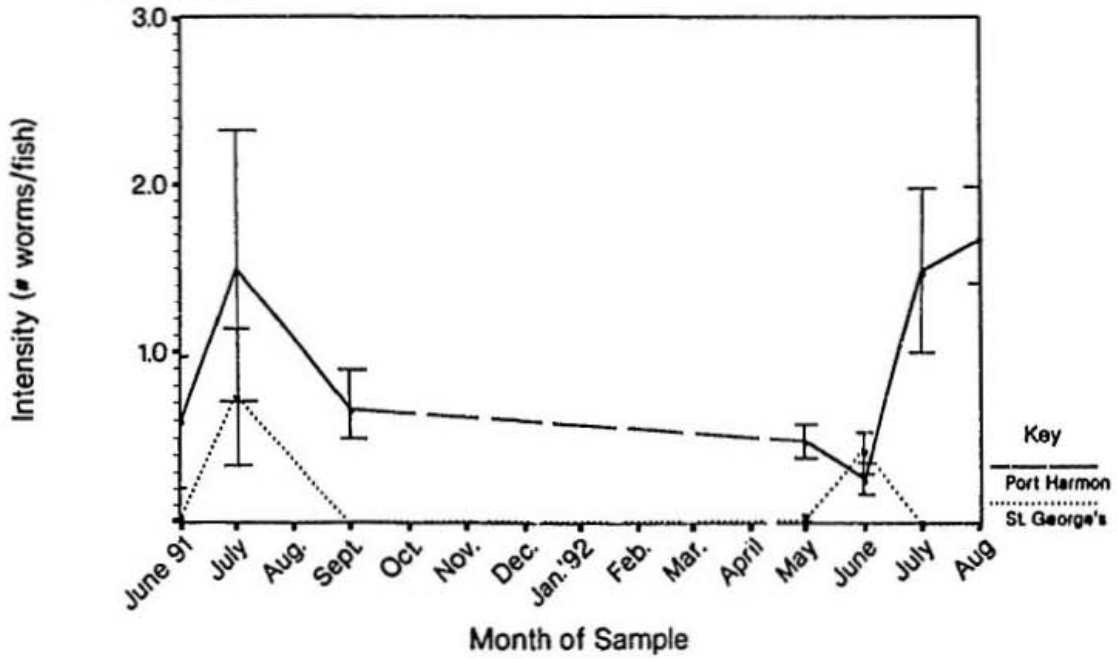


Figure 27: Mean (±s.e.) nematode intensity (#parasites/fish) in *P.americanus* collected from St. George's Bay during 1991-1992.



0.467) between both samples (Table 6b).

One other helminth parasitic taxonomic group, Cestoidea, was recorded from 5 of 11 winter flounder from St. George's collected in June 1991. These were actually post-larval (young adult) cestodes of the genus *Bothriomonus*, and might have been an accidental infestation.

The parasitic protozoan, *Glugea stephani*, was recorded from all samples of Port Harmon fish during 1992, but was only found in St. George's fish during May and June 1992 (Figure 30). During May, the prevalence of *G. stephani* was significantly higher ($0.025 < p < 0.05$) in St. George's fish, while no significant difference existed in June. The prevalence of *G. stephani* in Port Harmon fish showed a progressive increase from 2% in May to 20% in August. Cysts (pseudotumours) of *G. stephani* were located on the outer intestinal and stomach walls, and varied in diameter from 2-12 mm for infected fish from St. George's. Cysts of *G. stephani* of infected fish from Port Harmon were located on the outer intestinal and stomach wall as well as on the liver, spleen, kidneys, and testes, and varied in diameter from 2-28mm.

Virtually all fish from both Port Harmon and St. George's were infested with encysted metacercariae of *Cryptocotyle lingua* (Digenea). It was most common on the gills, right pectoral and caudal fins. The intensities of the other sites that were enumerated (pelvic fin, dorsal and anal fins, see

Figure 28: Digenean prevalence (%) in *P. americanus* collected from St. George's Bay during 1992.

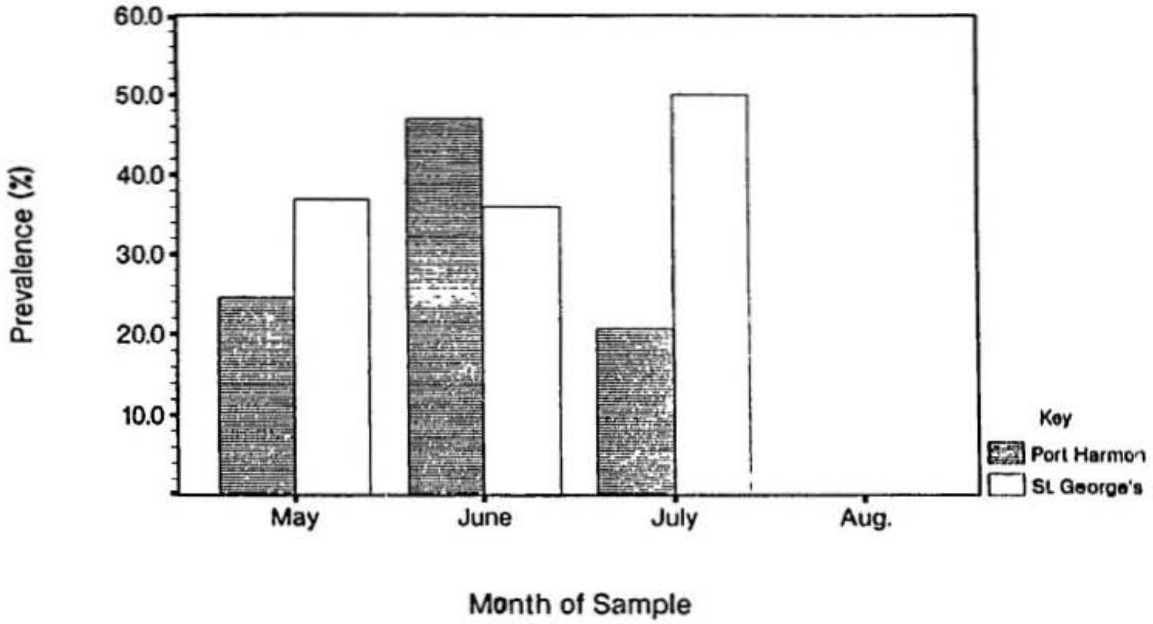


Figure 29: Mean (±s.e.) digenean intensity (#parasites/fish) in *P.americanus* collected from St. George's Bay during 1992.

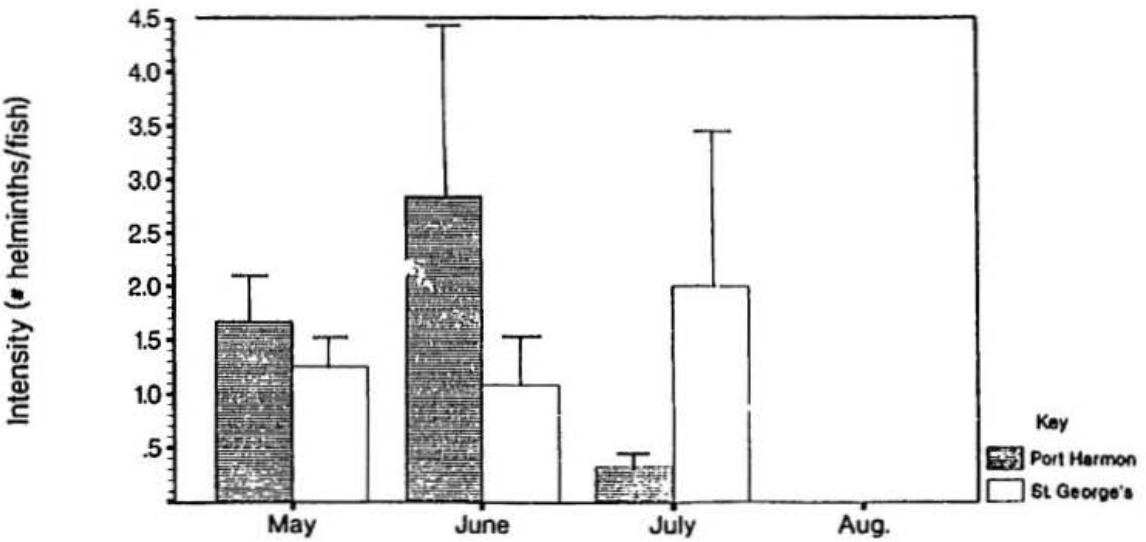
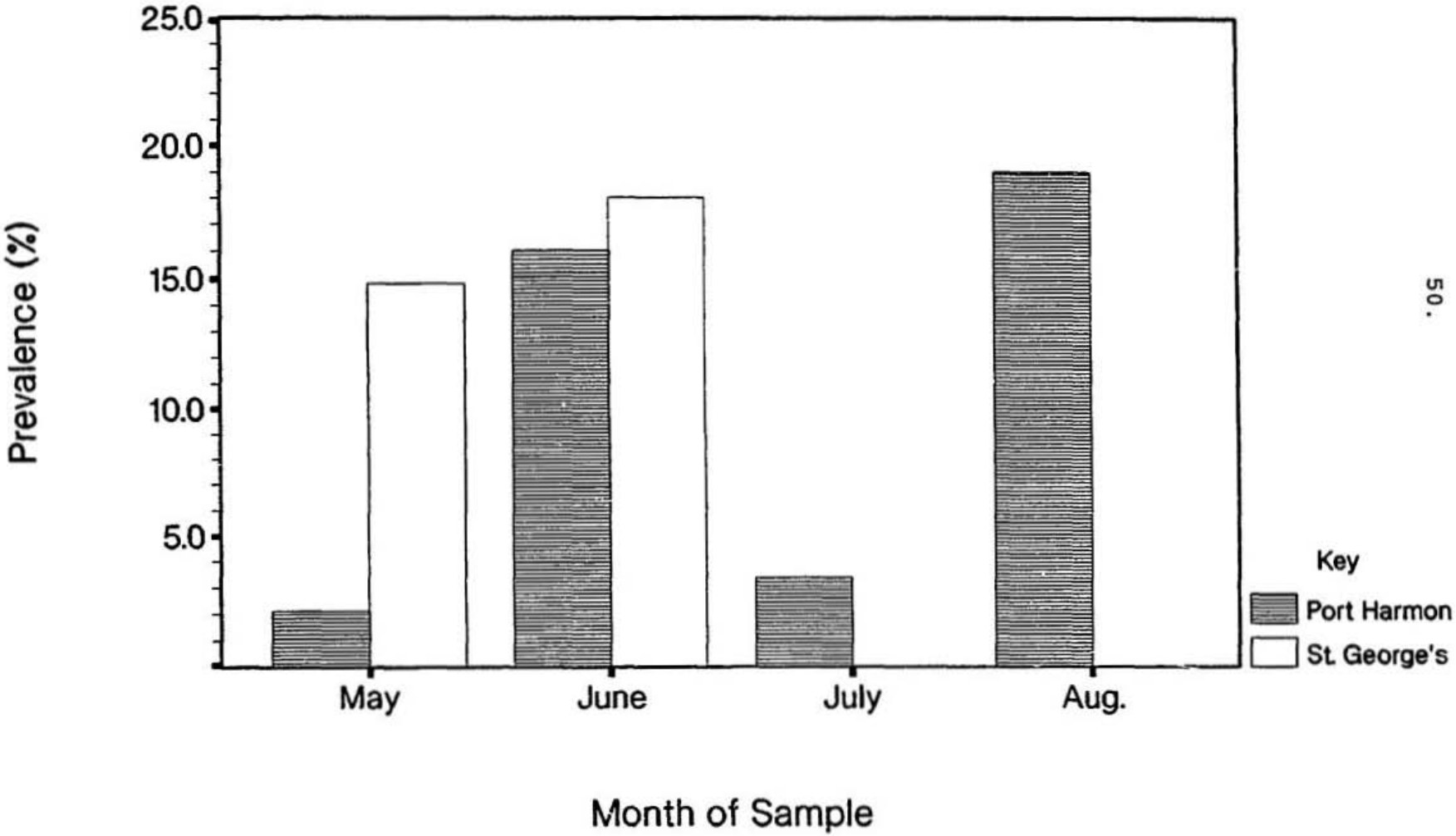


Figure 30: Prevalence (%) of *Glugea stephani* in *P. americanus* collected from St. George's Bay during 1992.



Appendix A) stated previously, showed very low and inconsistent numbers of metacercariae, and were omitted from statistical analyses.

There was a large variance in the number of *C. lingua* metacercariae on the fins of winter flounder from both sites (range:0 - 500+), with some fish from Port Harmon being "encrusted" with metacercariae too numerous to count accurately. These produced a thick black to grey coating on the fins, caudal peduncle and gills, a condition found only at Port Harmon. Fish that were infested with metacercariae greater than or equal to ($\geq$) 100 on the right pectoral fin were categorized as having a "heavy infestation". Winter flounder with such heavy infestations were observed at Port Harmon during all sampling periods of 1992, but were observed at St. George's only during May and June 1992 (Table 7, Figure 31). The pooled percentages of fish that were infected heavily were not significantly greater ($0.05 < p < 0.10$) in flounder from Port Harmon than in samples from St. George's.

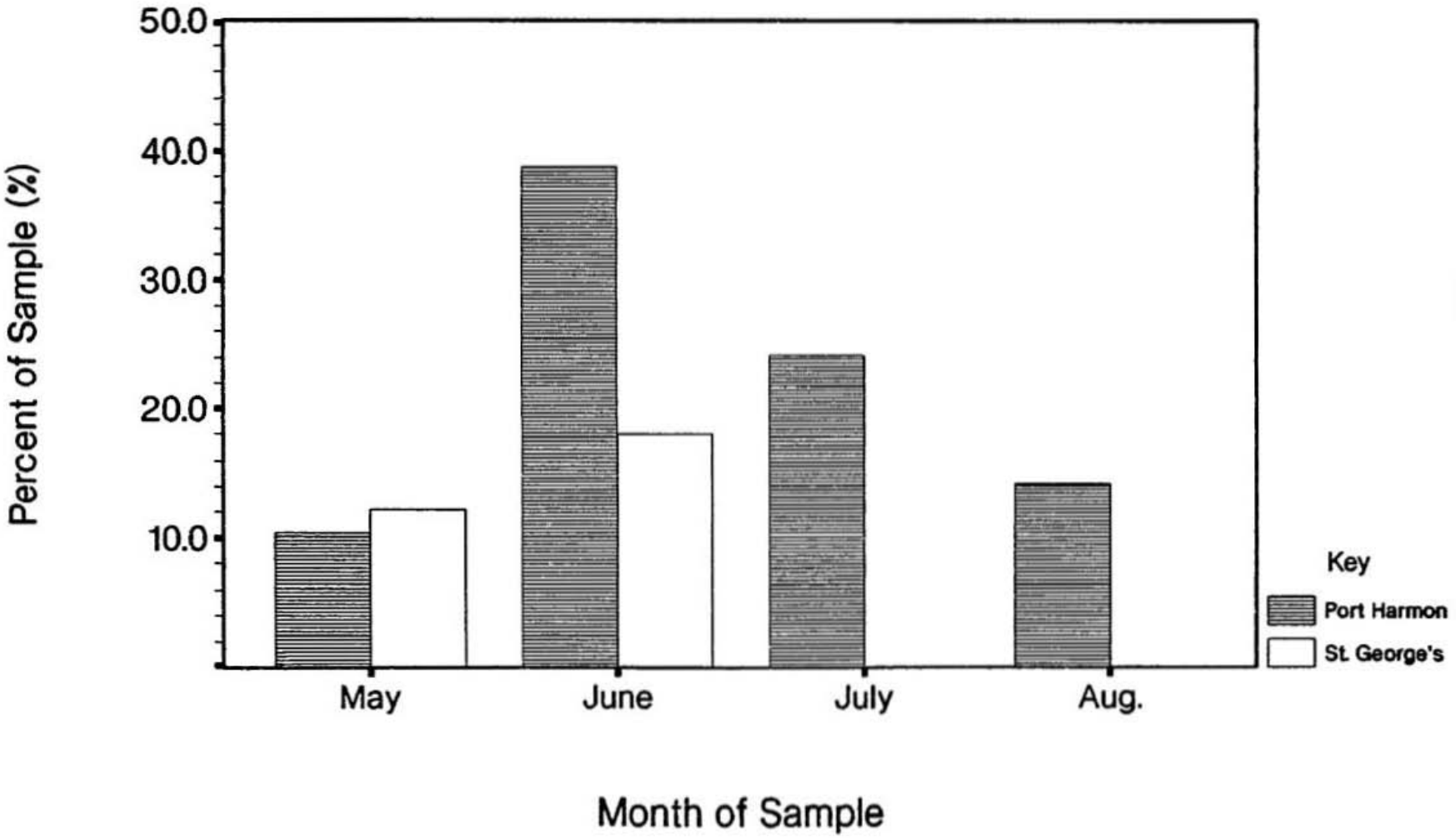
Values used to calculate intensity of metacercariae (mean number of *C.lingua* metacercariae/fin) were values less than 100 metacercariae/fin. A monthly comparison of mean intensity of metacercariae on the pectoral fin showed significantly higher intensities ($p < 0.05$) in Port Harmon fish during all months of 1992 except June (Table 7, Figure 32). The mean monthly intensity of metacercariae on the caudal fin was signif-

Table 7: Summary statistics of infestation analysis of *Cryptocotyle lingua* on *P. americanus* collected from St. George's Bay during 1992.

Month	Variable	Port Harmon	(n)	St. George's	(n)
May	% sample heavily infested	10	(96)	12	(82)
	Mean intensity pect. fin ($\bar{X} \pm$ s.e.)	31.5 ± 2.8	(85)	23.1 ± 2.8 **	(84)
	Mean intensity caud. fin ($\bar{X} \pm$ s.e.)	18.7 ± 2.1	(84)	16.8 ± 2.1	(70)
	(%) *C. lingua* on gills	50	(96)	38 *	(82)
June	% sample heavily infested	39	(18)	18	(11)
	Mean intensity pect. fin ($\bar{X} \pm$ s.e.)	40.1 ± 7.5	(11)	38.6 ± 6.7	(9)
	Mean intensity caud. fin ($\bar{X} \pm$ s.e.)	37.1 ± 4.8	(11)	31.9 ± 7.5	(9)
	(%) *C. lingua* on gills	79	(18)	73	(11)
July	% sample heavily infested	24	(63)	0 **	(8)
	Mean intensity pect. fin ($\bar{X} \pm$ s.e.)	29.3 ± 3.0	(47)	15.6 ± 3.1 **	(8)
	Mean intensity caud. fin ($\bar{X} \pm$ s.e.)	22.9 ± 2.7	(46)	4.3 ± 1.9 **	(8)
	(%) *C. lingua* on gills	59	(63)	38	(8)
Aug.	% sample heavily infested	14	(21)	0	(7)
	Mean intensity pect. fin ($\bar{X} \pm$ s.e.)	43.8 ± 6.1	(18)	25.9 ± 6.4 **	(7)
	Mean intensity caud. fin ($\bar{X} \pm$ s.e.)	25.9 ± 4.0	(17)	19.8 ± 5.0	(7)
	(%) *C. lingua* on gills	71	(21)	57	(7)
Pooled Totals					
	% sample heavily infested	18	(196)	11 *	(108)
	Mean intensity pect. fin ($\bar{X} \pm$ s.e.)	32.9 ± 1.9	(162)	24.2 ± 2.3 **	(105)
	Mean intensity caud. fin ($\bar{X} \pm$ s.e.)	22.4 ± 1.5	(187)	17.5 ± 1.9 **	(106)
	(%) *C. lingua* on gills	58	(196)	42 **	(108)

Note : "**" $p < 0.05$ "*" $0.05 < p < 0.10$
Intensities calculated for values < 100 metacercaria/fin
Heavily infested : > 100 metacercaria/fin

Figure 31: Percent (%) of P. americanus with a heavy infestation of Cryptocotyle lingua.



Note: Heavy infestation = > 100 metacercaria/pectoral fin

icantly greater (p = 0.0004) in Port Harmon fish only during July 1992 (Table 7, Figure 33). Pooled intensities show fish from Port Harmon to have significantly greater intensities (p = 0.0001) of metacercariae on both the pectoral and caudal fins (Table 7).

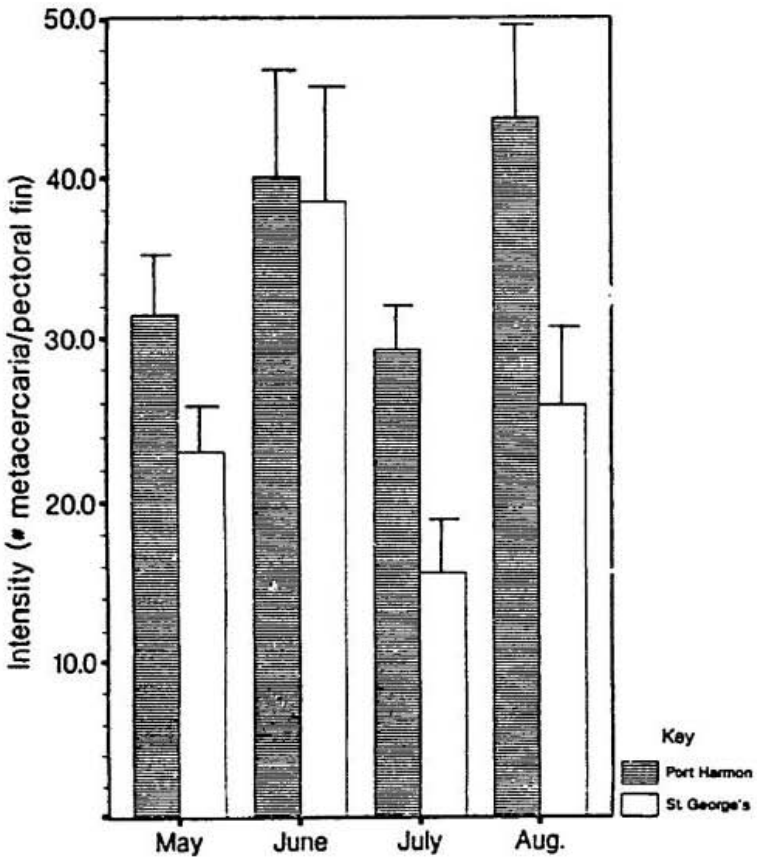
The monthly prevalence of *C. lingua* on the gills of winter flounder showed no significant differences in each monthly sample of 1992. (Table 7, Figure 34). An analysis of pooled prevalences for 1992, shows Port Harmon fish to have a significantly greater ($0.25 < p < 0.05$) prevalence of *C. lingua* on their gills, than St. George's fish (Table 7).

Water Parameters

Figure 35 depicts monthly mean water temperature (°C) for each site at the surface (0 meters) and at bottom (6 meters). Water temperatures for both sites were similar, beginning in May (0.5 to 3.1 °C) peaking in August (14 to 16 °C), and decreasing in November (5.5 to 3.0 °C). Little difference was observed between surface and bottom mean temperatures.

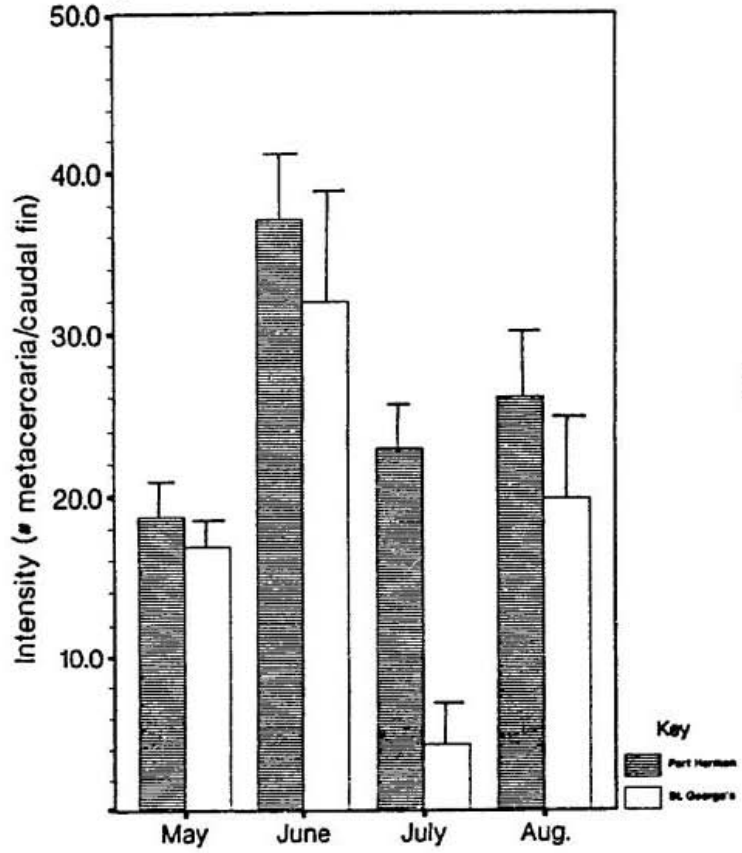
Salinity (‰) values showed a less consistent trend than temperature (Figure 36). At St. George's, surface water salinity peaked in August (27.1‰), while bottom salinity peaked in November (31.1‰). At Port Harmon, surface and bottom salinity both peaked in July (28‰ and 29.7‰ respectively), with a very distinct drop in salinity during November (21.7‰ and

Figure 32: Mean (±s.e.) intensity of *Cryptocotyle lingua* on the pectoral fin of *P. americanus* collected from St. George's Bay during 1992.



Note: values calculated < 100 metacercaria/pectoral fin

Figure 33: Mean (±s.e.) intensity of *Cryptocotyle lingua* on the caudal fin of *P. americanus* collected from St. George's Bay during 1992.



Note: values calculated < 100 metacercaria/ 1st 5 caudal fin rays

Figure 34: Prevalence of Cryptocotyle lingua on the gills of P. americanus from St. George's Bay during 1992.

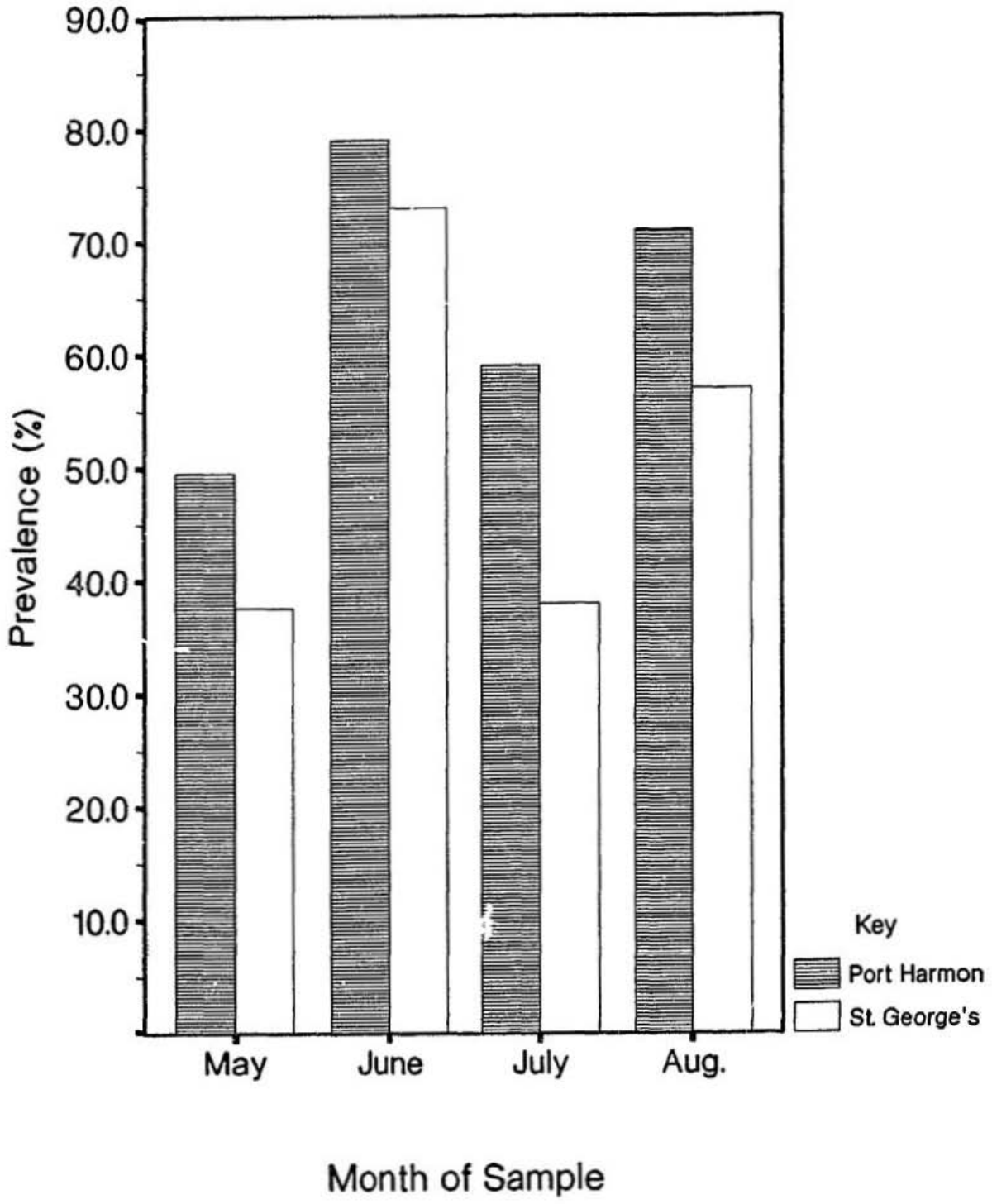


Figure 35: Mean monthly water temperature for sample study sites of St. George's Bay, during 1992.

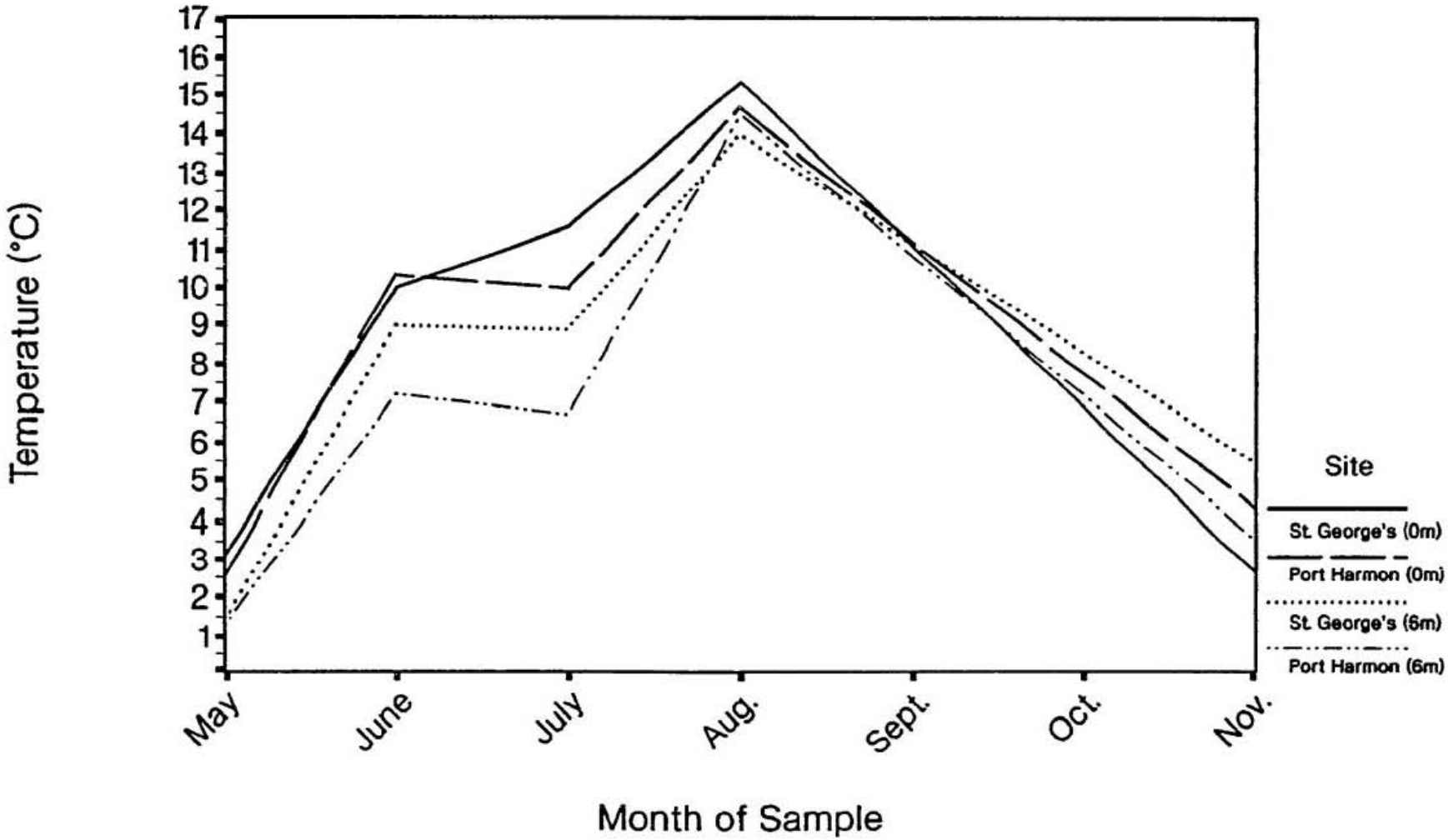
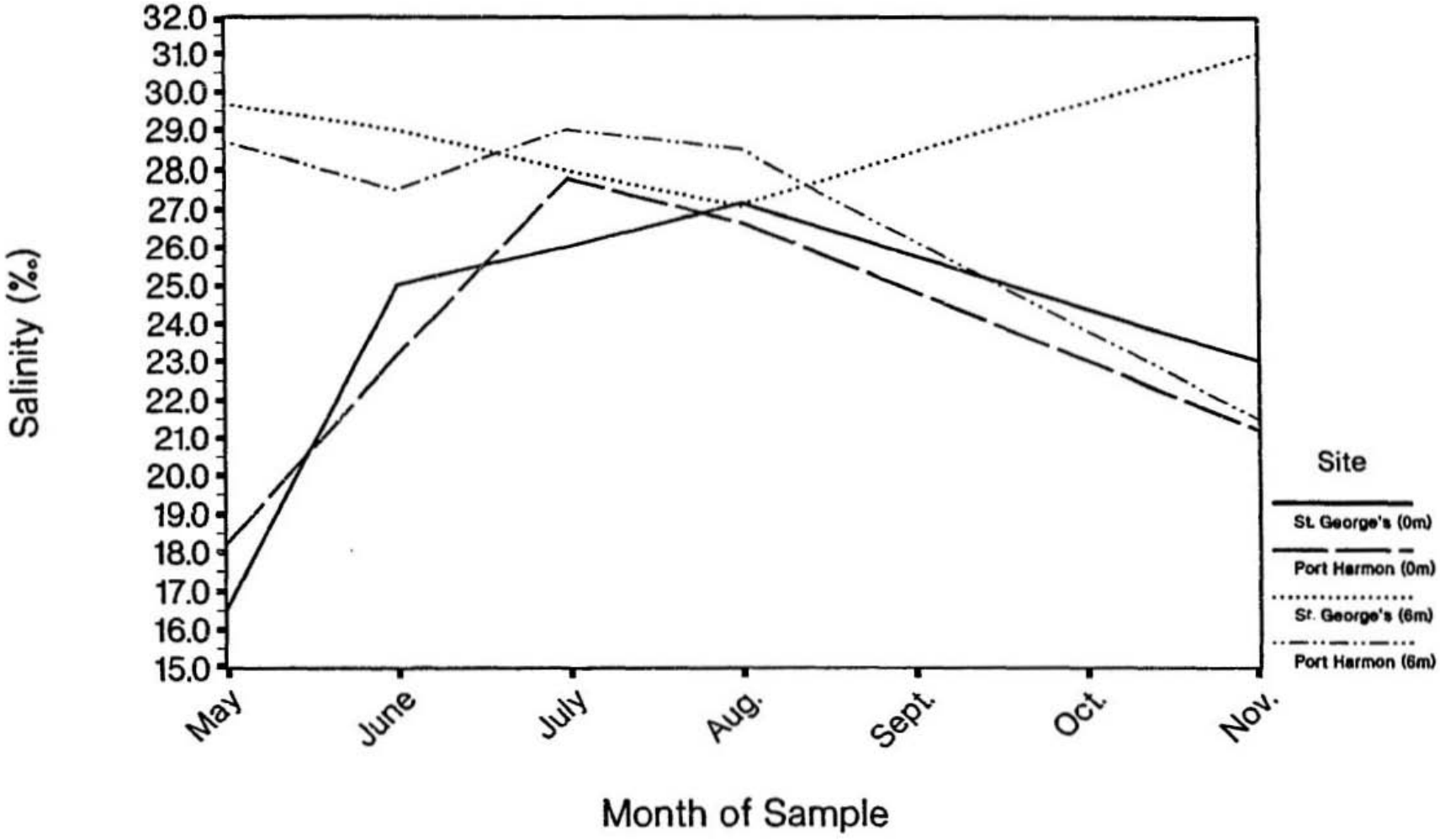


Figure 36: Mean monthly salinity values for sample study sites of St. George's Bay, during 1992.



28‰ respectively).

Conductivity (μohms/s) showed similar trends as salinity, except, both St. George's surface and bottom water peaked in August (300 μohms/s) (Figure 37). Again bottom water at St. George's had a higher mean conductivity value than bottom water at Port Harmon during November (280 and 195 μohms/s respectively).

Mean monthly pH values showed no significant difference between sites or between surface and bottom water. In terms of acidity content, the waters appear to be slightly alkaline with a mean pH in May of 7.8 to 8.2, which slowly increased over summer and into fall (November) with a mean pH of 9.0 to 8.5 (Figure 38).

Mean monthly dissolved oxygen levels (mg/l) varied considerably between sites and between depths. Mean dissolved oxygen levels for St. George's surface water peaked in May at 10.2 mg/l and never fell below 9.2 mg/l, while mean dissloved oxygen levels for Port Harmon surface waters peaked in August at 9.8 mg/l, and fell to 6.4 mg/l in November (Figure 39). Mean bottom water dissolved oxygen levels were very similar except during May when St. George's bottom water oxygen concentration was 9.7 mg/l and Port Harmon bottom water oxygen level was 6.3 mg/l. Both bottom water mean dissolved oxygen levels peaked in July (11.1 mg/l for Port Harmon and 10 mg/l for St. George's) and dropped in July to 5 mg/l (Figure 39).

Figure 37: Mean monthly conductivity values for sample study sites of St. George's Bay, during 1992.

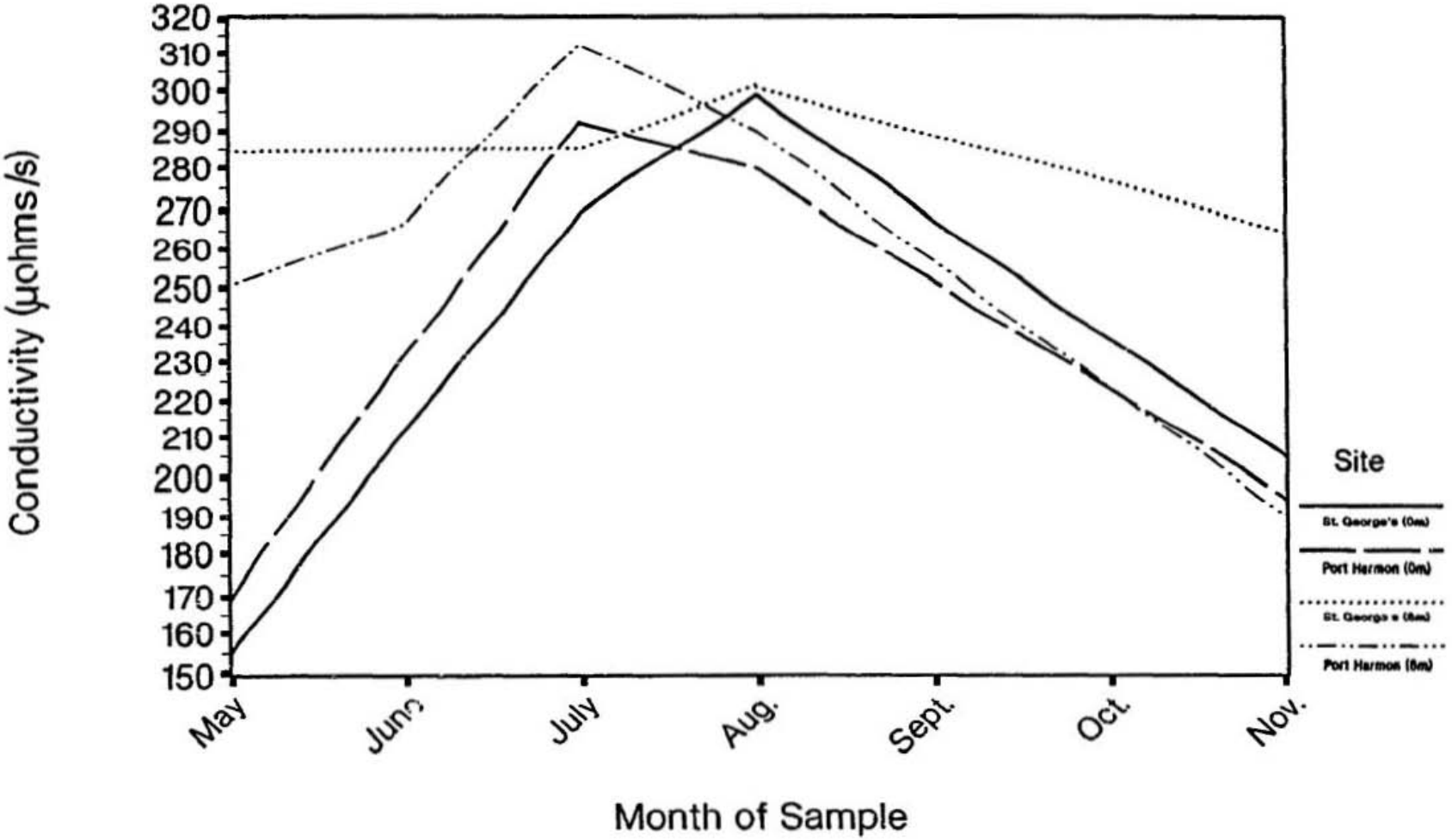


Figure 38: Mean monthly pH values for sample study sites of St. George's Bay, during 1992.

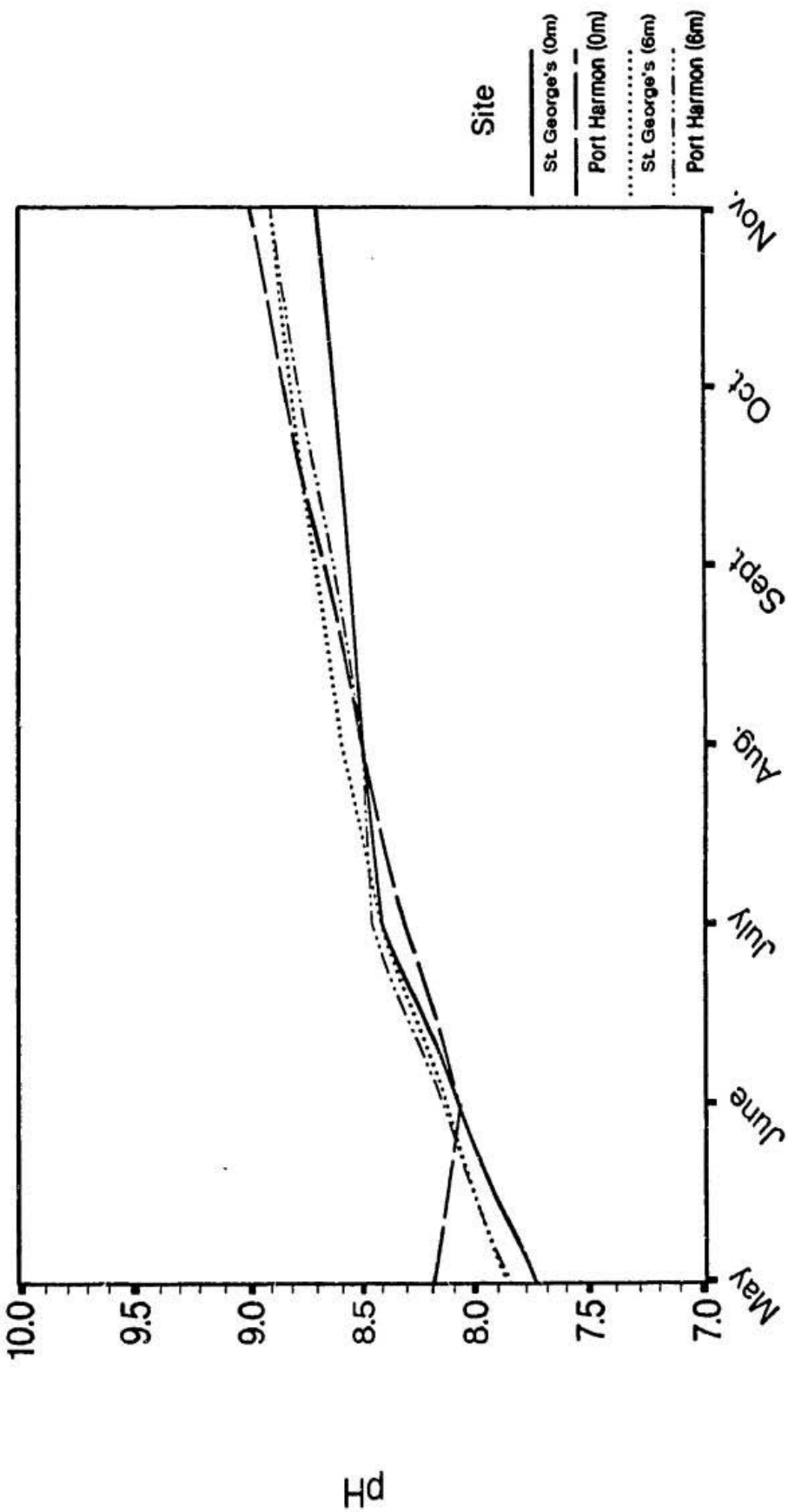
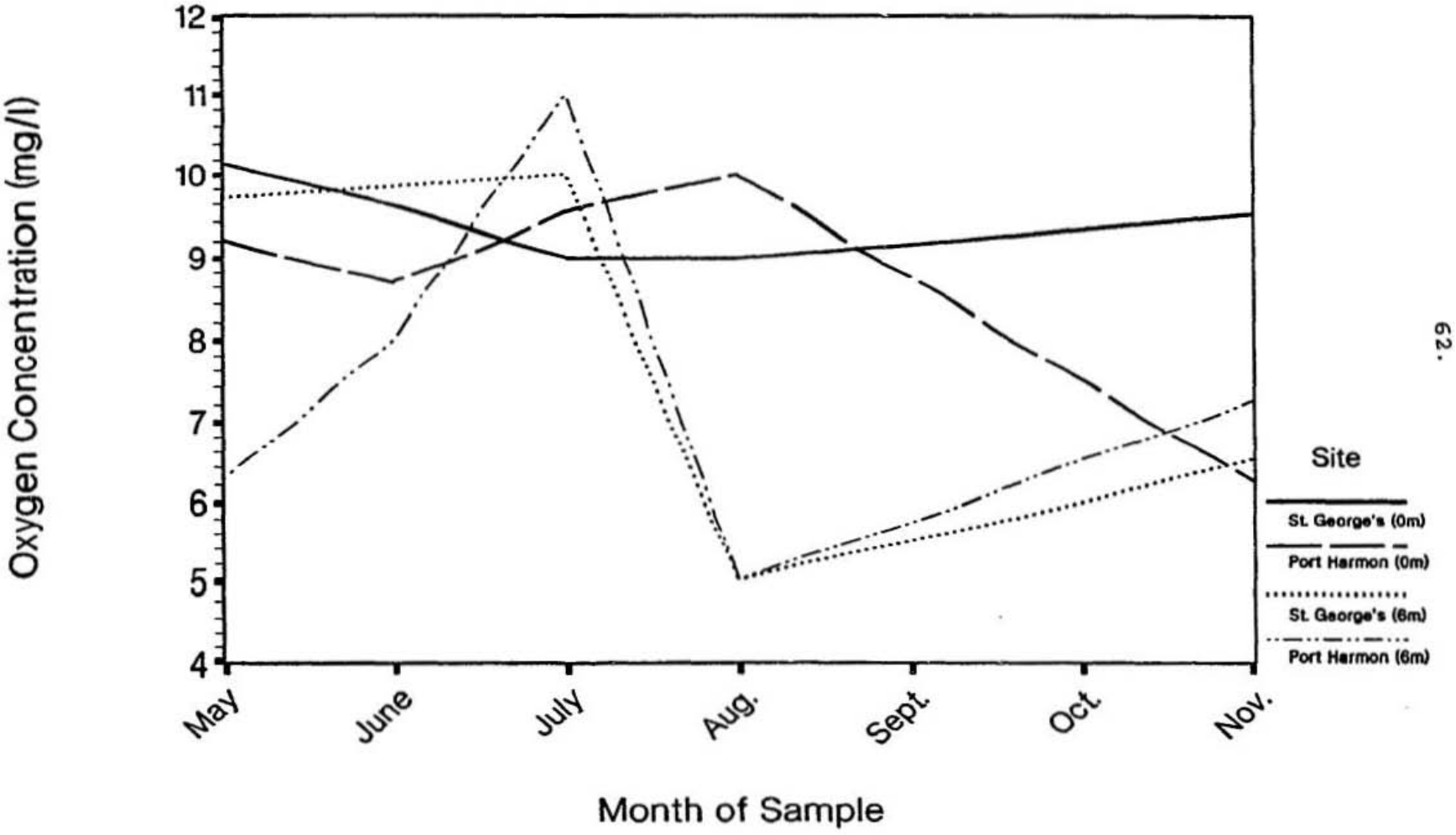


Figure 39: Mean monthly dissolved oxygen values for sample study sites of St. George's Bay, during 1992.



Discussion

Sample statistics

Winter flounder are found throughout the year in Newfoundland inshore waters of 2.0 to 20 meters (Kennedy, 1964; Kennedy and Steele, 1971). In the present study, it appears that more winter flounder reside in the Port Harmon area than in the St. George's area, and that there are more large fish (> 28cm) found at Port Harmon. It should be noted that an equal amount of effort was employed at both sites to capture fish (usually 2 dives per day, 1 at each site). The greater maximum depth and steeper bottom slope at Port Harmon may provide more suitable habitats for winter flounder in terms of refuge sites and temperature. Van Guelpen and Davis (1979) and Kennedy (1964) found that large winter flounder (>26cm) in Newfoundland will migrate to deeper water (>6m) during summer to avoid high temperatures (>14°C). This may account for the slightly larger mean length of winter flounder from Port Harmon. Throughout the sampling period, the largest winter flounder (>30cm) were caught during May (spawning time), while over summer (June,July,August) most winter flounder from both sites were less than 30 cm. No winter flounder greater than or equal to 30 cm were caught at St. George's during June, July, and August, but several fish from Port Harmon during these months were 30cm or greater.

Winter flounder in Newfoundland tend to submerge in sediment and cease feeding from October and November, as their gonads begin to ripen (Burton and Idler,1984; Kennedy, 1964). In November 1991, no winter flounder were caught, primarily due to unfavourable weather which prevented diving and necessitated the use of hook and line. A lack of feeding would account for no fish being caught. During November 1992, inclement weather again caused poor diving conditions and poor underwater visibility. Nevertheless, several dives were made but winter flounder were extremely difficult to find as they had submerged in the substrate. The few fish captured (5 from Port Harmon, 3 from St. George's) had empty stomachs and maturing gonads.

No differences in length and weight regression values might imply similar growth rates of flounder from both sampling sites. However, a large error occurs when using values at the extremes of the regression lines. This error may be the result of relatively few specimens at the extreme ends of the size range(<16 cm, >36cm). The fish were not aged due to time constraints, but otoliths and scale samples were retained for a subsequent study. McMaster et al. (1991) reported decreased growth rates in suckers (*Catostomus commersoni*) exposed to bleached kraft mill effluent.

The size range of the samples of winter flounder (11.0 to 45.7 cm) was similar to that of other studies (Kennedy, 1964)

and represents virtually the entire post larval size range for the species (Scott and Scott, 1988).

Body and Organismic Indicators

The condition factor (K-factor) is a widely used and useful indicator of energy metabolism. A decline in K-factor over a set time period can be indicative of a depletion in energy reserves, which is often the result of some disturbance in energy metabolism, i.e. some type of stress (Goede and Barton,1990; Hodson et al.,1992).

Winter flounder from Port Harmon, had significantly lower mean K-factor values compared to samples from St. George's except for July and September of 1991, and May of 1992. The reason for the lack of difference in 1991 are most likely due to the small sample sizes. When 1991 samples were pooled, mean K-factor values of fish at St. George's were significantly greater than others from Port Harmon. Although the fish from Port Harmon were significantly larger in length and weight, mean K-factor values were non-significant during May 1992. Perhaps spawning stress or some external stressor contributed to the depletion of energy reserves and ultimately lower K-factor values in the fish from Port Harmon. No comparison was made between data taken in the present study and that in 1978 or with a sample from Norris Point in 1990 because of relatively small "n" values of these samples.

Mean K-factor values increased over summer as winter

flounder feeding rates increased, as also noted by Tyler and Dunn (1976). However, fish from St. George's maintained significantly higher K-factors as compared to fish from Port Harmon. Due to small sample sizes from St. George's during June, July and August, data were pooled and collectively referred to as "summer 1992".

Goede and Barton (1990) cite several reports that demonstrate lower K-factor values in fish which resulting from one of several stressors: (i) lowered nutrient availability, (ii) sexual maturation, (iii) migration, (iv) capture and handling, (v) disease, and (vi) exposure to sublethal levels of pollutants. Hodson et al. (1992) and Khan et al. (1992) reported significantly lower K-factor values in fish living adjacent to pulp and paper mills compared with fish collected from reference (effluent-free) sites. In contrast, McMaster et al.(1991) reported increased K-Factor in white suckers (*Catostomus commersoni*) exposed to bleached kraft mill effluent and suggested that it was related to disruption in metabolic capability and altered energy allocation.

The liver is used for the detoxification and excretion of compounds that are toxic to the organism (Anderson, 1990; Bucher et al.,1992). An overall decrease in hepatosomatic index (HSI) is generally indicative of some physiological stress resulting in a depletion of energy reserves. Mean hepatosomatic indices (HSI) of flounder from Port Harmon were

greater than mean HSI values of flounder from St. George's in May 1992 perhaps because of differences in spawning times. Mean HSI values of flounder from Port Harmon collected in August 1992 were again significantly greater than mean HSI values of flounder from St. George's which may be a result of the fact that the August 1992 sample size from St. George's was small (n=7). When pooled summer 1992 (June, July, August) HSI values of flounder from both areas were compared, mean HSI was significantly greater in fish from St. George's. Lower HSI values in fish from Port Harmon collected during summer 1992, may be a direct result of the presence of large vacuolated hepatocytes (loss of stored glycogen) and pycnotic nuclei in the livers of flounder from Port Harmon as found in a histopathological study by Khan et al. (1993). Bucher et al. (1992) found similar results in liver morphology of bullheads (*Cottus gobio*) exposed to different levels of pulp mill effluent. Several authors reported an increase in mean HSI values in fish exposed to bleached kraft mill effluent (Andersson et al.1988; Hodson et al.,1992; Lehtinen, 1990; McMaster et al.,1991; Munkittrick et al., 1992). This increase was attributed to cellular hypertrophy and induction of liver detoxification enzyme systems. Furthermore, Lehtinen (1990) hypothesized that unbleached mill effluent had an inhibitory effect, while bleached mill effluent had a stimulatory effect on fish detoxification enzyme systems.

Mean splenosomatic indices (SSI) were not significantly different between flounder sampled at Port Harmon and St. George's. However, Anderson (1990) noted that enlarged spleens (splenomegaly), usually due to an increase in haemopoeisis and cellular hypertrophy caused by leukocyte proliferation, is common in fishes exposed to chronic stress. Khan et al. (1993) found multifocal sites of haemosiderosis in spleen samples of fish from Port Harmon. The expected difference in SSI values was not seen, possibly due to some type of compensatory mechanism, so that effects of chronic stress were more evident in other organs.

Mean visceral somatic indices (VSI) were not statistically different on a monthly comparison, but a pooled summer 1992 mean VSI of fish from St. George's was significantly greater than a pooled mean VSI of fish from Port Harmon. These VSI values, based on the presence of food in the digestive tract, imply that fish from St. George's were consuming more food (by weight) than fish from Port Harmon.

The gonadosomatic index (GSI) is an indicator of sexual maturity in fish, as high GSI values represent mature fish about to spawn (Goede and Barton, 1990). Fish from Port Harmon had significantly greater mean GSI values compared with fish from St. George's during May and June 1992. This difference might be a result of delayed spawning or delayed gonadal maturation in fish from Port Harmon. By late May, most of the

mature fish from St. George's had spawned, while many mature fish from Port Harmon had not spawned. By mid-June, all fish from St. George's had spawned, while several fish from Port Harmon had still not spawned. This condition was represented in the scatterplots of GSI and length for males and females (Figures 10 - 13). The relative percentages of mature fish and immature + post-spawned flounder also suggests delayed spawning in fish from Port Harmon. Andersson et al. (1988), McMaster et al.(1991), and Munkittrick et al.(1991;1992) reported low gonadosomatic indices (GSI), delayed age to maturation, and low levels of plasma sex steroids in fish exposed to bleached pulp mill effluent.

In an analysis of fecundity of female winter flounder from both areas, mean diameter and mean number of oocytes showed no differences. In addition, fish from both Port Harmon and St. George's collected in November 1992 showed maturing gonads. These two results might possibly rule out the idea of delayed maturation in fish from Port Harmon, but not delayed spawning. The mean diameters and numbers of oocytes produced by mature females recorded were in the same range of values recorded by Kennedy (1964) for winter flounder from Conception Bay, Newfoundland. Kennedy and Steele (1971) noted that larger winter flounder produce a greater number of oocytes per unit length. Since the fish from Port Harmon collected in May (1992) were significantly larger than the fish from St. George's, these

fish should have contained a greater number of eggs. In the present study, decreased fecundity might possibly be another sign of chronic stress in winter flounder from Port Harmon.

An external stressor, if involved, can act directly by impairing hormonal function, or indirectly by acting upon the nutritional resources available to the fish. Burton and Idler (1984) reported that a "non-reproductive" state in winter flounder was associated with low condition factors. In a series of experiments, mature-age winter flounder could be induced not to undergo gametogenesis by food deprivation (Burton and Idler, 1987). In nature, if conditions are unfavorable, winter flounder tend to adopt a strategy whereby egg production is sacrificed for somatic growth (Tyler and Dunn, 1976). The situation in this study seems to be of a different nature. The winter flounder matured, but either spawned later than the "normal" spawning period or did not spawn at all (atresia). Unfortunately, gonads were not examined histopathologically so no direct evidence of atresia in the fish from Port Harmon is available.

What are some of the implications for a fish that does spawn "late" in the spawning season ? Could it represent an adaptive strategy to avoid egg predation? The implications involved in delayed spawning present many questions that need be addressed in future studies. Additionally, the impact of delayed spawning on recruitment is unknown. Throughout the

summer months of July and August (1992) juvenile winter flounder (2.0 to 6.0 cm) were observed at each site in shallow water (0.6 to 0.8 meters), which might indicate spawning success at both sites. However, the relative numbers of these juveniles were not quantified, and whether or not they were transported by the current or migrated to these sites from elsewhere is unknown.

Haematological Values

Haematological variables such as haemoglobin (HB), haematocrit (HCRT), and lymphocyte number (LYM) have been widely used as indicators of fish health in field and laboratory settings (Blaxhall, 1972; Goede and Barton, 1990). The increase in haematological variables (HB, HCRT, LYM) over the summer months of June, July, and August, was probably a result of increased temperatures and increased feeding. Bridges et al. (1976) and Mahoney and McNulty (1992) found lower haematological values, than those of the present study, in winter flounder from Maine and the New York Bight. These differences may be a result of latitudinal, hence, climatological differences in sample sites.

Flounder from Port Harmon had consistently lower mean values of HB, HCRT, and LYM on a monthly and pooled basis than those from St.George's. Some of these low haematological values may be indicative of anaemia, which would ultimately

affect respiratory function, blood electrolyte level, and cellular immunity. Khan et al. (1992) reported lower blood haemoglobin levels in winter flounder living near pulp mill effluent as compared with flounder from a reference site. Mahoney and McNulty (1992) also found low levels of blood haemoglobin and haematocrit in winter flounder with fin rot disease from an industrially polluted area. McLeay and Gordon (1977) reported low levels of leucocytes and blood haematocrit in rainbow trout (*Salmo gairdneri*) exposed to various concentrations of bleached kraft mill effluent. Andersson et al. (1988) also found low levels of leucocytes in fish living downstream from a bleached kraft pulp mill.

Low leukocyte numbers is evidence of immunosuppression (Anderson, 1990; Andersson et al.,1988; Blaxhall, 1972). This suggests that fish from Port Harmon were exposed to some form of immunosuppressant. Immunosuppression reduces resistence to disease and pathogens so that diseases that are normally kept at a low level, proliferate, this appears to be the case in winter flounder from Port Harmon.

Fin Necrosis

Fin necrosis is a commonly reported disease of winter flounder and it proliferates with the increased levels of stress caused by pollutants and high temperatures (Mahoney and McNulty, 1992; Murchelano, 1975; Ziskowski and Murchelano,

1975). It begins as a wound or laceration, usually on the caudal, dorsal, or anal fins which aid in submergence in the substrate. The fins become infected by pathogenic bacteria or viruses which spread. If a fish's immune response is impaired, fin necrosis proliferates and causes extensive physical damage.

Although fin necrosis was evident in groups of winter flounder from both study sites, and its prevalence increased over summer in both groups, the fish from Port Harmon showed more severe signs of the disease. Only fish from Port Harmon showed haemorrhagic areas along the necrotic edge of eroded sites. In addition, some fish from Port Harmon had their caudal fin completely eroded and showed necrosis of both pectoral and pelvic fins. Also associated with fin necrosis was the presence of skin ulcers, which again were more severe at Port Harmon. Ziskowski and Murchelano (1975) reported a similar severity of infection in winter flounder from the highly polluted New York Bight region. Khan et al. (1992) reported a high prevalence (90%) of fin necrosis in winter flounder collected from Port Harmon in 1990. Several authors have reported high levels of fin necrosis in fishes such as rainbow trout (*Salmo gairdneri*), perch (*Perca fluviatilis*), and ruffe (*Gymnocephalus cernua*) exposed to bleached kraft mill effluents (Couillard et al., 1988; Lindesjoo and Thulin, 1990; Sandstrom and Thoresson, 1988). Additionally, Munkitt-

rick et al. (1992) reported "slash-like" lesions in whitefish (*Coregonus clupeaformis*) populations that were exposed to bleached kraft mill effluent. Both haemorrhagic fin necrosis and a proliferation of skin ulcers as reported in flounder from Port Harmon probably contributed to low haemoglobin and low haematocrit levels through blood loss.

Feeding and Parasitofauna

Winter flounder feed mostly during summer months in Newfoundland waters to build up energy reserves for maturation of the gonads over fall and winter for spawning in early spring (Kennedy, 1964; Kennedy and Steele, 1971; Tyler and Dunn, 1976). As they are sight feeders, they will consume a variety of benthic prey items diurnally but have been reported to forage non-selectively (Keats, 1990). Obviously, size constraints of prey and maximum gape, will limit the type of prey consumed.

The higher visceral somatic indices, noted in the present study in flounder from St. George's, suggests either that they were consuming a greater weight of food during the summer months of 1992 than at Port Harmon or that they were feeding continually at St.George's. Since both sample sites appeared to present ample foraging opportunities, an apparent reduction in food intake might be associated with stress or sensory impairment. McMaster et al. (1991) reported a decrease in

feeding in suckers (*Catosomus commersoni*) that were exposed to bleached kraft mill effluent.

The most dominant prey item in the diet of winter flounder from St. George's Bay was algae, although, winter flounder are not generally considered herbivorous. Algae were consumed in greatest amounts during May and early June, and was ingested incidentally with small epiphytic colonial hydrozoans and bryozoans, and juvenile molluscs (*Mytilis,Littorina spp.*). It was evident from stomach analyses that most of the algae were undigested in their passage through the alimentary canal. A greater frequency of amphipods was consumed by fish from St. George's than from Port Harmon. Although the density of amphipods at each site was not quantified, amphipods were observed at each site during all dives. Levings et al. (1976) reported increased mortality rates in amphipods (*Gammarus setosus, Anisogammarus confervicola*) exposed to increasing concentrations of bleached kraft mill effluent. Whether or not these are the same species of amphipods in St. George's Bay, and whether or not the pulp mill effluent from Port Harmon is lethal to the local amphipod populations is unknown at this time.

Perhaps the difference in frequency of prey items is associated with the more intense fishing activity from Port Harmon, resulting in fish offal (consisting of muscle tissue, viscera, and scales of *Gadus morhua, Clupea harengus, Scomber scombrus, Salmo salar,* and *Hippoglossus hippoglossus*) being readily

available to winter flounder. Obviously, the energy required to forage on amphipods is greater than that required to forage on fish offal. This might account for a greater frequency of fish offal being consumed by flounder from Port Harmon. However, a greater frequency of fish eggs (*Pleuronectes americanus, Myoxocephalus sp.*) was consumed by fish from St. George's, mostly in May (1992). This might possibly be further evidence of delayed spawning in winter flounder from Port Harmon, if eggs were available for consumption by flounder at St. George's during May, but were not in Port Harmon. All other food items were consumed at similar frequencies by both groups of fish.

With such a varied diet, winter flounder are hosts to a range of parasites, including helminth and protozoan parasites. Three general trends were evident in the examination of endoparasites : (i) an increase in prevalence and intensity with a close proximity to pulp mill effluent (Nematoda, Microspora), (ii) a decrease in prevalence and intensity with a close proximity to pulp mill effluent (Acanthocephala), and (iii) no difference in prevalence and intensity (Digenea).

Nematoda

All nematodes collected from winter flounder were anisakid larvae, which possibly were acquired either from ingestion of fish offal, which was abundant at Port Harmon, or from crus-

taceans. Interestingly, with the exception of two sample periods (June 1991 and June 1992), flounder from St. George's had low infestations of nematodes. These two annual peaks in nematode prevalence and intensity correspond to two peak fishing times (thus peak fish offal disposal times) at the public wharf in St. George's. Fishing in St. George's is done on a more local basis, not of the same scale as the fishing activity at Port Harmon. Another possible explanation is that immunosuppression in the winter flounder from Port Harmon enabled such intense nematode infestations (acquired from eating benthic crustaceans) to become established.

Ronald (1963) reported nematode prevalences in winter flounder from the Gulf of St. Lawrence at a maximum of 10%. Nematode prevalences in winter flounder in the present study were much higher, varying from 0 to 57%. Khan et al. (1992) reported that a 40% prevalence of anisakid nematodes in winter flounder from Port Harmon, collected in 1990, was higher than that in fish from a reference study site.

Microspora

The prevalence of the microsporidan, *Glugea stephani*, in winter flounder from Port Harmon was also higher than in samples from St. George's. As noted previously, the infection was not found in fish from St. George's during July and August, 1992, and the infection was much more severe in fish from Port

Harmon. This may be a direct result of immunosuppression. Evans and Gratzek (1989) reported that infections of *G. stephani* in winter flounder were responsible for immunosuppression of humoral antibodies. This, combined with the very low lymphocyte numbers in flounder from Port Harmon, possibly allowed the cysts (pseudotumours) of *G. stephani* to enlarge, metastasize, and proliferate throughout the viscera of the infected fish as noted in the present study. Takvorian and Cali (1984) reported monthly prevalences of *G. stephani* in winter flounder that varied between 0.63 and 25%. Also, *G. stephani* was present throughout the year, but proliferated only at water temperatures above 15°C. Furthermore, they noted that these cysts were found on the intestinal wall and rarely on the outer stomach wall. In the present study, infections were found at water temperatures as low as 3°C and infections increased with increasing temperatures (which peaked at 16°C), also, cysts were observed on all body organs of flounder from Port Harmon.

Acanthocephala

Acanthocephalan infestations, unlike nematode and microsporidan infections, were maintained at a higher level in flounder from St. George's, and were often absent in flounder from Port Harmon. Amphipods and copepods are the intermediate hosts of acanthocephalans (Möller and Anders, 1986). Possibly, the differences in acanthocephalan prevalence and intensity

occurred because a higher frequency of amphipods were eaten by fish from St. George's. However, during the sample months when no acanthocephalans were found in fish from Port Harmon, amphipods were still noted in the stomach and intestine of these fish. Möller and Anders (1986) and Ronald (1963) gave seasonal prevalences of acanthocephalan infestations in flounder, which were very similar to the acanthocephalan infestations in flounder from St. George's in the present study. Khan et al. (1992) observed no acanthocephalans in flounder collected from Port Harmon in 1990, compared with a 41.2% prevalence of acanthocephalans in flounder collected from the same site in 1978 (shortly after the linerboard mill had closed). Acanthocephalans have been used in many studies as bioindicators of pollution (Khan and Thulin, 1991). Khan and Kiceniuk (1983) reported a decrease in acanthocephalan infestation in Atlantic cod, *Gadus morhua*, exposed to sublethal concentrations of petroleum hydrocarbons. Khan and Thulin (1991) cited the work of Valtonen and Koskivaara (1989), who reported an increase in acanthocephalan infestation in roach, *Rutilus rutilus*, living in a freshwater lake exposed to bleached kraft mill effluent.

Marine fish drink seawater to osmoregulate, and perhaps these parasites were voided after their host ingested some toxin(s). There is also the possibility that these parasites were voided as the host's physiology changed as a result of stress. Acanthocephalans absorb nutrients through their

tegument while nematodes have a complete alimentary canal, with a thick protective outer cuticle (Möller and Anders, 1986); such structural differences would make acanthocephalans more susceptible to changes in their environment within the host. Another possible explanation accounting for a decrease in intensity is that the toxin(s) acts on the intermediate host, perhaps altering larval viability, thereby preventing transmission of the parasite. Unfortunately, none of these hypotheses has been tested at this time.

Digenea - Endoparasites

Digenetic trematodes were observed only in flounder caught in 1992. Freezing might have affected samples collected in 1991. Schmidt (1988) noted that trematodes degenerate in frozen stomach samples. In the present study, digenetic trematode infestations were similar in flounder from both sites. Perhaps the adult parasites are unaffected by any of the host's physiological alterations. Interestingly, one of the common digeneans (*Podocotyle atomon*) found in fish from both sample sites, uses an amphipod as the second intermediate host in its life cycle (Möller and Anders, 1986). However, the differences in observed frequencies of amphipods consumed by winter flounder, did not affect prevalences and intensities of digenes in specimens from both sample sites.

Scott (1982; 1984) reported in winter flounder from the

Gulf of St. Lawrence, seasonal digenean prevalences and intensities similar to the values obtained in the present study. In addition, Scott (1984) reported a decrease in digene infestations in late summer, as was also noted in this study. Khan and Thulin (1991) cited the findings of Valtonen and Koskivaara (1989) who reported a 0% prevalence of digenetic trematodes in ruffe, *Rutilus rutilus*, in a freshwater lake exposed to bleached kraft mill effluent. Khan and Kiceniuk (1983) reported a decrease in digenean infestation in winter flounder exposed to sublethal concentrations of petroleum hydrocarbons.

Cestoda

Cestodes were found only in the winter flounder collected in June 1992. The infestation was most likely accidental following ingestion of recently disposed fish offal at St. George's. The occurrence of post-larval parasites (recently metamorphosed), and their absence in other fish at any other time supports the view of accidental consumption.

Digenea - Ectoparasites

The only ectoparasite observed in winter flounder in the present study was metacercariae of *Cryptocotyle lingua*, encysted in the skin. The life cycle of *C. lingua* is typical of most digenetic trematodes in that the eggs shed by the

adult parasite are first picked up by a snail (*Littorina sp.*) in which they undergo rapid metamorphosis and emerge as free swimming cercariae that eventually encyst (as metacercariae) in the skin of fish (Möller and Anders, 1986). Once the fish is eaten by a bird, the adult parasite develops in the intestine, completing its life cycle. The parasite was commonly found on the gills, right pectoral fin, and caudal fin of winter flounder from St. George's Bay. A high prevalence of *C. lingua* on the gills and pectoral fin of winter flounder probably occurred when the preceding larval stage (cercariae) were taken in the buccal cavity (during respiration), expelled through the opercula, and immediately deposited on the pectoral fins. The metacercariae found on the caudal fin were probably acquired when flounder became submerged in the substrate, using their caudal fin which would stir up cercariae that were deposited on the substrate. The results in the present study have demonstrated that *C. lingua* is a common ectoparasite of winter flounder from both Port Harmon and St. George's, but infestations of *C. lingua* were much more pronounced, in terms of higher prevalences and intensities, in fish from Port Harmon. Khan et al. (1992) reported a high prevalence (90%) of *C. lingua* metacercariae on winter flounder collected from Port Harmon in 1990 that was significantly greater than that from a reference site.

With respect to the life cycle of *C. lingua*, a fish that

spends more time in shallow water (where more *Littorina sp.* reside), should increase the likelihood of becoming infected. Port Harmon is deeper than St. George's, yet infestations were more pronounced in fish from Port Harmon. This might again be consequence of immunosuppression in winter flounder from Port Harmon.

Theoretically, infestations of *C. lingua* metacercariae should only increase over time. The parasite will excyst only if ingested by the definitive host (Möller and Anders, 1986). However, the prevalences of *C. lingua* metacercaria in flounder from St. George's Bay decreased somewhat during July and August, suggesting that new uninfected winter flounder had immigrated from deep water into the area.

Recently, Khan and Thulin (1991) reviewed the use of parasites as biological indicators of pollution. Generally, most ectoparasites on the gills and skin of fishes tend to increase in prevalence and intensity when the host is exposed to pollution, whereas endoparasites decrease in prevalence and intensity (Khan and Thulin, 1991). When using parasites as biological indicators of pollution, in interpretation of the results, caution should be exercised because of differences in host diet, physiological variability of hosts, and physiological variability of parasites. Most studies using parasites as bio-indicators appear to be more valid when supported with data from organ somatic indices, haematological variables,

and/or tissue histopathology. With increased knowledge of parasites and their hosts, parasites will become useful as indicators of pollution.

Water Parameters

The purpose for measuring water parameters from both sample sites was to determine if differences between flounder from St. George's and Port Harmon, could be attributed to differences in water characteristics. Port Harmon has a greater maximum water depth and steeper bottom topography than St. George's. Both sample sites were shown to have very similar water characteristics in terms of temperature, salinity, conductivity, pH, and dissolved oxygen. A difference between the sites was observed in November 1992, when salinity, conductivity, and dissolved oxygen values at Port Harmon were significantly lower than at St. George's. Pulp mill effluent has been reported to have a high biochemical and biological oxygen demand, since chemicals in pulp effluent combine with dissolved oxygen in the water (Gibbons et al.,1992; Kukkonen,1992; and Oleszkiewcz et al.,1992). This decrease in salinity, conductivity, and dissolved oxygen at Port Harmon was probably due to the discharge of freshwater during paper production at the mill, rather than to local river runoff.

Future Considerations

The present study did not demonstrate a direct causal relationship between any particular toxin(s) and physiological stress in winter flounder. However, it provides evidence of chronic stress in winter flounder living adjacent to a pulp and paper mill at Port Harmon. These effects appear to be very severe especially since chlorine was used in the past, dioxins and furans may have accumulated in the sediment. When flounder submerge in the substrate over the winter months there is a high risk of exposure to any pollutants that have accumulated in the sediment. Dredging in the area would also presumably bring such dioxins to the surface, increasing the risk of exposure.

Pulp mill effluents contain a multitude of compounds which could be toxic singly or in combination with other compounds (Bettis, 1991; Gibbons et al. 1992). The first step in any control effort would be to change the type of bleach used or reduce the quantity currently used. Abitibi-Price at Port Harmon has attempted to rectify the problem currently by using thermo-mechanical pulp (TMP), which essentially cooks the wood pulp and reduces the overall amount of bleaching chemicals employed. The TMP process still uses bleaching agents however, and, Abitibi-Price at Port Harmon uses sodium hydrosulfite as its primary bleaching agent. Many authors have reported several alternative, "less harmful" bleaching techniques ranging

from the use of ozone (O_3), alkali extraction, electrolysis, to biopulping, using enzymes from a fungus, *Trichoderma reesei* (Bettis, 1991; Buchert et al., 1992; Pulliam, 1991). Two main disadvantages of using such alternative methods (from the viewpoint of the paper industry) are high cost and reduced brightness and durability of the paper produced. The second treatment step would be to treat the effluent before it is discharged into the marine ecosystem. Fishermen working from the Port Harmon area have confirmed seeing a overflow of effluent from the treatment pond into Port Harmon. Furthermore, they have confirmed that the effluent seeps through the bottom of the effluent pond directly into Port Harmon.

Several authors have reported a reduced toxicity in pulp mill effluents that were subject to biological treatment using a series of aerated lagoons, a more efficient system than a single lagoon (Gibbons et al.,1992; Oleszkiewicz et al.,1992). Further studies in this area should concentrate on the chemical composition and quantity of compounds in the discharged effluent and in the sediment at Port Harmon. Another study should involve transplanted fish (collected elsewhere in St. George's Bay) that are either individually tagged and released or placed in cages at Port Harmon, to determine the relationship between exposure times and signs of stress. A caged experiment was attempted at Port Harmon and St. George's in 1992 (by the author), but, unfortunately, vandalism precluded

any collection of data. Finally, future studies should investigate further the concept of parasites as bioindicators of pollution, closely examining the transmission stages and intermediate host(s) involved in the life cycle of each respective parasite.

In conclusion, the bioindicator methods used in this study have clearly demonstrated evidence of chronic stress in winter flounder, *Pleuronectes americanus*, living adjacent to a pulp and paper mill at Port Harmon, Stephenville, Newfoundland. Furthermore, it has shown that the winter flounder is an ideal specimen to use as a bioindicator of pollution in a marine ecosystem because of its benthic lifestyle and parasitofauna.

Summary

(i) Port Harmon, Stephenville, Newfoundland, the site of a pulp and paper mill since 1981, supports a larger (both numerically and physically) annual assemblage of winter flounder, *Pleuronectes americanus*, than St. George's, a reference site, situated 12 km from Port Harmon. These differences might be due to differences in maximum depth, bottom topography, and availability of food.

(ii) Winter flounder from Port Harmon show significantly lower condition factors (K-factors) and hepatosomatic indices than fish from St. George's. This implies a depletion of energy reserves, as a result of physiological impairment (i.e. chronic stress). No differences were observed between splenosomatic indices (SSI) between flounder from both sites.

(iii) Delayed spawning was observed in winter flounder from Port Harmon as indicated by significantly higher gonadosomatic indices (GSI) and a significantly greater percent of mature vrs. non-mature flounder beyond the "normal" spawning period. No differences were observed in oocyte mean diameter and mean number for mature females from Port Harmon and St. George's. Based on prior studies of winter flounder reproduction, female fish from Port Harmon may be producing fewer eggs per

unit length of fish.

(iv) Haemoglobin (%g) and haematocrit (%) values were significantly lower, approaching anaemic levels, in winter flounder from Port Harmon compared with values in samples from St. George's. Blood lymphocyte numbers (per 1000 erythrocytes) were also significantly lower, indicating immunosuppression, in winter flounder from Port Harmon.

(v) Prevalence and degree of severity (i.e. haemorrhaging) of fin necrosis of the caudal, dorsal, and anal fins were significantly greater in winter flounder from Port Harmon than from St. George's. In addition, winter flounder from Port Harmon had a significantly higher prevalence of skin ulcers.

(vi) Winter flounder from St. George's appear to have consumed a significantly greater weight of food than fish from Port Harmon during the summer (June, July, August) of 1992. Diets of both groups of flounder were similar, consisting mainly of algae, amphipods, bivalves, fish offal, fish eggs, and polychaetes. Winter flounder from St. George's consumed a higher frequency of amphipods and fish eggs, while winter flounder from Port Harmon consumed a higher frequency of fish offal.

(vii) In an analysis of endoparasites of winter flounder, both the prevalence and intensity of nematodes were significantly greater in samples from Port Harmon. The prevalence and degree of proliferation of a parasitic protozoan, *Glugea stephani*, was significantly greater in winter flounder from Port Harmon. The prevalence and intensity of acanthocephalans were significantly greater in winter flounder from St. George's. No differences were observed in prevalence and intensity of endoparasitic digenetic trematodes. These differences in parsitemias might possibly be due to stress and immunosuppression in fish at Port Harmon, rather than due to differences in diet.

(viii) The only ectoparasite recorded was the metacercaria of the digenetic trematode, *Cryptocotyle lingua*, which had a significantly higher prevalence and intensity among winter flounder from Port Harmon.

(ix) The primary water characteristics of both sample sites were very similar, implying that the signs of chronic stress noted in winter flounder were associated with exposure to pulp effluent (and/or accumulated toxins in the sediment), and not simply a result of differences in water characteristics. During November 1992, increased paper production, and increased effluent disposal, might have been the reason for a notable decrease in salinity

(ix) and dissolved oxygen at Port Harmon.

(x) Finally, this study has provided evidence of chronic stress in winter flounder living adjacent to a pulp and paper mill by using various bioindicator methods that will be useful in future ecological assessment studies.

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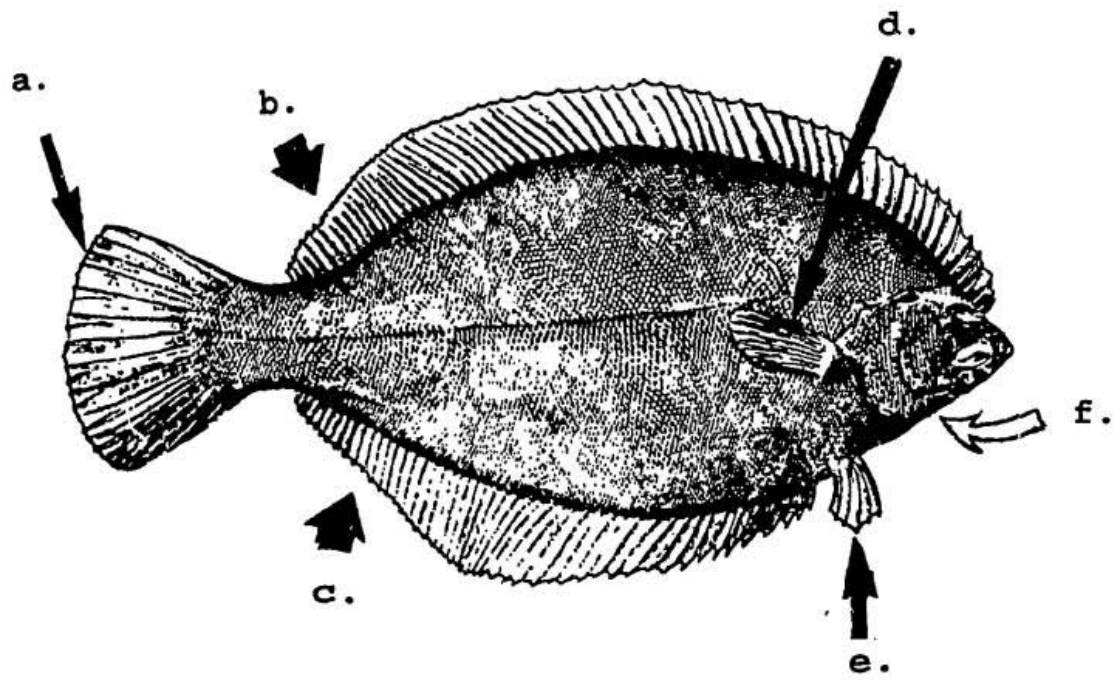
Appendix A: Enumeration sites of encysted *Cryptocotyle lingua* metacercaria on the right side (eyed side) of winter flounder, *Pleuronectes americanus*.

(modified from Pitt, 1984)



a. 1st 5 dorsal caudal fin rays

b. 1st 10 posterior dorsal fin rays

c. 1st 10 posterior anal fin rays

d. Entire pectoral fin

e. Entire pelvic fin

f. Gills : presence/absence