Seasonal Variations in the Concentration of *Oikopleura* spp. (Tunicata: Appendicularia) in Conception Bay, Newfoundland

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SEASONAL VARIATIONS IN THE CONCENTRATION OF *OIKOPLEURA* SPP. (TUNICATA:APPENDICULARIA) IN CONCEPTION BAY, NEWFOUNDLAND

by

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ABSTRACT

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A study was conducted to determine the concentration of the developmental stages of Oikopleura spp. at sampling stations in Conception Bay, Newfoundland, between November 1980 and July 1981. O. labradoriensis (Lohmann) was present in all samples while O. vanhöffeni (Lohmann) was only present from March through July. Maximum concentration of combined Oikopleura spp. (80-97 individuals . m^{-3}) occurred in July. The enigmatic appearance of a large morph of Oikopleura with a red tail (ca. 29 mm long), a house diameter of ca. 50 mm and subchordal cell characteristics of O. vanhöffeni occurred in May and July. The presence of these latter individuals in inshore Newfoundland waters coincided with the appearance of mucus ("slub") which clings to gillnets.

Key words: Appendicularia, larvacea, Newfoundland fisheries, net-fouling, Oikopleura, slub.

RÉSUMÉ

Mahoney, E.M., and R.G. Buggeln. 1983. Seasonal variations in the concentration of Oikopleura spp. (Tunicata:Appendicularia) in Conception Bay, Newfoundland. Can. Tech. Rep. Fish. Aquat. Sci. 1155: iv + 12 p.

Une étude pour déterminer la concentration des stages de developpment des essp. Oikopleura dans les diverses stations d'échantillonnage de la baie de Conception, Terre-Neuve, a été entreprise durant la période de november 1980 a juillet 1981. La larve O. labradoriensis..(Lohman) était presente dans tout les échantillons, tandis que la larve O. vanhofferi (Lohmann) était seulement presente durant le periode de mars a juillet. La concentration maximum des éspeces combinées Oikopleura (80-97 individus m^{-3}) a eu lieu au mois de juillet. L'apparance enigmatique d'une large varieté de Oikopleura ayant une queue rouge (ca 29 mm de long) et un diàmètre de maison de ca 50 mm, et des cellules subchordales characteristique de L'éspece O. vanhoffeni, a eu lieu durant les mois de mai et juillet. La présence de ces derniers individus dans le eaux territorial de Terre-Neuve, coincidait avec l'apparance de mucus (slub) qui se collé aux filets.

INTRODUCTION

Over the years inshore fishermen along the Atlantic coast of Nova Scotia, around the island of Newfoundland and along the southern coast of Labrador as far north as Battle Harbour, have experienced the accumulation of a sticky opaque mucus on fishing nets. According to Newfoundland fishermen the recent incidence (1974 to present) of this net-fouling material, locally referred to as slub, seems to be more prevalent during the spring and early summer than they recollect as the case in former times. Unfortunately, it is difficult to give concrete substance to these comparative impressions. The diary of a fishermen in Louisbourg, N.S., contains notations of slime (=slub) in some years, between the early 1960s and present, but not in others (Buggeln 1980). Historically, the spring salmon fishery of earlier decades had been punctuated by "years of the great slime" (J. Bagnell, Louisbourg, Nova Scotia, pers. comm.). In every maritime community of fishermen there is an empirically derived profile of conditions which bring mucous-laden water into the inshore environment. The mucous coating on the mesh makes nylon (single or multi-strand) highly visible in the water and fishermen claim that the catching efficiency of slub- or slime-laden gear is greatly reduced.

The source of the mucus remained obscure until R. Hooper (pers. comm., Biol. Dept., Memorial University of Newfoundland, St. John's, Nfld.) suggested the discarded houses of the larvacean genus, _Oikopleura_, as one possibility. Laboratory tests showed that houses of a very large, red-tailed taxon of _Oikopleura_ spp. were tenaciously adsorbed onto pieces of monofilament twine fishnet, while the ctenophores, _Beroe_ and _Bolonopsis_, clearly lacked this adhesive characteristic (Mahoney 1981). In addition, microscopic examination of patches of mucus collected from the sea surface near the mouth of St. John's harbour were found to contain an abundance of filter screens from _Oikopleura_ spp. houses (Mahoney 1981).

Udvardy (1954) reported that coastal stations in the Atlantic Ocean east of Newfoundland contained a mixed appendicularian fauna of both _O. vanhöffeni_ (Lohmann) and _O. labradoriensis_ (Lohmann). He considered the two species characteristic of mixed and unmixed Labrador Current water, respectively (see also Thompson and Frost 1934). The giant, red-tailed taxon noted above was distinguished from _O. vanhöffeni_ and _O. labradoriensis_ by a much larger trunk, a bluntly rounded tail but more especially by its striking red tail. The subchordal cell arrangement, however, was similar to that in _O. vanhöffeni_ (Mahoney 1981). Although Udvardy (1954) mentioned a giant morph of _O. vanhöffeni_ in Newfoundland waters, he did not describe these creatures. Rather, a comparative reference was made to normal and giant morphs of _O. valvidiae_ (Lohmann), an Antarctic species.

We have conducted a study on the seasonal age class structure of _O. labradoriensis_ and _O. vanhöffeni_ in Conception Bay, Newfoundland, and have related the sudden appearance of the giant, red-tailed morph of _Oikopleura_ in May-July with the occurrence of slub.

MATERIALS AND METHODS

Between November 1980 and July 1981 inclusive, six cruises (Nov. 25, Dec. 11, Feb. 24, Mar. 27, May 28 and July 27) were made aboard the R.V. Karl and Jackie to the following stations in Conception Bay, Newfoundland: Station 1 near Bell Island (47°39.2'N, 52°59.6'W); Station 2 (47°45.5'N, 52.5°54.0'W) and Station 3 near Cape St. Francis (47°52.5'N, 52°54.0'W) (Fig. 1). Surface water temperatures were obtained at each station during every cruise (Table 1).

At each station, two vertical plankton tows were made with a cone-shaped plankton net equipped with a flow meter (210 µm mesh size; 700 mm net mouth diameter). One tow was made between a uniform depth of 27 m and the surface; the depth of the second tow varied due to water depth at the stations and was initiated at 55 m at Station 1 and 150 m at Stations 2 and 3.

Plankton brought to the surface were preserved with 3% formalin in 0.1 M phosphate buffer. Key characters in Oikopleura taxonomy are the shape, number and placement of subchordal cells in the tail. The handling, staining and mounting procedures for light microscopic determination of these characteristics frequently destroyed the specimens. Although it would have been technically feasible to separate O. vanhöffeni from O. labradoriensis in the plankton samples using the latter methodology, limitation on manpower and time precluded this approach in favor of the following protocol.

The total number of Oikopleura in each plankton sample was first determined under a binocular dissecting microscope. A Motoda Box plankton splitter was used to reduce each plankton collection to 1/16 of its initial volume. From this latter portion, every Oikopleura was removed with fine forceps and put into a numbered plexiglass petri dish. A random sample of 15 animals was selected from the dishes. Each of the 15 Oikopleura was first identified to species and the trunk and tail lengths were measured. On the basis of gonad development, each Oikopleura was then assigned to one of the following age classes using Bückmann (1970; Fig. 12 for O. labradoriensis and Fig. 14 for O. vanhöffeni), i.e. early stage A (ESA), late stage A (LSA), stage B (SB) or stage C (SC).

RESULTS

SIZE OF OIKOPLEURA SPP. FROM CONCEPTION BAY, NEWFOUNDLAND

The trunk size and approximate house diameters of the three Oikopleura taxa from Conception Bay (Table 2) were compared with values reported by Lohmann (1896). The average tail length of mature O. vanhöffeni was 20.1 ± 0.3 mm SD (n = 10) while the mature red-tailed morph had a tail length of 29.2 ± 0.6 mm SD (n = 10). The average trunk length of each class of O. labradoriensis increased from November to July, e.g. for age class SC, 1.6 ± 0.16 mm SD (n = 6) to 3.1 ± 0.52 mm SD (n = 6), respectively.

SEASONAL TRENDS IN OIKOPLEURA SPP. ABUNDANCE IN CONCEPTION BAY

Generally the concentrations of Oikopleura at the three stations could be ranked in an increasing progression from inshore to offshore, i.e. stations 1 2 3 (Fig. 2). The low concentration of Oikopleura spp. at Station 1 between November and February and the subsequent 25-fold increase between February and July were particularly striking. An unexplained peak in February occurred at Stations 2 and 3 when the concentration difference between Stations 1 and 3 was about 10-fold. Maximum larvacean concentrations were recorded at Stations 2 and 3 during July. The late May and early July sampling dates bracket the period when the slub nuisance was at its height during the commercial gillnet salmon fishery along the east coast of Newfoundland.

On October 2, 1980, SCUBA dives to 25 m were conducted at Stations 1 and 3 in order to visually estimate the Oikopleura house abundance (every 3 m) and the ratio of occupied:abandoned houses. At Station 1, there were about 20-30 houses m^{-3}, and the occupied:abandoned ratio was 1:1. At Station 3, there were about 100 houses . m^{-3}, and the occupied:abandoned ratio was 1:9.

COMPARATIVE SEASONAL OCCURRENCE OF DEVELOPMENTAL STAGES OF O. LABRADORIENSIS AND O. VANHOFFENI

From November through February, O. vanhöffeni was absent (Table 3). During this period there was a trend toward the presence of early developmental stages of O. labradoriensis in inshore water (Station 1 > Station 3) while later developmental stages appeared with greater frequency in offshore water (Station 3 Station 1). In March, later developmental stages of O. labradoriensis appeared with about equal frequency at the three stations.

In May, the general decrease in abundance of O. labradoriensis was paralleled by an increase in O. vanhöffeni. Early developmental stages of former species were present at all stations at this time. The sudden predominance of O. vanhöffeni in May, including the presence of the red-tailed giant morph at all stations, coincided with the spring increase occurring in the total Oikopleura spp. population (Fig. 2). In July, the red-tailed morph was recorded from Stations 2 and 3 but not from inshore Station 1.

An extensive examination of the O. vanhöffeni present in the March through July plankton tows revealed only a single size grouping within each of the immature and mature stages (Fig. 3). Thus the appearance of sexually mature individuals of the giant, red-tailed taxon in May-July was intriguing. The red-tailed morph had also been collected in June 1979 (Buggeln 1979b) and in May 1980 (Mahoney 1981). Tail coloration is not apparently a characteristic in Oikopleura taxonomy (R. Fenaux, pers. comm., Station Zool. de Villefranche-sur-Mer, France). Nevertheless, it is curious that Udvardy (1954) made no reference to tail color in his notation on the occurrence of a giant morph of O. vanhöffeni unless, of course, he was examining perserved material in which case leaching and/or destruction of the red pigment could have occurred. It is difficult to comment on the statistical significance of much of the data as sample sizes for the developmental stages are small.

DISCUSSION

Perhaps the most interesting questions arising from this study pertain to the red-tailed Oikopleura taxon. If the red-tailed Oikopleura is, in fact, a giant morph of O. vanhöffeni, then how may one explain the coincident occurrence ···in the same plankton samples ···of both the normal and the giant morph, in sexually mature stages, between May and July? It may be possible to produce different sizes of O. vanhöffeni by culturing the animals in the laboratory under a range of environmental and nutritional conditions as Paffenhöffer (1973) has reported for O. dioica. In Conception Bay, the (separate?) water masses in which the normal O. vanhöffeni and the giant red-tailed morph grew and became sexually mature may have become mixed in the spring (May-July), bringing the two forms together. This tentative hypothesis is based on indirect evidence. The presence of the giant, red-tailed morph in the plankton coincides with the peak in the slub occurrence on the east and south coasts of Newfoundland (Buggeln 1979a and b). A visual cue used by Newfoundland inshore fishermen for recognizing water which is likely to cause slub accumulation on gillnets is contained in the paradoxical saying: "when the water is clear, it is dirty". In biological oceanographic terms this statement means that the water column over the fishing grounds is cold Labrador Current water, and this water mass is apparently carrying the large, red-tailed Oikopleura taxon. The inshore presence of Labrador Current water is the result of upwelling of deep offshore water, usually brought about by the interaction of local wind, tide and current patterns (Essenberg 1926). The relationship between seasonal variations in (1) the physical oceanographic parameters of temperature and salinity (depth profiles) in inshore and offshore (Labrador Current) water masses, and (2) the developmental biology of inshore and offshore populations of O. vanhöffeni should be examined in detail to test the mixed water mass hypothesis. Temperature may be one of the factors which regulates the different seasonal occurrences of the Oikopleura taxa. Both Oikopleura spp. are cryophyllic; however, O. labradoriensis can reportedly tolerate variations in temperature from -1.3 to 20.1 C, while O. vanhöffeni has a more restricted temperature range of -1.7 to 11.7 C (Forneris 1957).

If the annual spring phytoplankton bloom is followed by an increase in the concentration of particulate matter ···and associated bacterial flora ···in the sea, it is reasonable to expect that the population of Oikopleura, which feeds to a considerable extent on bacteria and small phytoplankton, would subsequently increase (Fig. 2). The greater trunk length in O. labradoriensis (July > November) may be partially related to increased food availability as well as elevated growth rates at a higher water temperature (Table 1). However, trunk length of sexually mature individuals also seems to depend on egg number because the trunk becomes distended as the number of eggs increases (R. Fenaux, pers. comm). Alldredge (1976) found a positive relationship between total particulate carbon in the seawater and the percentage of abandoned appendicularian houses. The maximum value for the total number of discarded (89%) and occupied (11%) appendicularian houses in the Gulf of California was reportedly 1,130 . m^{-3} (Alldredge 1976). From our SCUBA observations in October 1980, we estimated that there were nearly 100 abandoned Oikopleura houses per m^3 at Station 3. The Oikopleura concentration (Fig. 2) in November was about 10-fold lower than the concentration in July; therefore, the discarded house density in June-July — assuming the November rate of house building and discarding — may reach ca. 1,000 . m^{-3} as a minimum value. If a significant number of these houses are contributed by the red-tailed morph (house diameter ca. 50 mm; Table 1) then an enormous amount of gelatinous material, i.e. potential slub, will be present in the water. It is likely, however, that slub will not occur with equal severity every year as seasonal and yearly variations in current patterns have been reportedly correlated with variations in range and abundance of oikopleurids (Frost et al. 1933).

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REFERENCES

Alldredge, A. L. 1976. Discarded appendicularian houses as sources of food, surface habitats and particulate organic matter in planktonic environments. Limnol. Oceanogr. 21: 14-23.

Bückmann, A. 1970. The distribution of the cold water and warm water fauna of appendicularians in the Northern North Atlantic Ocean during late winter and late summer 1958. Mar. Biol. 5: 35-56.

Buggeln, R. G. 1979a. Slub and the salmon fishery. Final Report to the Newfoundland Department of Fisheries, Development Branch. 8 p.

1979b. Net cleaning procedures for removing slub. Final Report to the Newfoundland Department of Fisheries, Development Branch. 11 p.

1980. The correlation of "slub" on fishing nets with the zooplankton species, Oikopleura vanhöffeni, in coastal Newfoundland. Final Report to the Newfoundland and Labrador Department of Fisheries. 9 p.

Essenberg, C. E. 1926. Copelata of the San Diego Region. Univ. of Calif. Publ. Zool. 28: 399-521.

Forneris, L. 1957. The geographical distribution of the copelata. Acad. Brasil. Cien. Anan. 29: 273-284.

Frost, N., S. T. Lindsay, and H. Thompson. 1933. Hydrographic and biological investigations. Report of the Newfoundland Fishery Research Comm. 2: 58-74.

Lohmann, H. 1896. Die Appendicularien der Plankton-expedition. Zoologische Ergebnisse Bibliotheca Zoologica XX: 4-44.

Mahoney, E. M. 1981. Observations of Oikopleura (Tunicata, Appendicularia) and the contribution of discarded larvacean houses to the slub problem in selected Newfoundland inshore locations. B.Sc. Honors Thesis (Memorial University of Newfoundland). 56 p.

Paffenhöfer, G. A. 1973. The cultivation of an appendicularian through numerous generations. Mar. Biol. 22: 183-185.

Thompson, H., and N. Frost. 1934. Hydrographic and biological investigations. Report of the Newfoundland Fishery Research Comm. 2: 19-23.

Udvardy, M. D. F. 1954. Distribution of appendicularia in relation to the Strait of Belle Isle. J. Fish. Res. Board. Can. 11: 431-453.

Table 1. Surface water temperatures (°C) at Stations 1-3 in Conception Bay, Newfoundland, November 1980 - July 1981.

Date	Station 1	Station 2	Station 3
November	6.0	-	5.4
December	4.2	3.5	-
February	0.5	0.2	0
March	2.1	3.8	-0.2
May	5.5	5.3	5.2
July	11.0	10.0	8.5

Table 2. Comparison of trunk and house sizes of Oikopleura spp. from Conception Bay, Newfoundland.

	Trunk size (mm) (Lohmann 1896)	Trunk size (mm) (present study)	House diameter (mm) approx. (present study)
O. labradoriensis	1.5 - 2.0	1.8 ± 0.3 SD (n = 10)	15.0 ± 4 (n = 10)
O. vanhöffeni	2.0 mm⁺	3.9 ± 0.3 SD (n = 10)	25.0 ± 3 (n = 10)
Red-tailed morph	-	7.8 ± 0.7 SD (n = 10)	52.0 ± 4 (n = 10)

Table 3. Seasonal appearance of developmental stages of *O. vanhöffeni* and *O. labradoriensis* in Conception Bay during 1980-81.

Date	Species	Station 1				Station 2				Station 3			
		ESA[a]	LSA[b]	SB[c]	SC[d]	ESA	LSA	SB	SC	ESA	LSA	SB	SC
NOV	VAN[e]	0	0	0	0			ND		0	0	0	0
1980	LAB[f]	4	4	6	1			ND		0	2	7	6
DEC	VAN	0	0	0	0	0	0	0	0			ND	
1980	LAB	3	7	4	1	0	3	5	7			ND	
FEB	VAN	0	0	1	1	0	0	0	0	0	0	0	0
1981	LAB	0	7	3	3	3	4	5	3	2	4	2	7
MAR	VAN	0	0	0	0	0	1	0	1	0	1	1	2
1981	LAB	2	1	7	5	1	1	5	6	0	2	5	4
MAY	VAN	0	3	2	2*	2	0	2	5*	1	1	1	1*
1981	LAB	3	2	3	0	1	2	3	0	6	2	1	2
JULY	VAN	0	5	4	0	0	1	5	2*	3	0	4	2*
1981	LAB	1	1	1	3	2	3	2	0	1	1	0	3

[a] ESA = early stage A

[b] LSA = late stage A

[c] SB = stage B

[d] SC = stage C

[e] VAN = *O. vanhöffeni*

[f] LAB = *O. labradoriensis*

* Red-tailed giant morph present

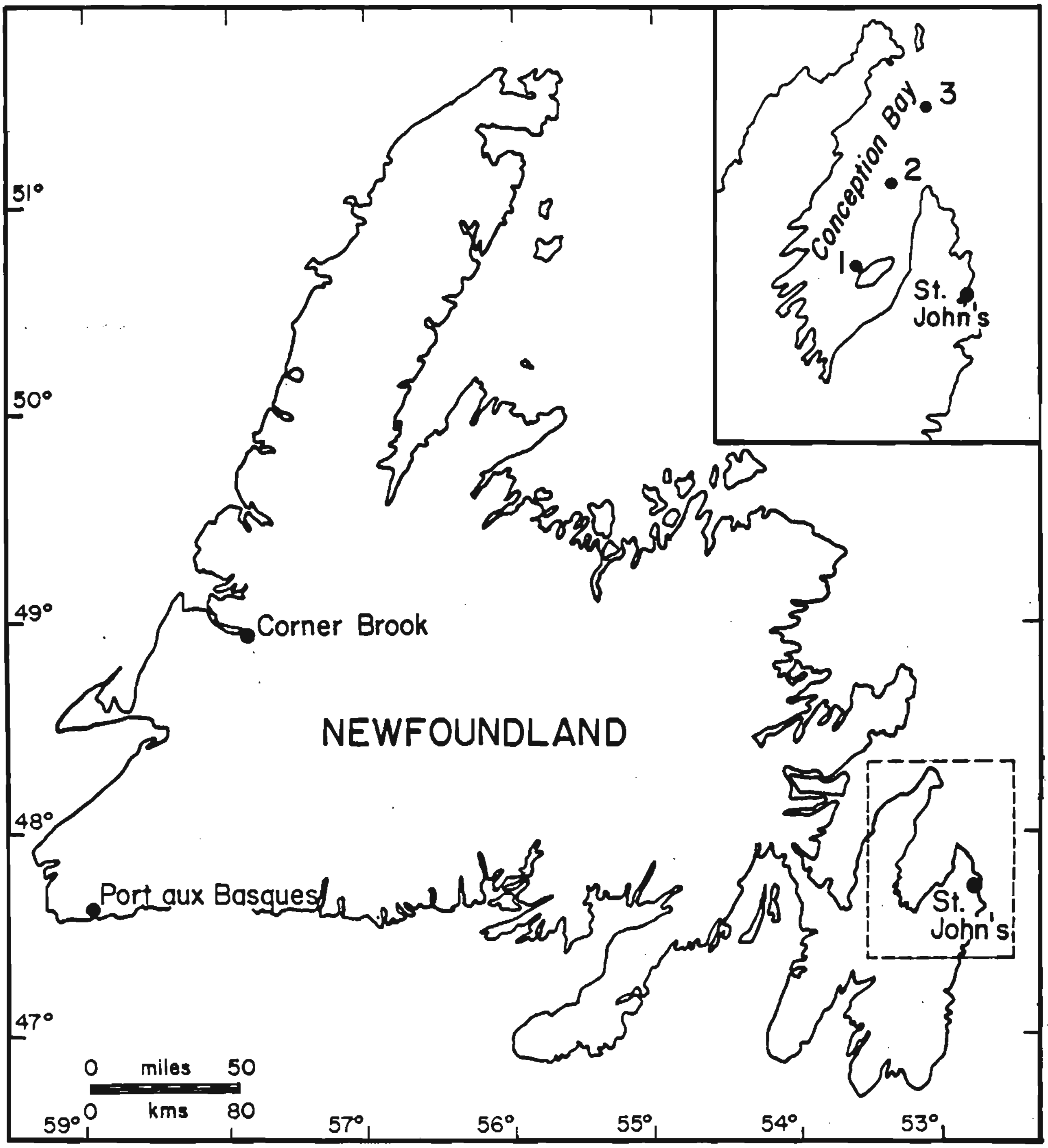

Fig. 1. Map of the island of Newfoundland with inset showing Conception Bay and the three Oikopleura sampling stations.

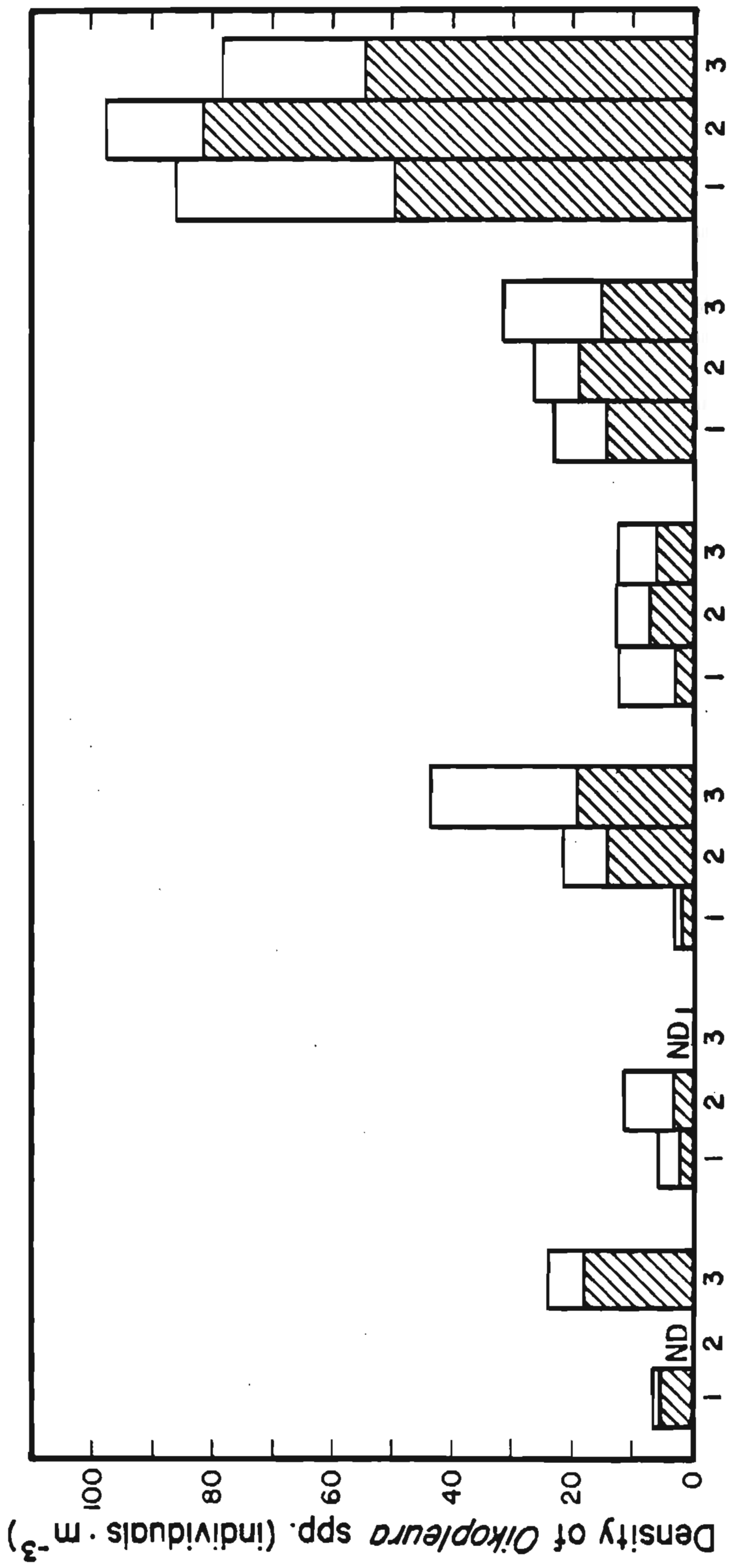

Fig. 2. Seasonal variations in the density of Oikopleura spp. at the three sampling stations in Conception Bay, [hatched box], 27 m to surface; [open box], 55 m to surface at Station 1 and 150 m to surface at Stations 2 and 3. ND, no data.

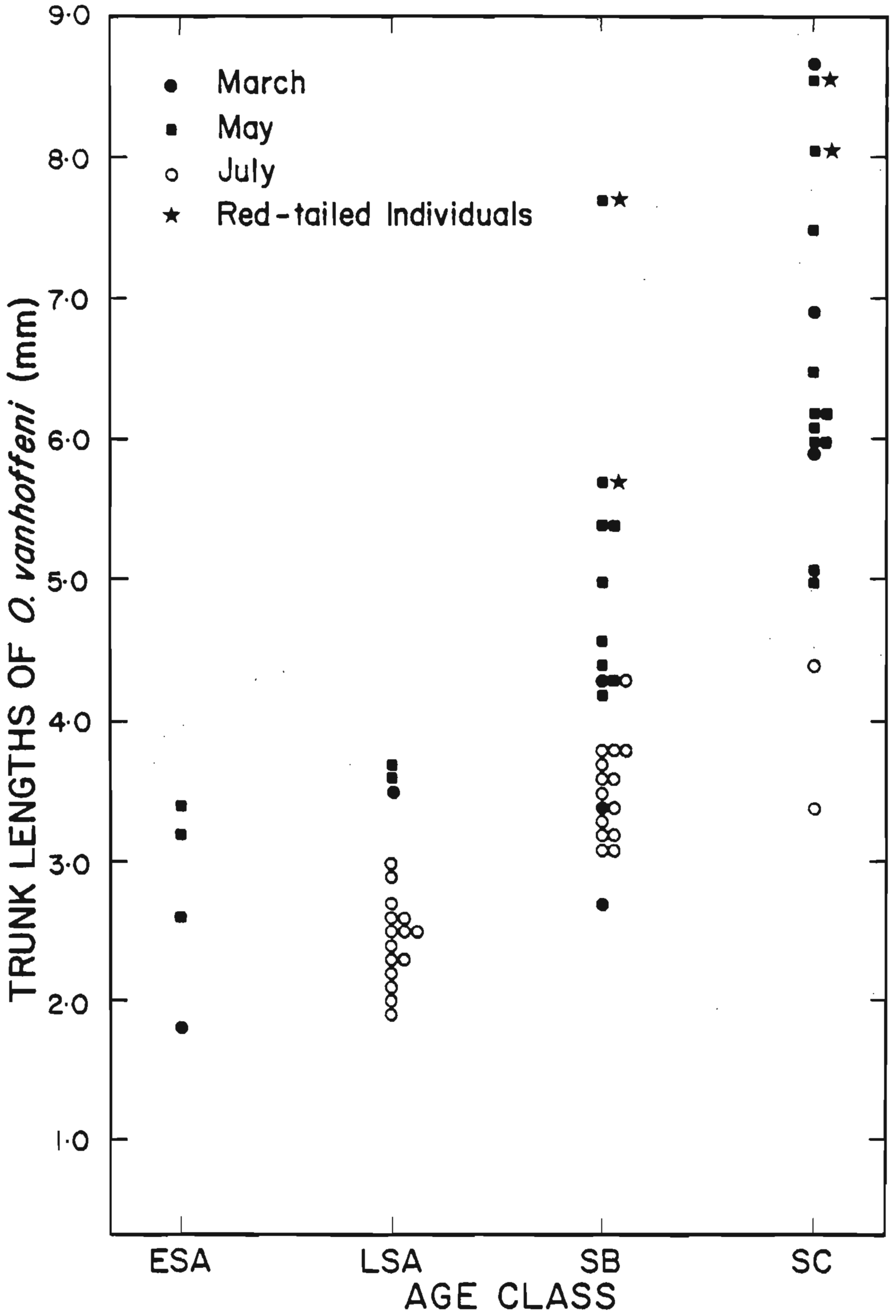

Fig. 3. Trunk lengths for the four developmental stages of O. vanhöffeni in plankton tows made in March, May and July 1981. The data for each age class have been pooled from all tows and all stations in each month. ESA (early stage A); LSA (late stage A); SB (stage B); SC (stage C) (cf. Fig. 14 in Buckmann 1970).