AN ECOLOGICAL STUDY OF TASMANIAN FLOUNDER

by

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I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any university, and that, to the best of my knowledge and belief, the thesis contains no copy or paraphrase of material previously published or written by another person, except when due reference is made in the text.

C. Crawford.

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SUMMARY

The ecology of the juveniles of two species of Tasmanian flounder, *Rhombosolea tapirina* and *Ammotretis rostratus*, which occur sympatrically on nursery grounds was investigated in order to determine which environmental parameters are important in habitat selection and resource partitioning. The reproductive strategies of adults also were examined and methods were developed for the cultivation of flounder. A third species, *Ammotretis lituratus*, was caught in low numbers and some aspects of the ecology of this species are also discussed.

Field studies showed that both *R. tapirina* and *A. rostratus* juveniles were abundant on estuarine sandflats and were concentrated mostly in shallow water (0-1 m depth). They apparently partially partitioned the spatial and trophic resources of the habitat but were not segregated temporally. Newly-metamorphosed juveniles of both species occurred in the highest densities from late winter to early summer. Although they were widely distributed within the estuary, *A. rostratus* was most abundant at the mouth and *R. tapirina* on the extensive shallow sandflats. *A. rostratus* juveniles also were caught more frequently in deeper water (1 m) than in the shallows whilst *R. tapirina* did not show a clear pattern of depth distribution over 0-1 m depth. Newly-metamorphosed juveniles of both species were daytime feeders and consumed the same food organisms - predominantly amphipods, harpacticoids and polychaetes. However, the relative proportions of each food type eaten differed between the species.

A. lituratus juveniles were caught only on semi-exposed beaches. They, therefore, were segregated spatially from the major populations of the other two flounder species.

Experimental studies indicated that the field distributions of *R. tapirina* and *A. rostratus* juveniles were related to their differing swimming abilities, preferred substrate types and possibly levels of turbulence. Temperature and salinity preferences were not considered to be as important.

The results also suggested that the larvae of *R. tapirina* and *A. rostratus* are dependent on water movements to transport them towards nursery grounds. An ontogenetic change in preferred salinity was observed in both species, and position in the water column in *R. tapirina*, at metamorphosis. These factors, in association with a preference for fine sand and probably shallow water, would play a role in guiding larvae towards settling on estuarine sandflats.

R. tapirina and *A. rostratus* adults appeared to have a similar reproductive strategy of a prolonged spawning season, serial spawning, relatively high fecundity and both species were mature for the first time at approximately the same length.

These two species were cultivated in the laboratory to the post-metamorphosis stage. The high survival rates obtained indicate that both species could be readily cultured using the techniques developed. Developmental stages of eggs and larvae were described and were used to identify planktonic stages.

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CHAPTER 1

GENERAL INTRODUCTION

Studies on the ecology of fishes, particularly those of commercial importance, have traditionally centred on adults or those age groups which are important to the fishery. Although a large amount of information has been amassed on the biology of some fish stocks, the mechanisms controlling the size of fish populations have generally remained obscure. This has been particularly emphasised when recruitment failures have occurred, or some important fish stocks have declined in abundance, independently of the rate of exploitation. In recent years there has been an increasing interest in the ecology of the pre-recruit stages, especially the mechanisms which control year class strength, i.e. survival from egg to recruitment. It is now generally accepted that predictions of recruitment to an exploitable parent stock require a fuller understanding of the stages before recruitment (Cushing, 1975; Pitcher and Hart, 1982).

Many marine fishes have life history strategies that result in the spatial segregation of eggs and/or larvae, juveniles and adults. Thus, the factors affecting the survival rates of each stage in the life cycle often differ. The highest mortalities generally occur during the planktonic egg and larval stages although fluctuations in survival during the period on the nursery ground may be substantial (Cushing, 1975).

Estuaries and shallow coastal waters are utilized by many species of fish as nursery grounds. There have been numerous studies on the factors affecting distributions and abundances of juveniles in these areas, but relatively few on habitat selection, or habitat partitioning amongst co-existing species. Investigations on the partitioning of resources of sympatric species in various taxonomic groups indicate, however, that such studies are important to an assessment of the factors affecting the survival of a species (see MacArthur, 1972; Schoener, 1974; Diamond, 1978; Pianka, 1978).

Preliminary observations of fish populations in estuaries and shallow coastal waters around south-eastern Tasmania indicated that the juveniles of three species of flatfish, *Rhombosolea tapirina*, *Ammotretis rostratus* and *A. lituratus* provided an ideal group for ecological study

because they comprise a guild of closely related species which presumably must partition the structurally-simple sedimentary environment. These three species present, therefore, an opportunity to examine patterns of habitat utilization and co-existence amongst juvenile flatfish. Moreover, the adults of the three species are commercially important and a knowledge of the factors affecting the survival of juveniles would be useful in any attempt to manage these species. This is especially important for *R. tapirina* and *A. rostratus* juveniles because they apparently extensively utilize estuarine sandflats as nursery grounds; areas which are threatened by the activities of man (e.g. by siltation, pollution, changes in salinity regime, foreshore development).

Seven species of pleuronectids, five species of bothids and one soleid species have been recorded in Tasmanian waters (Table 1.1). Of these, only three are of commercial importance (viz. *R. tapirina, A. rostratus* and *A. lituratus*); the other species do not reach an edible size. A key for the identification of the thirteen species of Tasmanian flatfish, descriptions and general information on their biology are given by Last *et al.* (1983). Notes on the systematic positions of six species of Tasmanian Rhombosoleinae are provided by Last (1978).

Although the three commercial species of flounder caught in Tasmania have high market acceptability, they are captured only in relatively low numbers. The annual weight of flounder sold to fish merchants in Tasmania for the years 1978/79 to 1982/83 constituted only 0.01-0.14% of the total annual sales of all finfish (Table 1.2). The weights of the three species have been recorded in the one category of flounder, however *R. tapirina* comprise the bulk of the commercial catch and *A. rostratus* are caught more frequently than *A. lituratus* (unpublished data, Tasmanian Fisheries Development Authority).

Flounder are caught mostly as an incidental by-catch of Danish-seine trawling for predominantly school whiting (*Sillago bassensis*) and tiger flathead (*Platycephalus richardsoni*), although some target fishing for flounder does occur. They are taken mostly during the winter months as in other seasons, particularly summer, Danish-seine trawling for tiger flathead is conducted in deeper water. Furthermore, some Danish-seine boats transfer to the rock lobster fishery. Commercial

TABLE 1.1 Flatfish species occurring in Tasmania (Order Pleuronectiformes)

Scientific Name	Common Names	Comment
Family Pleuronectidae		
Subfamily Rhombosoleinae		
Rhombosolea tapirina (Günther, 1862)	Greenback flounder	Most abundant, caught commercially
Ammotretis rostratus (Günther, 1862)	Sole, long-snouted flounder	Caught occasionally in commercial quantities
Ammotretis lituratus (Richardson, 1843)	Spotted sole or flounder	Caught infrequently, of commercial size
Ammotretis macrolepis (McCulloch, 1914)	Large-scaled flounder	Very rare
Ammotretis elongatus (McCulloch, 1914)	Elongated flounder	One specimen only, from Bass Strait
Taratretis derwentensis (Last, 1978)	Derwent flounder	Not commercial
Azygopus pinnifasciatus (Norman, 1926)	Banded-fin flounder	Not commercial
Family Bothidae		
Arnoglossus andrewsi (Kurth, 1954)	Andrew's flounder	Not commercial
Arnoglossus armstrongi (Scott, 1975)	Armstrong's flounder	One specimen only
Arnoglossus bassensis (Norman, 1926)	Bass Strait flounder	Not commercial
Arnoglossus muelleri (Klunzinger, 1872)	Mueller's flounder	Not commercial
Lophonectes gallus (Günther, 1880)	Crested flounder	Not commercial
Family Soleidae		
Zebrius fasciatus (Macleay, 1882)	Many-banded sole	Not commercial, rare

TABLE 1.2 Total annual weight of flounder, and of all finfish, sold to fish merchants in Tasmania for the years 1978/79 to 1982/83 (unpublished data, Tasmanian Fisheries Development Authority)

Year	Flounder Weight (kg)	Total Finfish Weight (kg)
1978/79	347	6,803,455
1979/80	1,430	6,829,666
1980/81	10,639	7,594,074
1981/82	10,581	15,865,932
1982/83	7,736	18,158,845

spearfishing for flounder also occurs in many sheltered bays and estuaries around Tasmania. Adult *R. tapirina*, in particular, are common in the intertidal zone in all months of the year. However, no statistics are collected on the quantity of flounder caught by this method. Similarly, the impact of amateur spearfishmen on the flounder population is not known. Nevertheless, as the coastline of Tasmania is regularly inhabited, except on the west and south coasts where few suitable embayments occur, the numbers taken by spearing are probably considerable.

Few studies have been conducted on the ecology of flounder in Australia, probably because of their relatively low commercial importance. The two most comprehensive studies have been by Kurth (1957) on the biology of adult *R. tapirina* in Tasmania and by Burchmore (1982) on the comparative ecology of nine species of sympatric flatfish in Botany Bay, New South Wales. Of the nine species studied by Burchmore, only *A. rostratus* also occurs in Tasmania.

Aspects of the biology of *R. tapirina* examined by Kurth (1957) include growth rates and reproduction. He calculated that the mean length of I+ flounder at approximately three months after the nominal birthday of 31 July was 10.5 cm, II+ 19.4 cm and III+ 26.6 cm. Kurth (1957) also observed that *R. tapirina* had a prolonged spawning season and were probably serial spawners.

Burchmore (1982) found that small samples of *A. rostratus* juveniles showed clear growth patterns, and in the warm waters of Botany Bay were

approximately 20 cm in length after one year. However, only one fish less than 5.5 cm total length was caught. The smallest fish were taken in October and the larger I+ juveniles disappeared from the catches after December when about 24 cm in length. *A. rostratus* juveniles were caught most frequently on shallow sandy and/or vegetated areas of the bay.

A study by Last (1983) of the fish communities inhabiting inshore Tasmanian sedimentary habitats included some information on the distributions of juveniles of the three species. *A. lituratus* were most abundant on exposed and semi-exposed beaches, *A. rostratus* at the mouths of estuaries and *R. tapirina* further up estuaries.

A greater number of investigations on the biology of flatfish has been conducted in New Zealand, particularly on the two most important commercial species *Rhombosolea plebia* and *R. leporina* (e.g. Tunbridge, 1966; Coleman, 1972, 1973, 1974a,b, 1978 and Webb, 1972, 1973). *R. tapirina* also occur in New Zealand but are only of minor commercial importance and little is known about the biology of adults. However, juvenile *R. tapirina* have been studied in some detail by Roper (1979) and Roper and Jillett (1981). They were most abundant in shallow water over tidal sandflats and sandy beaches during the summer but few juvenile fish were caught in winter. Growth rates of juveniles could not be determined because the population was continually turning over. They concentrated in shallow water at low tide and migrated onto tidal sandflats at high tide. *R. tapirina* juveniles possessed an endogenous circatidal activity rhythm and tidal changes in hydrostatic pressure was probably the Zeitgeber for this rhythm. Coastal inlets were considered to be of vital importance to *R. tapirina* populations because the juveniles are dependent on these areas as nursery grounds (Roper and Jillett, 1981).

Investigations on the ecology of juveniles of commercially important species of flatfish in the northern hemisphere have increased during the last two decades. These studies have been conducted for several reasons. As part of the predictions of recruitment and hence management of a fishery, factors affecting survival of the pre-recruit stages have been examined. An example of such research is a series of papers summarized by Steele and Edwards (1970), on the ecology of 0-group plaice, *Pleuro-*

nectes platessa. They evaluated the relative importance of factors which determined the population size after one year on the nursery ground and concluded that food supply and mortality were the controlling factors rather than initial numbers of fish. Similarly, Bannister *et al.* (1974) examined the survival of different year classes of plaice from the egg to O-group stage in relation to environmental variables and population density. They suggested that the density-dependent mortality of larvae was most important in some years and environmental variables, particularly temperature, in other years for controlling year class strength.

Studies on the ecology of juvenile flatfish have also been conducted to evaluate the importance of estuaries or shallow coastal waters in their life histories. These areas are becoming increasingly subjected to alteration by man and it is feared that any intervention may have deleterious effects on flatfish populations which use these areas as nursery grounds. For example, Zilstera (1972) concluded that the Wadden Sea was a major nursery ground of plaice and sole, *Solea solea*, and if this area was reduced then flatfish population numbers in the North Sea would also decrease.

Techniques for rearing larval and juvenile flatfish have also progressed in the last two decades. Concomitant with these developments has been the requirement for further knowledge on the biology of natural populations to provide a basis for rearing methods. Studies on the ecology of juvenile plaice by Riley and Corlett (1966) and on juvenile turbot (*Scophthalmus maximus*) by Jones (1973a), for example, were conducted to provide this background information.

Most studies on juvenile flatfish, however, have concentrated on one particular species and there have been relatively few investigations on the comparative ecology or habitat partitioning of co-existing species. The factors which influence the distributions of O-group fish, including several species of flatfish, in shallow coastal waters were examined by Gibson (1973) and Riley *et al.* (1981). They both found that each species occupied a distinct depth zone which differed from other species. These depth preferences were correlated with other environmental variables. Macer (1967) and Edwards and Steele (1968) observed that juvenile plaice and dabs (*Limanda limanda*) occurred sympatrically on nursery grounds but

showed distinct differences in time of settlement, depth distribution and food organisms eaten. Similarly, Roper (1979) suggested from field and experimental studies that differences in depth and dietary preferences were the most important factors in segregating juvenile *R. tapirina* and *Peltorhamphus latus*. The co-existing species of flatfish studied by Burchmore (1982) were found to partition mainly the spatial and trophic resources of the habitat.

Thus, in view of the paucity of information concerning the juvenile stages of Tasmanian flounder and on the co-existence of juvenile flatfish species in general, as well as the importance of ecological studies overseas, the present investigation was initiated with four specific objectives. These were:

(1) to investigate the ecology of juvenile *R. tapirina*, *A. rostratus* and *A. lituratus* on estuarine sandflats and sandy beaches and to evaluate the importance of these areas as nursery grounds;
(2) to determine which environmental parameters are important in habitat selection and resource partitioning by juveniles on the nursery grounds;
(3) to examine the reproductive strategies of adult flounders and to compare them with abundances of larvae and juveniles; and
(4) to develop methods for the artificial cultivation of flounder, to describe the egg and larval stages, and to assess the possibility of commercial farming.

It should be noted, however, that because *A. lituratus* were caught infrequently, this study has been concerned mainly with *R. tapirina* and *A. rostratus*.

CHAPTER 2

DISTRIBUTIONS AND SEASONAL ABUNDANCES OF JUVENILE FLOUNDER

2.1 INTRODUCTION

The importance of estuaries and shallow coastal waters as nursery grounds for juvenile flatfish has been well documented and there have been numerous studies overseas on the ecology of juveniles of commercially important species, (e.g. Edwards and Steele, 1968; Jones, 1973a; Kuipers, 1977; Riley *et al.*, 1981; Roper, 1979). In Australia nearly all information on juvenile flounder has resulted from general studies of fish communities in selected geographical areas, (e.g. Lenanton, 1974, 1977). Last (1983) has provided some information on the distributions of juvenile flatfish in the shore zone around Tasmania and Burchmore (1982) studied the ecology of juveniles of several flatfish species in Botany Bay, N.S.W.

In this Chapter the seasonal abundances and distributions of larvae and juveniles of *Rhombosolea tapirina* and *Ammotretis rostratus* around south-eastern Tasmania are reported in some detail and those of the third species *Ammotretis lituratus* only briefly. From these results, the ability of *R. tapirina* and *A. rostratus* to co-exist on the nursery grounds was investigated. Habitat partitioning on a spatial and a temporal level was examined.

2.2 METHODS

2.2.1 Planktonic Eggs and Larvae

Planktonic samples were collected from two stations (DP1, 2) in the Derwent Estuary and four stations (FP3,4,5,6) in Frederick Henry Bay (Figure 2.1) during most months from August 1980 to December 1981. An unencased high-speed plankton net (Lockwood, 1974a) with mouth diameter 20 cm, mesh 250 μm and a flowmeter in the mouth was used. At each station the net was towed for 15 min during flood to high tide. Initially, samples were collected during the day by oblique tows.

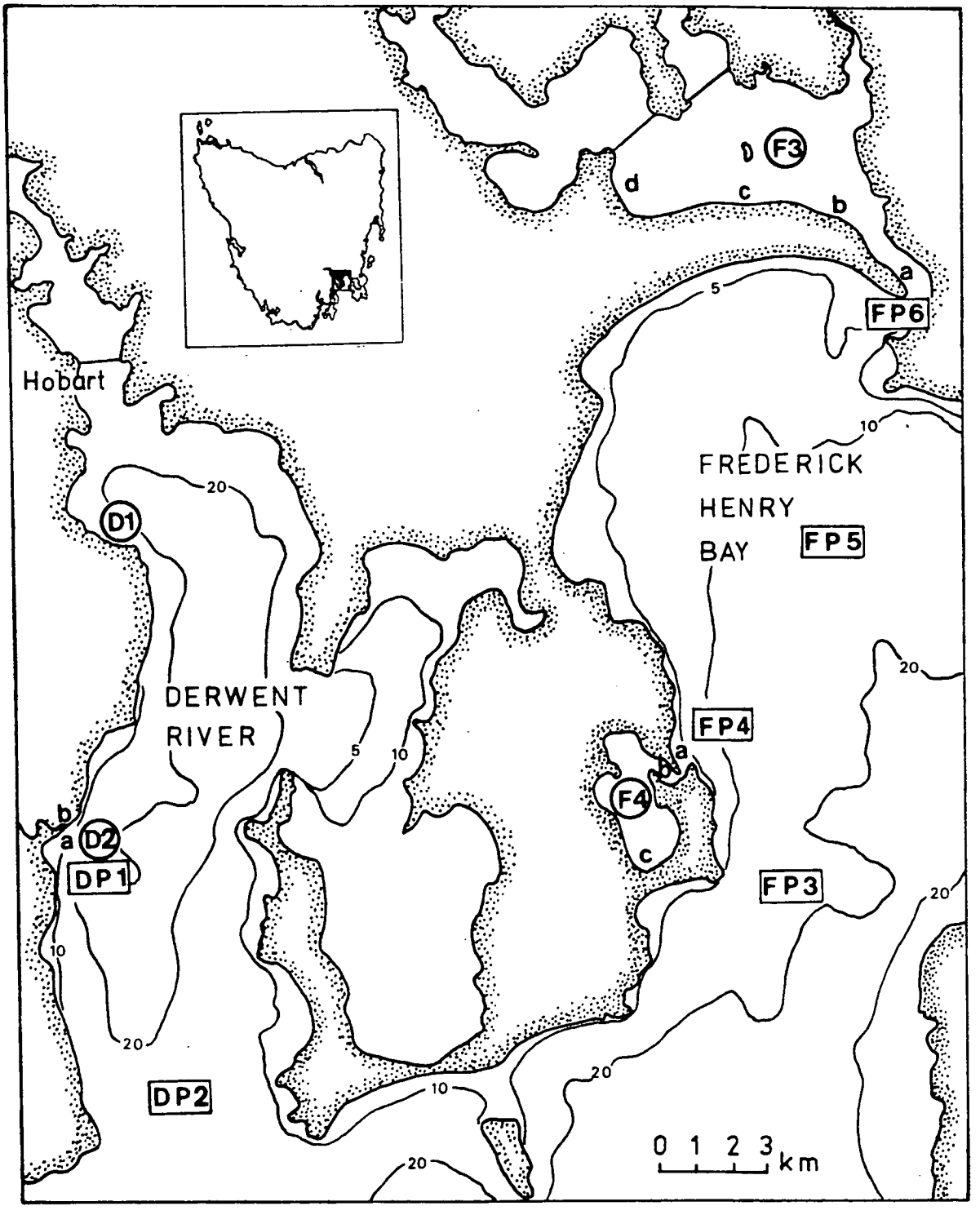


FIGURE 2.1 Map of the Derwent River and Frederick Henry Bay estuaries showing the position of sampling sites for plankton ▭ DP1, DP2, FP3, FP4, FP5, FP6 and juvenile flounder ○ D1, D2a,b, F3a,b,c,d and F4a,b,c. The location of this area in south-eastern Tasmania is shown in the inset.
Depth contours are in metres.

However, as few larvae of either species were caught, horizontal tows near the surface at night were made from June to December 1981 because Roper (1979) reported that he obtained the largest catches of *R. tapirina, R. plebia* and *Peltorhamphus latus* in this way. The plankton samples were preserved immediately in 5% v/v buffered formalin in seawater. Large samples were divided for counting using a whirling subsampler (Kott, 1953).

2.2.2 Juvenile Flounder

(i) Sampling Sites

Juvenile flounder were sampled monthly for 15-17 months in shallow water at two sites (D1, D2) in the Derwent Estuary and two sites (F3, F4) in Frederick Henry Bay. One station at site D1, two at site D2, four at site F3 and three at site F4 were sampled each month (Figure 2.1).

Site D1, Nutgrove Beach, is a small sheltered beach with a greater slope than the other sites. At approximately 2 m below low tide mark the bottom drops off rapidly to a depth of about 20 m. This site receives low energy wave action, mostly from northerly winds.

Station D2a at Kingston Beach is a semi-exposed beach which receives moderate wave action, especially from southerly or south-easterly winds. Station D2b is at the mouth of a freshwater creek, Brown's Rivulet, which flows into the Derwent Estuary across Kingston Beach. It is a small area (*c.* $10^4/m^2$) of sheltered sandflats, which are mostly exposed at low tide, and one deeper channel. This area is flushed with seawater during high tide; at low tide the freshwater outflow is dominant. Twice during the study period this area was dredged by local authorities to increase the rate of flushing with seawater.

Site F3, at Pittwater, is an open estuarine lagoon into which run four small rivers; it is connected to Frederick Henry Bay by a narrow, deep channel. The lagoon has extensive sheltered sandflats. Station F3a, at the entrance to the lagoon, is characterized by strong current velocities and a narrow platform of sandy beach at high tide mark which drops off steeply to the channel. Station F3b is located at the beginning of the sandflats and closer to the channel than Station F3c

which consists of extensive unvegetated sandflats protected from northerly winds by Woody Island. Station F3d has a softer substrate with patches of tunicates and seagrasses.

Site F4 at Cremorne is a marine inlet with large sheltered sandflats; it opens into Frederick Henry Bay via a narrow channel at one end of a semi-exposed beach. This semi-exposed beach, station F4a, receives moderate wave action from northerly to easterly winds. Within the marine inlet, station F4b is a gently-sloping sandflat which is close to the channel and station F4c is part of an extensive area of shallow sandflats.

(ii) Sampling Procedure

All samples were taken around the time of a high tide. In shallow water (maximum depth 120 cm) a 1.5 m wide push-net (Riley, 1971) with 5 mm mesh knot to knot was used. This net was either pushed by hand for 100-300 m or towed behind a dinghy with a 15 h.p. outboard motor at 35-40 m min^{-1} for 300-400 m. Four trials were conducted to test for any differences in numbers of fish caught by pushing or by towing the push-net over 100 m at 50-70 cm depth. The numbers of *R. tapirina* and *A. rostratus* caught by the two methods were not significantly different (t-test, $P>0.05$). The area swept by the net was determined from the distance covered and the width of the net.

At stations D1, D2b, F3c and F4b two to four samples were collected each month in different depths up to 100 cm and mean abundances per 500 m^2 were calculated. At stations D1 and F3c these samples were taken at depths of 10-30 cm, 50-70 cm and 90-110 cm to examine for depth preferences of *R. tapirina* and *A. rostratus*. In the estuarine lagoon, site F3, samples were also collected monthly at stations F3a,b and d in 50-70 cm depth to compare the distributions of the two species within the lagoon. Distributions of juvenile flounder at the marine inlet, site F4, were also investigated by sampling at stations F4a and c in addition to station F4b, as mentioned above. Sampling in shallow water at station F4a, however, was not always possible due to strong wave action. One or two tows were made each month at station F4c where the depth never exceeded 50 cm.

The abundances of newly-metamorphosed juveniles of *R. tapirina* and *A. rostratus* in three depths at different times of the day and stages of tide were investigated at site D1 in January 1982. The push-net was pushed for 100 m along the beach at depths of 20-30 cm, 50-70 cm and 90-110 cm every 3 h for 27 h.

Juvenile flounder were also sampled in deeper water (1.5 - 4.0 m) adjacent to several push-net sampling stations. A miniature Granton trawl slung to and held open by a 3 m beam pole in a similar way to Japanese beam trawls was used. This net, which was designed by Mr. D. Wolfe, Tasmanian Fisheries Development Authority, consisted of 16 mm mesh, knot to knot, and a codend of 7 mm mesh. It was towed behind a dinghy at 35-40 m min^{-1} for 15 min; the area swept was calculated from the time and speed of each tow. One or two tows were made at stations D1, F3b, c and F4,b, c in and on the edge of the channel and at stations D2a and F4a close to the shore. Tows could not be made at station D1 from November 1980 to January 1981 due to obstructions on the bottom.

Temperatures and salinities were recorded at each station from near the bottom. Substrate samples were collected at all stations on two occasions and analysed for sand grain sizes using the methods of Buchanan and Kain (1971).

The fish caught were preserved immediately in 5% v/v formalin; those required for gut content analysis were first anaesthetized in 0.2% w/v tricaine methanosulfonate solution to prevent regurgitation of food. The total lengths of all fish were measured to the nearest mm and subsamples were weighed to 0.001 g for fish <3 cm and 0.01 g for fish ≥3 cm, within two weeks of capture. As changes in weight and length occurred due to preservation in formalin (Appendix 1), measurements were adjusted to correspond to fresh lengths and weights.

2.2.3 Statistical Analysis

A model I two-way analysis of variance (ANOVA) was performed to examine the effects of different factors on abundances of each species separately. Mean monthly abundances were used, as the number of samples taken each month were unequal, and they were grouped into three monthly

periods (seasons). Before analysis abundances were transformed by ln(x+1) to normalize the distributions (Zar, 1974); after transformation Bartlett's test for different error variance and tests for skewness and kurtosis were not significant.

If the Anova showed a significant difference between levels of a factor then the specific levels between which differences occurred were determined using the Student-Newman-Keuls test (SNK test) (Zar, 1974). This test compares the levels (overall means) of each factor by ranking the means in order of magnitude and testing for significant differences between pairs of means. If the interactions of two factors were significant, subgroup means were tested separately for significant differences using the SNK test, i.e. the means of all levels of one factor were compared at each level of the second factor.

The results of the SNK test using P = 0.05 level of significance are shown by ranking the levels of a factor in order of increasing magnitude of their means and by underscoring levels which had non-significant differences between their means.

2.3 RESULTS

2.3.1 Planktonic Eggs and Larvae

Although samples and descriptions of developmental stages of *R. tapirina* eggs were available (Chapter 6) it was not possible to positively identify and separate these eggs from others in the plankton samples. Undescribed eggs of similar characteristics from other species of fish were common in the samples. Also, very few *A. rostratus* eggs were collected. Thus, only larval densities at each site are given (Table 2.1).

The number of flounder larvae caught in the plankton tows was low. Seventy *R. tapirina* and 4 *A. rostratus* larvae were caught, with a maximum of 8 *R. tapirina* in one tow. The numbers of larvae caught and volume of water filtered per tow are given in Appendix 2.

As the larval numbers were low, the data were not analysed statistically. However, Table 2.1 shows that *R. tapirina* larvae were present

TABLE 2.1 The number of larvae per 100 m^3 (N) and mean length in mm ($\bar{X}$) at the River Derwent and Frederick Henry Bay sites. (- not sampled, 0 - no larvae caught)

R. tapirina

Date		Derwent River				Frederick Henry Bay							
		DP1		DP2		FP3		FP4		FP5		FP6	
		N	($\bar{X}$)	N	($\bar{X}$)	N	($\bar{X}$)	N	($\bar{X}$)	N	($\bar{X}$)	N	($\bar{X}$)
1980	August	0		1.4	(2.9)	0		4.9	(2.0)	1.6	(3.8)	4.2	(3.1)
	September	0		0		1.4	(3.3)	0		4.5	(3.8)	4.0	(2.4)
	October	0		3.7	(3.2)	4.2	(2.6)	11.1	(2.6)	1.3	(3.1)	0	
	November	1.6	(3.8)	4.4	(3.4)	5.9	(5.1)	0		0		0	
	December	-		-		-		-		-		-	
1981	January	0		0		0		0		0		0	
	February	0		3.5	(2.1)	0		0		0		0	
	March	0		0		0		0		0		0	
	April	0		0		0		0		0		0	
	May	0		0		0		3.2	(2.5)	2.6	(3.0)	2.2	(6.9)
	June	0		0		0		0		5.8	(3.7)	0	
	July	2.7	(6.1)	2.5	(2.6)	8.6	(3.3)	2.8	(6.6)	2.9	(4.9)	0	
	August	0		2.8	(7.0)	5.3	(5.1)	6.8	(3.0)	6.9	(6.9)	4.1	(8.0)
	September	-		-		-		-		-		-	
	October	2.7	(4.3)	6.4	(3.8)	3.3	(5.7)	1.8	(7.3)	0		1.8	(6.8)
	November	0		0		0		0		0		0	
	December	0		0		0		0		0		0	

A. rostratus: Larvae were caught only on three occasions

Date		Site	N	($\bar{X}$)
1980	November	FP3	1.2	(10.7)
1981	February	FP1	1.3	(3.1)
	October	FP5	2.2	(3.0)

in the plankton from the start of sampling in August to November 1980 and May-October 1981; two larvae only were caught in other months. The numbers of larvae caught each month were generally higher at the entrances to the Derwent Estuary and to Frederick Henry Bay than at other sites in each area. The results also show a trend towards smaller larvae being caught each month at the entrances of the two estuaries.

Differences in day and nighttime catches of larvae on any one day were not compared. However, the numbers of larvae caught during the day in August, September and November 1980 were not significantly different to the numbers caught at night in the same months of 1981 (t-test, $P>0.05$).

2.3.2 Juvenile Flounder

(i) Physical and Chemical Parameters of the Four Sites

The maximum and minimum temperatures recorded during the study period were 25.5°C and 6.1°C respectively. Yearly ranges in temperature were greatest on the shallow sandflats, e.g. stations D2b and F4c and least on the semi-exposed beaches, e.g. stations D2a and F4a (Figure 2.2). Temperature differences between sites in each month were generally low; a maximum difference of 7.1°C was recorded between sites F3c and F4b in January 1982. Similarly, temperatures generally varied by only 1-2°C at the stations within site F3 and site F4.

In contrast, salinities varied considerably between sites (Figure 2.2). A minimum of 2‰ and a maximum of 38.9‰ were recorded during the study period. Monthly variations in salinities were low at the open beach stations (D2b, F4a) and at the stations within the marine inlet (F4b,c) and the estuarine lagoon (F3a,b,c,d). Salinities were generally lower in winter at site D1 due to freshwater runoff. At site D2b salinities fluctuated markedly with the stage of the tide.

Substrate grain size analysis (Figure 2.3) showed that the substrate at site D1, low tide mark, was coarser than at all the other sites. The substrate consisted mainly of medium sand (0.25 - 0.5 mm) at this site and of fine sand (0.125 - 0.25 mm) at the other sites. There was little variation in percentage grain sizes at the three stations of site D2.

FIGURE 2.2 Water temperatures and salinities recorded at each site in different months. At sites F3a-d and F4b-c, the mean (circle) and range (vertical bar) of temperatures and salinities at stations a-d and b-c, respectively, are given.

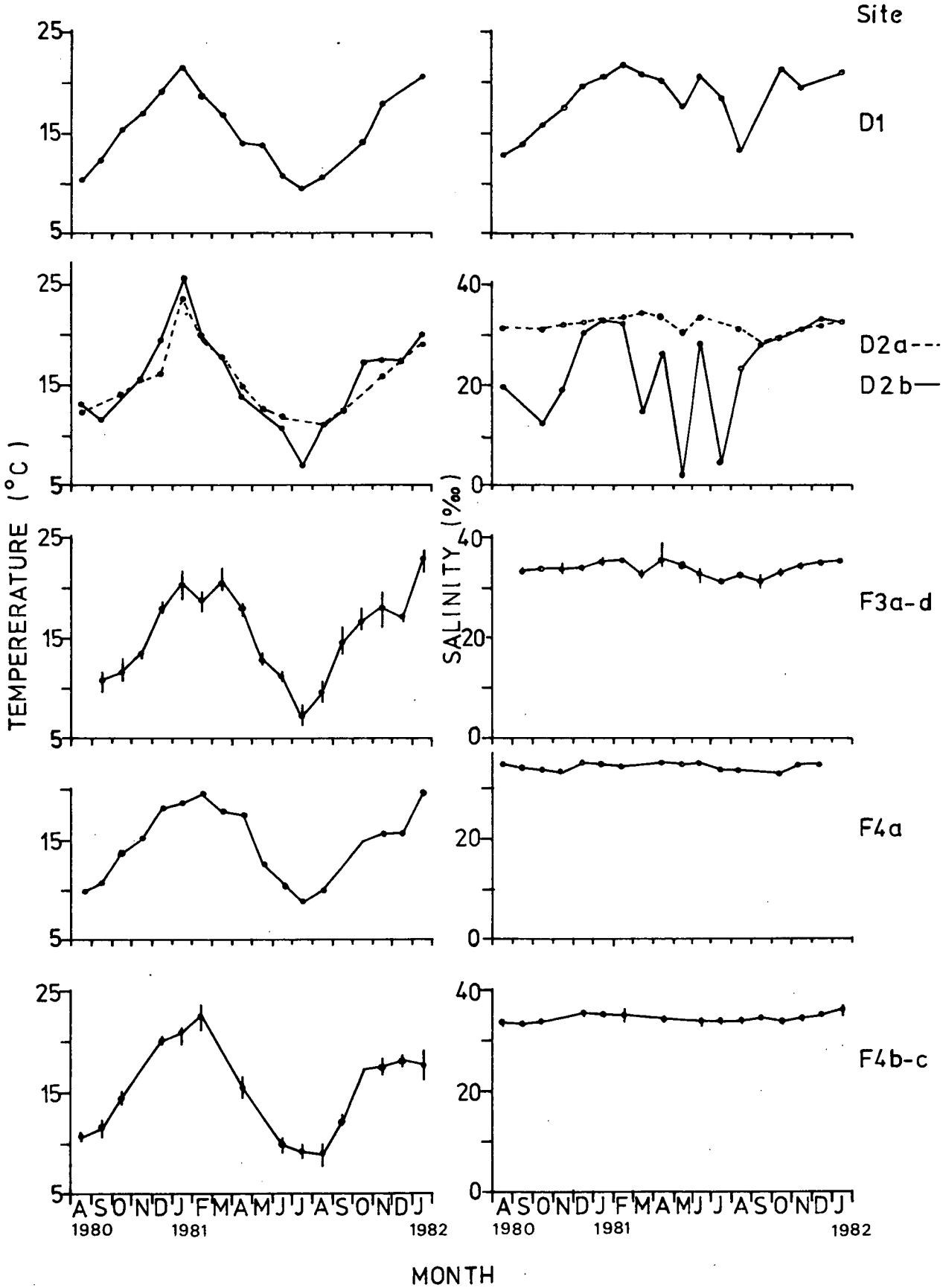

Site
D1
D2a---
D2b—
F3a-d
F4a
F4b-c
TEMPERERATURE (°C)
SALINITY (‰)
25
15
5
40
20
0
A S O N D J F M A M J J A S O N D J
1980
1981
1982
MONTH

FIGURE 2.3 Percentage frequency distribution of substrate particle size at

Site D1: (i) HWNT, (ii) LWNT

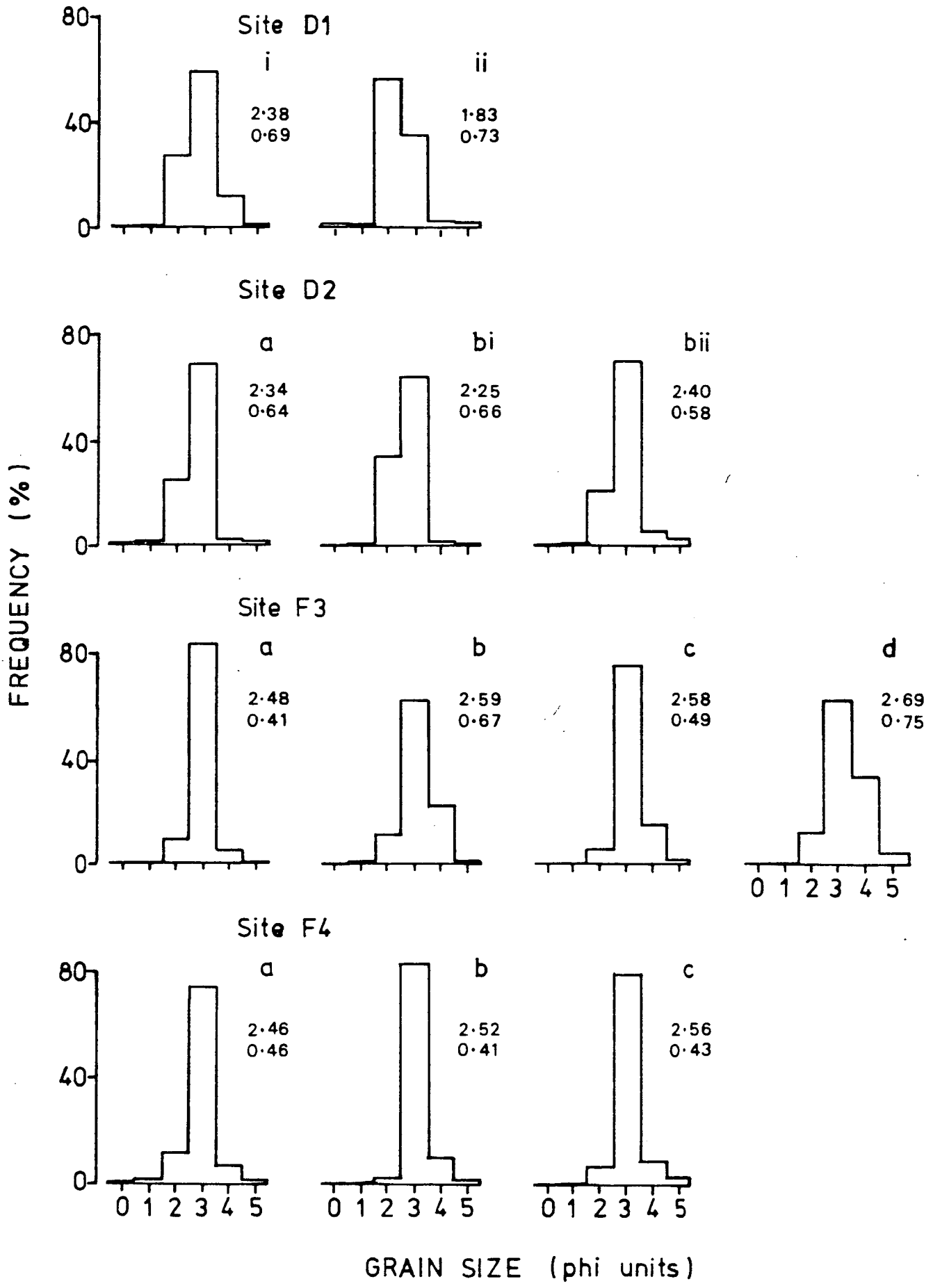
Site D2: Station a HWNT, station b - (i) HWNT, (ii) LWNT

Site F3: stations a,b,c,d HWNT

Site F4: stations a,b,c HWNT

phi units: (0) $\geqslant$1.00 mm, (1) 1-0.5 mm, (2) 0.5-0.25 mm, (3) 0.25-0.125 mm, (4) 0.125-0.062 mm, (5) $\leqslant$0.062 mm.

Of the two numbers in each histogram, the top number is the mean grain size (MdØ) and the bottom number is the sorting coefficient (QDØ)


Site D1
i
ii
2·38
0·69
1·83
0·73
Site D2
a
bi
bii
2·34
0·64
2·25
0·66
2·40
0·58
Site F3
a
b
c
d
2·48
0·41
2·59
0·67
2·58
0·49
2·69
0·75
Site F4
a
b
c
2·46
0·46
2·52
0·41
2·56
0·43
80
40
0
0 1 2 3 4 5
FREQUENCY (%)
GRAIN SIZE (phi units)

At site F3 the percentage of very fine sand and silt generally increased from stations F3a - F3d and at site F4 the percentage of coarse and medium sand was greatest at station F4a.

(ii) Seasonal Abundance in Shallow Water

Juvenile *R. tapirina* and *A. rostratus* were abundant in the push-net catches in 0-100 cm depth at all four sites during most months of the year (Figures 2.4 and 2.5 respectively). In some months abundances varied markedly with depth over 0-100 cm, resulting in large standard deviations; variation with depth is discussed in Section 2.3.2(iii). *R. tapirina* juveniles clearly occurred in higher densities than *A. rostratus* at all four sites. The numbers of each species caught per area covered at all sites are listed in Appendix 3.

Mean monthly abundances of each species separately at the four sites in five seasons were compared by analysis of variance. Abundances of *R. tapirina* were significantly different between sites and between seasons, and there was a significant site x season interaction (Table 2.2). Over all seasons, densities of *R. tapirina* were highest at, and not significantly different between, sites F3c and D2b, although the greatest densities on any one date were recorded in the River Derwent. For all sites, densities were significantly higher in August 1981 - October 1981 and November 1981 - January 1982 than in other seasons, and were significantly lower in February 1981 - April 1981 and May 1981 - July 1981. Abundances in November 1980 - January 1981 were significantly different to all other seasons.

As the site x season interaction was significant, means of abundances each season were compared for each site separately. The results of the SNK tests indicate seasonal differences in abundances between the Derwent River and Frederick Henry Bay sites. Abundances at the Derwent River sites were significantly higher in August 1981 - October 1981 and November 1981 - January 1982 than in other seasons, including November 1980 - January 1981. However, at the Frederick Henry Bay sites there was little

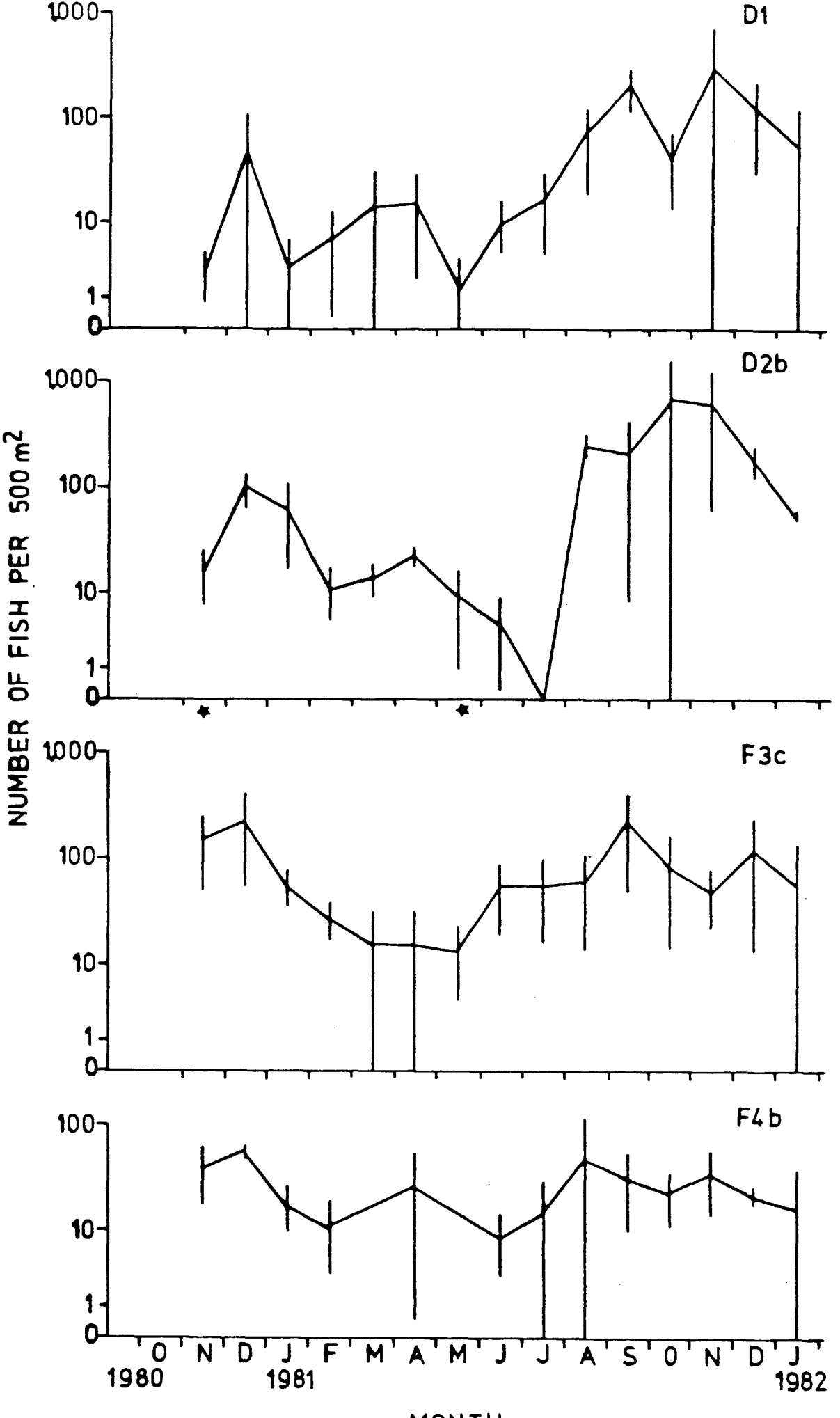


FIGURE 2.4 Mean monthly abundances (± S.D.) of *R. tapirina* juveniles in 0-1 m depth at sites D1, D2b, F3c and F4b.
★- Site D2b was dredged in this month

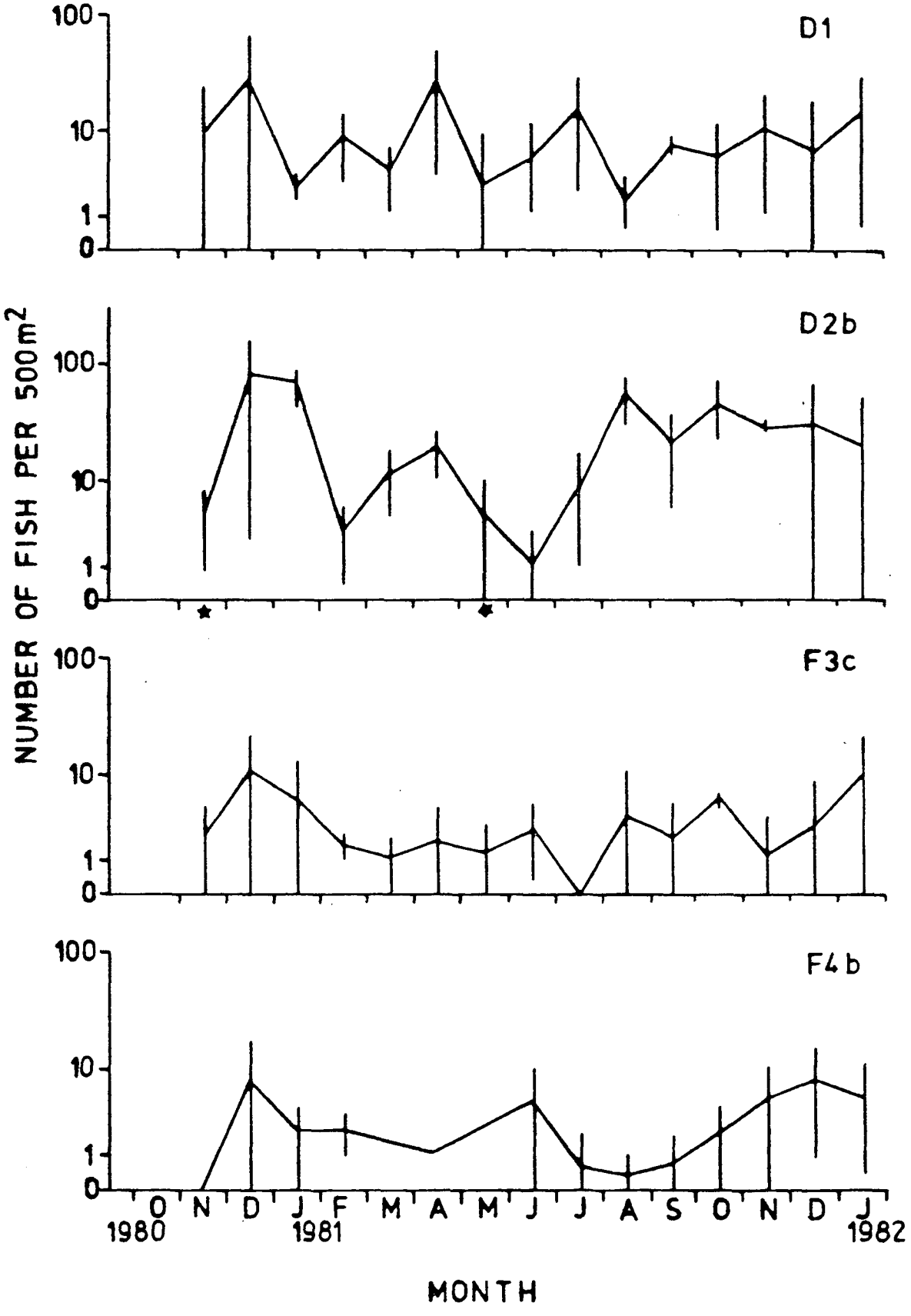


FIGURE 2.5 Mean monthly abundances (± S.D.) of *A. rostratus* juveniles in 0-1 m depth at sites D1, D2b, F3c and F4b
★- Site D2b was dredged in this month

TABLE 2.2 Results of two-way ANOVA comparing ℓn(x+1) transformed mean abundances of *R. tapirina* at four sites in five seasons (seasons: 1 = November 80 - January 1981, 2 = February 81 - April 1981, 3 = May 81 - July 1981, 4 = August 81 - October 1981, 5 = November 81 - January 1982)

ANOVA Table

	SS	DF	MS	F	P
Site	9.3319	3	3.1106	4.9880	P<0.01
Season	47.0912	4	11.7728	18.8782	P<0.001
Site x season	25.4248	12	2.1187	3.3975	P<0.01
Error	23.6975	38	0.6236		
Total	105.5454	57	1.8517		

SNK Test

Sites	F4b	D1	D2b	F3c
Seasons	3 2	1	5	4

Site x season interaction — Seasons

Site					
D1	3	1	2	5	4
D2b	3	2	1	5	4
F3c	2	3	5	4	1
F4b	3	2	5	4	1

Sites

Season					
1	D1	F4b	D2b	F3c	
2	D1	D2b	F4b	F3c	
3	D2b	D1	F4b	F3c	
4	F4b	D1	F3c	D2b	
5	F4b	F3c	D1	D2b	

TABLE 2.3 Results of two-way ANOVA comparing ℓn(x+1) transformed mean abundances of *A. rostratus* at four sites in five seasons (seasons: 1 = November 80 - January 1981, 2 = February 81 - April 1981, 3 = May 81 - July 1981, 4 = August 81 - October 1981, 5 = November 81 - January 1982)

ANOVA Table

	SS	DF	MS	F	P
Site	29.0577	3	9.6859	17.2996	P<0.001
Season	8.1479	4	2.0370	3.6382	P<0.05
Site x season	8.7039	12	0.7253	1.2955	P>0.05
Error	21.2758	38	0.5599		
Total	67.1853	57	1.1787		

SNK Test

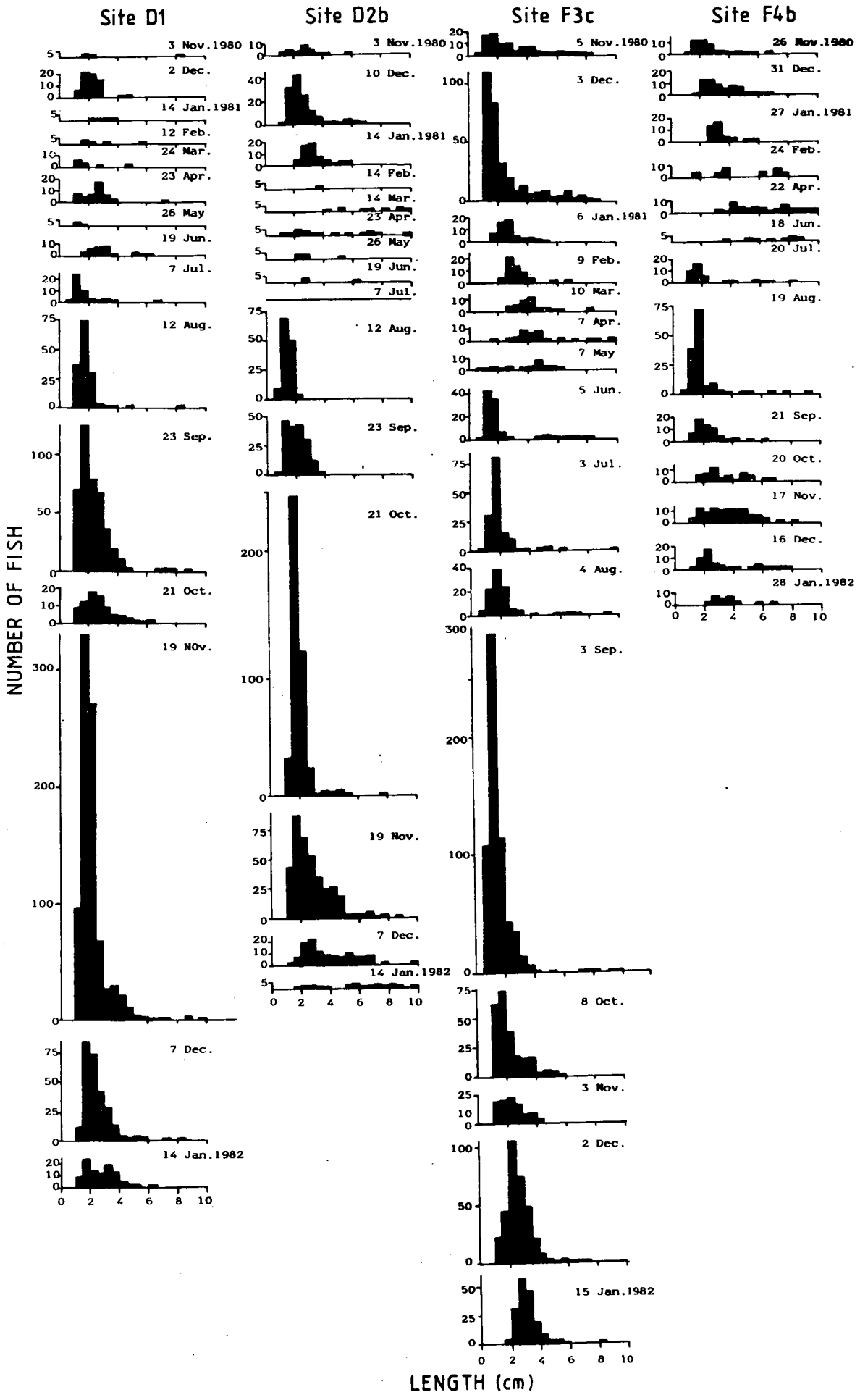
Sites	F4b	F3c	D1	D2b	
Seasons	3	2	4	1	5

difference in the densities of fish between seasons; in particular densities in November 1980 - January 1981 were not significantly different from November 1981 - January 1982. However, a comparison of abundance means at the four sites in each season separately showed that generally abundances did not differ markedly between sites in each season.

Abundances of *A. rostratus* were significantly different between sites and between seasons but the interaction of site x season was not significant. They occurred in significantly higher densities at the Derwent River sites than in Frederick Henry Bay, densities at site D2b being significantly greater than at site D1. For catches over time, the SNK test implied subsets of significantly different seasons. Abundances were significantly different between May - July 1981 and November to January in both 1980-81 and 1981-82 (Table 2.3).

Patterns of recruitment of juvenile *R. tapirina* into shallow water were examined from seasonal abundances (Figure 2.4) and from length frequency histograms (Figure 2.6). At sites D1 and D2b a small influx of newly-metamorphosed juveniles occurred in December 1980. From

FIGURE 2.6 Length frequency histograms of *R. tapirina* juveniles caught in the push-net in 0-1 m depth at sites D1, D2b, F3c and F4b from November 1980 to January 1982.


Site D1
Site D2b
Site F3c
Site F4b
NUMBER OF FISH
LENGTH (cm)
3 Nov.1980
2 Dec.
14 Jan.1981
12 Feb.
24 Mar.
23 Apr.
26 May
19 Jun.
7 Jul.
12 Aug.
23 Sep.
21 Oct.
19 NOv.
7 Dec.
14 Jan.1982
3 Nov.1980
10 Dec.
14 Jan.1981
14 Feb.
14 Mar.
23 Apr.
26 May
19 Jun.
7 Jul.
12 Aug.
23 Sep.
21 Oct.
19 Nov.
7 Dec.
14 Jan.1982
5 Nov.1980
3 Dec.
6 Jan.1981
9 Feb.
10 Mar.
7 Apr.
7 May
5 Jun.
3 Jul.
4 Aug.
3 Sep.
8 Oct.
3 Nov.
2 Dec.
15 Jan.1982
26 Nov.1980
31 Dec.
27 Jan.1981
24 Feb.
22 Apr.
18 Jun.
20 Jul.
19 Aug.
21 Sep.
20 Oct.
17 Nov.
16 Dec.
28 Jan.1982

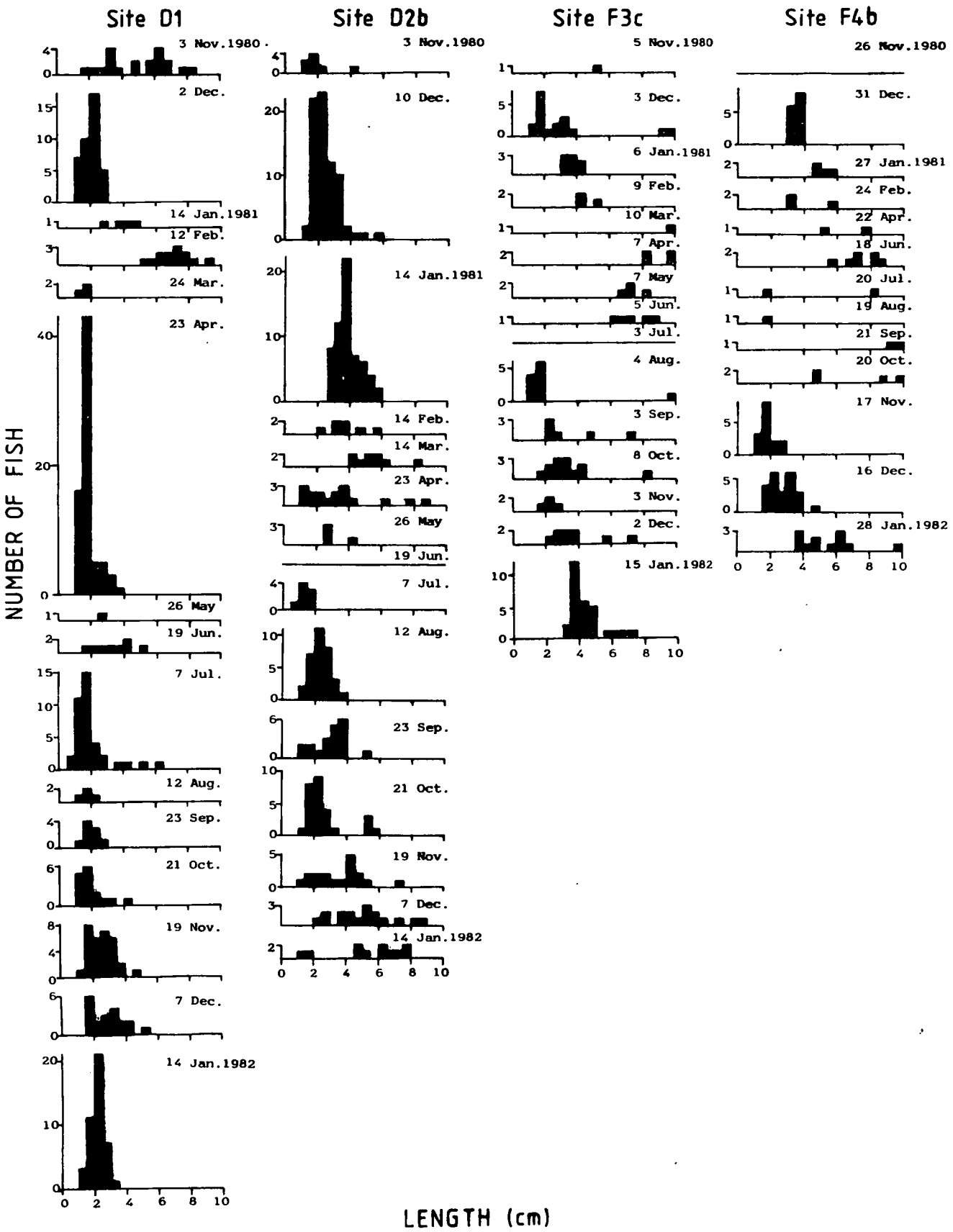
January until June 1981 at site D1, and until July 1981 at site D2b, fewer fish were caught and they were generally larger in size. The densities of recruits at site D1 increased from July to November 1981 (except for October) and from July to October at site D2b. The numbers then declined at both sites until January and a greater percentage of larger fish were caught, especially at site D2b.

At the Frederick Henry Bay sites, *R. tapirina* recruits were more abundant in November 1980 than at the Derwent River sites. The numbers of fish caught increased in December due to an influx of recruits at site F3c and an increase in the number of larger fish at site F4b. From December 1980 until May 1981 at site F3c and until June 1981 at site F4b, abundances generally decreased and the size of fish progressively increased. Modal lengths at site F3 increased from 1 - 1.5 cm to 4.5 - 5 cm from December to May, and at site F4 from 1 - 2 cm to 4 - 4.5 cm during November to April. The densities of recruits increased at site F3c from June to September 1981 and from July to August 1981 at site F4d. Densities then generally declined to January 1982 at both sites and overall the mean length of fish increased.

Seasonal abundances (Figure 2.5) and length frequency histograms (Figure 2.7) of *A. rostratus* show that at sites D1 and D2b the numbers of recruits increased in December 1980. They then declined at site D1 in January 1981 but remained high at site D2b. Densities remained low at site D1 until March 1981; in April an influx of recruits occurred but abundances were low again in May and June. At site D2b densities were low from February to June 1981 with only a minor increase in the numbers of recruits in April. Abundances peaked again in July 1981 at site D1; they dropped in August and then generally increased to January 1982 with both recruits and larger juveniles being caught. At site D2b densities increased in July and were highest in August and October 1981. The numbers of recruits decreased from November 1981 to January 1982 and the mean length of fish increased.

At the Frederick Henry Bay sites abundances of *A. rostratus* juveniles were high in December 1980. From January until July 1981 at site F3c and until October 1981 at site F4b abundances were generally low and larger fish were caught. Newly-metamorphosed juveniles were caught at site F3c in August 1981 and at site F4b in November 1981. Densities of

FIGURE 2.7 Length frequency histograms of *A. rostratus* juveniles caught in the push-net in 0-1 m depth at sites D1, D2b, F3c and F4b from November 1980 to January 1982


Site D1
Site D2b
Site F3c
Site F4b
NUMBER OF FISH
LENGTH (cm)
3 Nov.1980
2 Dec.
14 Jan.1981
12 Feb.
24 Mar.
23 Apr.
26 May
19 Jun.
7 Jul.
12 Aug.
23 Sep.
21 Oct.
19 Nov.
7 Dec.
14 Jan.1982
3 Nov.1980
10 Dec.
14 Jan.1981
14 Feb.
14 Mar.
23 Apr.
26 May
19 Jun.
7 Jul.
12 Aug.
23 Sep.
21 Oct.
19 Nov.
7 Dec.
14 Jan.1982
5 Nov.1980
3 Dec.
6 Jan.1981
9 Feb.
10 Mar.
7 Apr.
7 May
5 Jun.
3 Jul.
4 Aug.
3 Sep.
8 Oct.
3 Nov.
2 Dec.
15 Jan.1982
26 Nov.1980
31 Dec.
27 Jan.1981
24 Feb.
22 Apr.
18 Jun.
20 Jul.
19 Aug.
21 Sep.
20 Oct.
17 Nov.
16 Dec.
28 Jan.1982

juveniles generally increased to January 1982 at site F3c with a progressive monthly increase in mean length from 1.5 - 2 cm in August to 3.5 - 4 cm in January. At site F4b densities and mean length increased in December 1981; in January 1982 fewer, larger fish were caught.

Monthly rates of decline in numbers of both species at the four sites were determined for the four month periods after peaks in recruitment as the maximum decline in numbers generally occurred during this time, or until the time of the next recruitment if this occurred within four months. The rates of decline in numbers (Z) were calculated from the slope of the regression line of $\log_e$ mean abundances against time. The values of Z for *R. tapirina* varied from -0.14 at site F4b to -0.86 at sites D1 and D2b in November 1981 - January 1982 and September 1981 - January 1982 respectively. The values of Z for *A. rostratus* were, overall, marginally higher; they varied from -0.24 at site D2b in October 1981 - January 1982 to -0.92 at this site in December 1980 - March 1981 (Table 2.4). These values of Z are, however, only approximate because small numbers of recruits were caught in many months.

(iii) Depth Distributions

In months of relatively low abundances at both sites D1 and F3c, *R. tapirina* juveniles were generally distributed over the three depths (0-100 cm) and the variation in mean length of fish at each depth was high (Figure 2.8). At site F3c, in particular, larger fish occurred in deeper water. However, during months of peak recruitment, newly-metamorphosed juveniles were most abundant in deeper water (50 - 70 cm and 90 - 110 cm) but the mean lengths were similar at each depth.

Few *A. rostratus* juveniles were caught at the shallowest depth at either site (Figure 2.9). At site D1 they were abundant at depths of 50 - 70 cm and 90 - 110 cm in most months; at site F3c the highest densities mostly occurred in 90 - 110 cm depth. The mean lengths of *A. rostratus* at both sites were generally higher during months of low densities than in months of recruitment.

Analysis of the data by ANOVA showed that abundances of *R. tapirina* were significantly different between depths and between seasons at site

TABLE 2.4 Monthly mortality rates (Z) with 95% confidence limits and percentage monthly mortality (%) for *R. tapirina* and *A. rostratus* at the four sites

Site	*Rhombosolea tapirina*				*Ammotretis rostratus*			
	Months	Z	95% C.L.	%	Months	Z	95% C.L.	%
D1	Dec. 1980 - Mar. 1981	-0.29	±2.65	25.2	Dec. 1980 - Mar.1981	-0.47	±2.05	37.5
	Nov. 1981 - Jan. 1982	-0.86	±0.12	57.7	Apr. 1981 - Jun.1981	-0.78	±18.03	54.2
D2b	Dec. 1980 - Apr. 1981	-0.46	±0.76	36.9	Dec. 1980 - Mar.1981	-0.92	±2.63	60.1
	Oct. 1981 - Jan. 1982	-0.86	±0.82	57.7	Oct. 1981 - Jan.1982	-0.24	±0.28	21.3
F3c	Dec. 1980 - Apr. 1981	-0.68	±0.46	49.3	Dec. 1980 - Apr.1981	-0.52	±0.55	40.5
	Sep. 1981 - Jan. 1982	-0.24	±0.55	21.3				
F4b	Dec. 1980 - Apr. 1981	-0.14	±1.18	13.1	Dec. 1980 - Apr.1981	-0.43	±0.62	34.9
	Aug. 1981 - Dec. 1981	-0.15	±0.28	13.9	Dec. 1981 - Jan.1982	-0.43		34.9

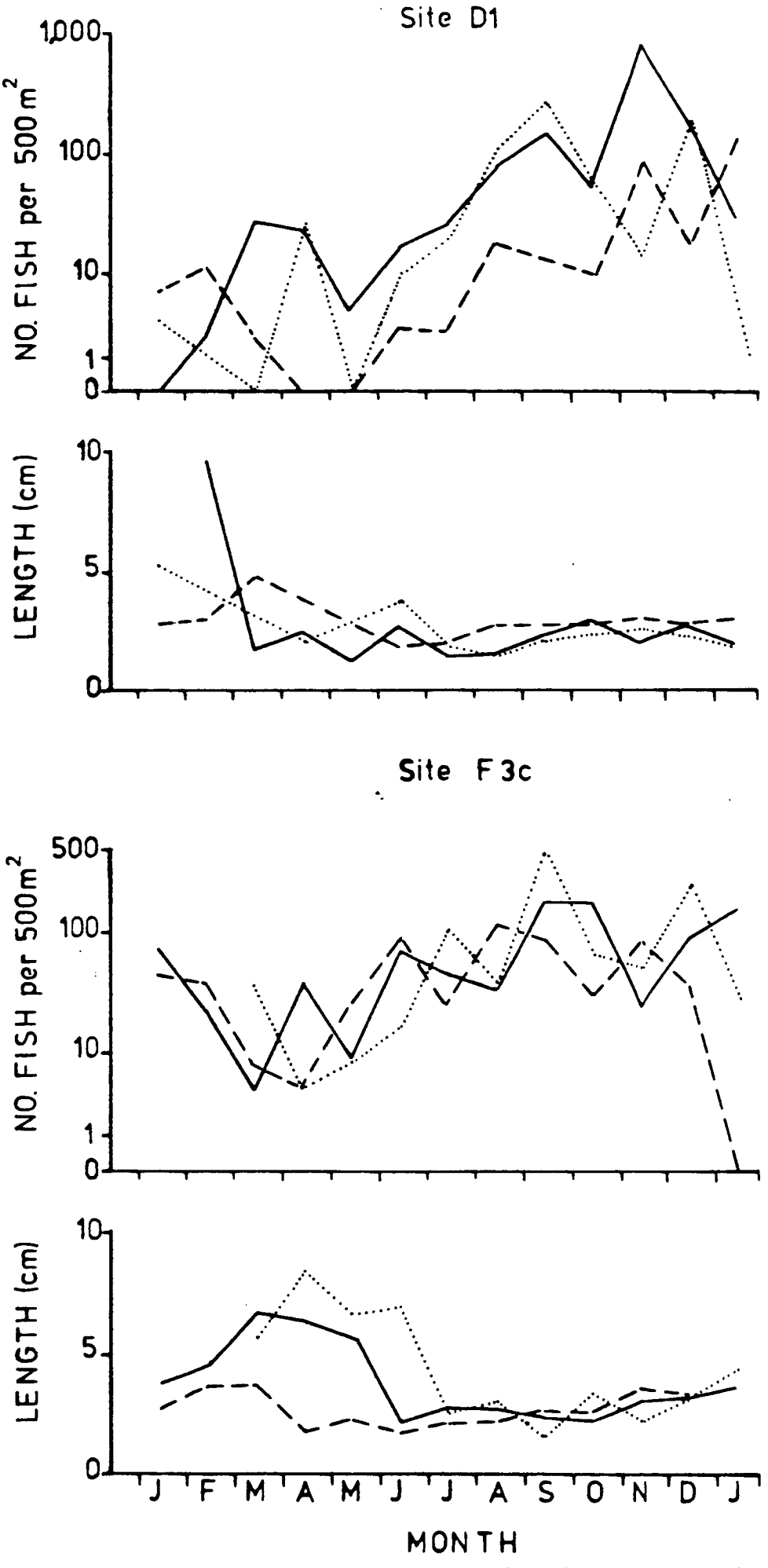


FIGURE 2.8 Monthly abundances and mean lengths of *R. tapirina* juveniles in three depths at site D1 and site F3c
Depths ---- 10-30 cm, ____ 50-70 cm, ····· 90-110 cm

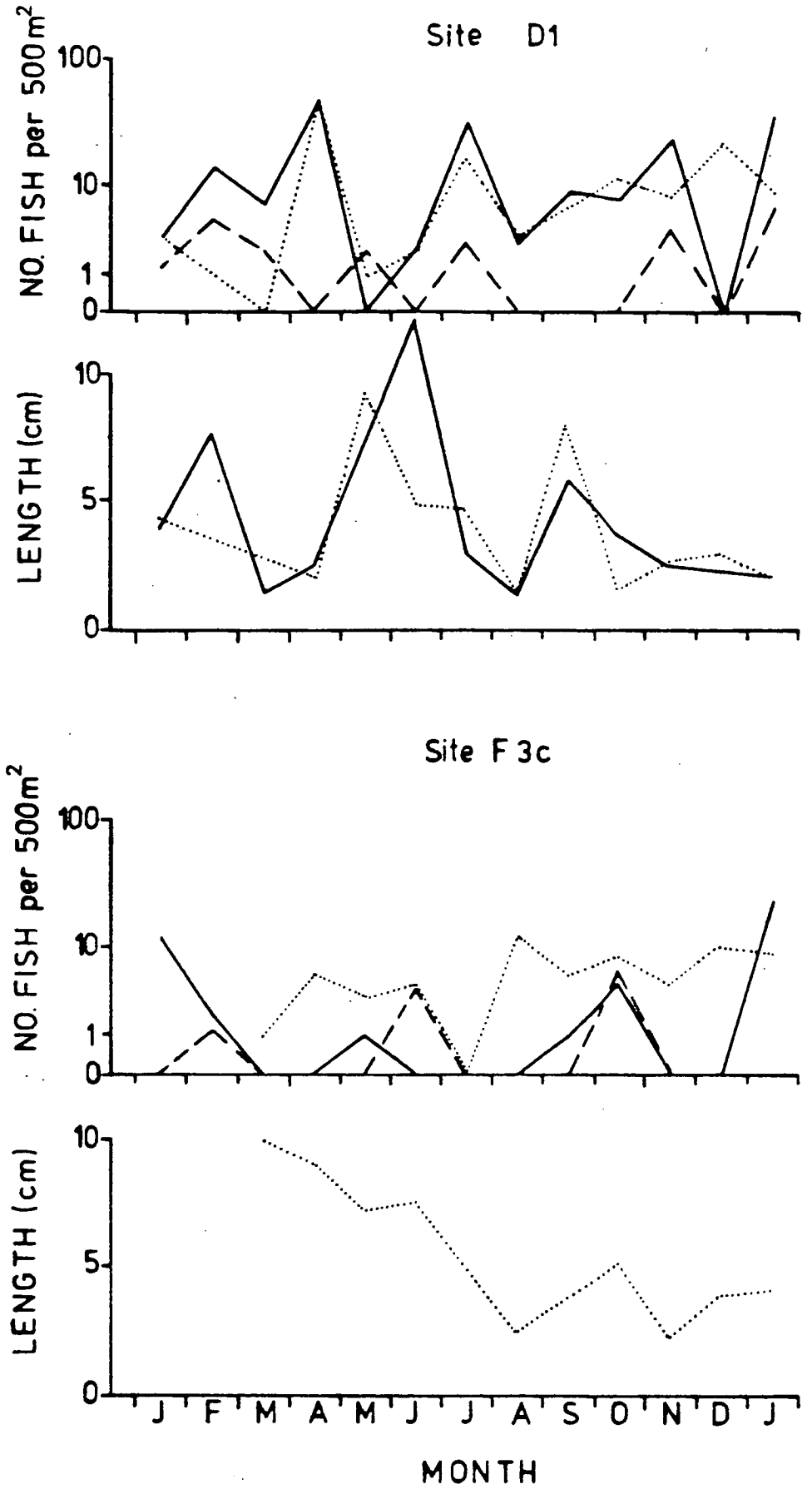


FIGURE 2.9 Monthly abundances and mean lengths of *A. rostratus* juveniles in three depths at site D1 and site F3c
Depths ---- 10-30 cm, —— 50-70 cm, ····· 90-110 cm

D1 but only between seasons at site F3c. The depth x season interaction was not significant at either site (Table 2.5). Abundances of *R. tapirina* at site D1 were significantly higher at 50 - 70 cm than at 10 - 30 cm, but were not different between 10 - 30 cm and 90 - 110 cm or between 90 - 110 cm and 50 - 70 cm. They were significantly higher in August 1981 - October 1981 and November 1981 - January 1982 than in February 1981 - April 1981 and May 1981 - July 1981. At site F3c, densities of *R. tapirina* were significantly different between August 1981 - October 1981 and March 1981 - April 1981 but did not differ between other seasons.

Densities of *A. rostratus* at both sites D1 and F3c differed significantly with depth but not with season, and the depth x season interaction was not significant (Table 2.6). They were significantly more abundant at depths of 90 - 110 cm and 50 - 70 cm than at 10 - 30 cm at site D1, and at 90 - 110 cm than at 10 - 30 cm and 50 - 70 cm at site F3c.

The relative number of recently metamorphosed *R. tapirina* and *A. rostratus* juveniles at each depth over 27 h indicates that both species moved up and down the shore with the tide but occupied different depths during the sampling period (Figure 2.10). *R. tapirina* juveniles were most abundant at the shallowest depth (10 - 30 cm) at high tide and during the night, and at medium depth (50 - 70 cm) at other times. *A. rostratus* were caught in the highest numbers at the medium depth except around the time of low tide at night when they were most abundant in shallow water. These results indicate that although both species have tidal-related movements, the depths they occupy are apparently more related to the time of day than the stage of the tide. The numbers of fish caught at different depths during the day and the night were compared using ANOVA for each species separately. Abundances of each species were not significantly different between day and night over all depths, and the interaction of time and depth was not significant (Table 2.7). Thus, although relative abundances at 10 - 30 cm and 50 - 70 cm depths differed between day and night, the variation in actual numbers of fish caught resulted in a non-significant interaction. Abundances of each species, however, were significantly different between depths, for day and night combined. The numbers of *R. tapirina* were significantly higher at 50 - 70 cm and 10 - 30 cm depth than at 90 - 110 cm. *A. rostratus* were significantly more abundant at 50 - 70 cm than at 90 - 110 cm depth but were not different between 90 - 110 cm and 10 - 30 cm or 10 - 30 cm and 50 - 70 cm depth.

TABLE 2.5 Results of two-way ANOVA comparing ℓn(x+1) transformed mean abundances of *R. tapirina* at different depths in different seasons at sites D1 and F3c; (seasons: 1 = February 81 - April 1981 Site D1, March 81 - April 1981 Site F3c, 2 = May 81 - July 1981, 3 = August 81 - October 1981, 4 = November 81 - January 1982).

Site D1

ANOVA Table	SS	DF	MS	F	P
Depth	17.1919	2	8.5960	5.0889	P<0.05
Season	40.6208	3	13.5403	8.160	P<0.01
Depth x season	10.8190	6	1.8032	1.0675	P>0.05
Error	33.7830	20	1.6891		
Total	101.4395	31	3.2722		

SNK Test

Depth (cm)	10-30		90-110	50-70
Season	2	1	3	4

Site F3c

ANOVA Table	SS	DF	MS	F	P
Depth	1.7262	2	0.8631	0.5190	P>0.05
Season	18.7626	3	6.2542	3.7604	P<0.05
Depth x season	7.6545	6	1.2758	0.7671	P>0.05
Error	34.9269	21	1.6632		
Total	63.0366	32	1.9699		

SNK Test

Season	1	2	4	3

TABLE 2.6 Results of two-way ANOVA comparing ℓn(x+1) transformed abundances of *A. rostratus* at different depths in different seasons at sites D1 and F3c; (Seasons: 1 = February 81 - April 1981 site D1, March 81 - April 1981 Site F3c, 2 = May 81 - July 1981, 3 = August 81 - October 1981, 4 = November 81 - January 1982).

Site D1

ANOVA Table

	SS	DF	MS	F	P
Depth	16.0013	2	8.0007	5.4591	P<0.05
Season	2.2533	3	0.7511	0.5125	P>0.05
Depth x season	1.9089	6	0.3182	0.2171	P>0.05
Error	29.3114	20	1.4656		
Total	49.2791	31	1.5896		

SNK Test

Depth (cm)	10-30	90-110	50-70

Site F3c

ANOVA Table

	SS	DF	MS	F	P
Depth	9.8872	2	4.9436	6.7362	P<0.01
Season	2.3408	3	0.7803	1.0632	P>0.05
Depth x season	2.3128	6	0.3855	0.5253	P>0.05
Error	15.4115	21	0.7339		
Total	30.4461	32	0.9514		

SNK Test

Depth (cm)	10-30	50-70	90-110

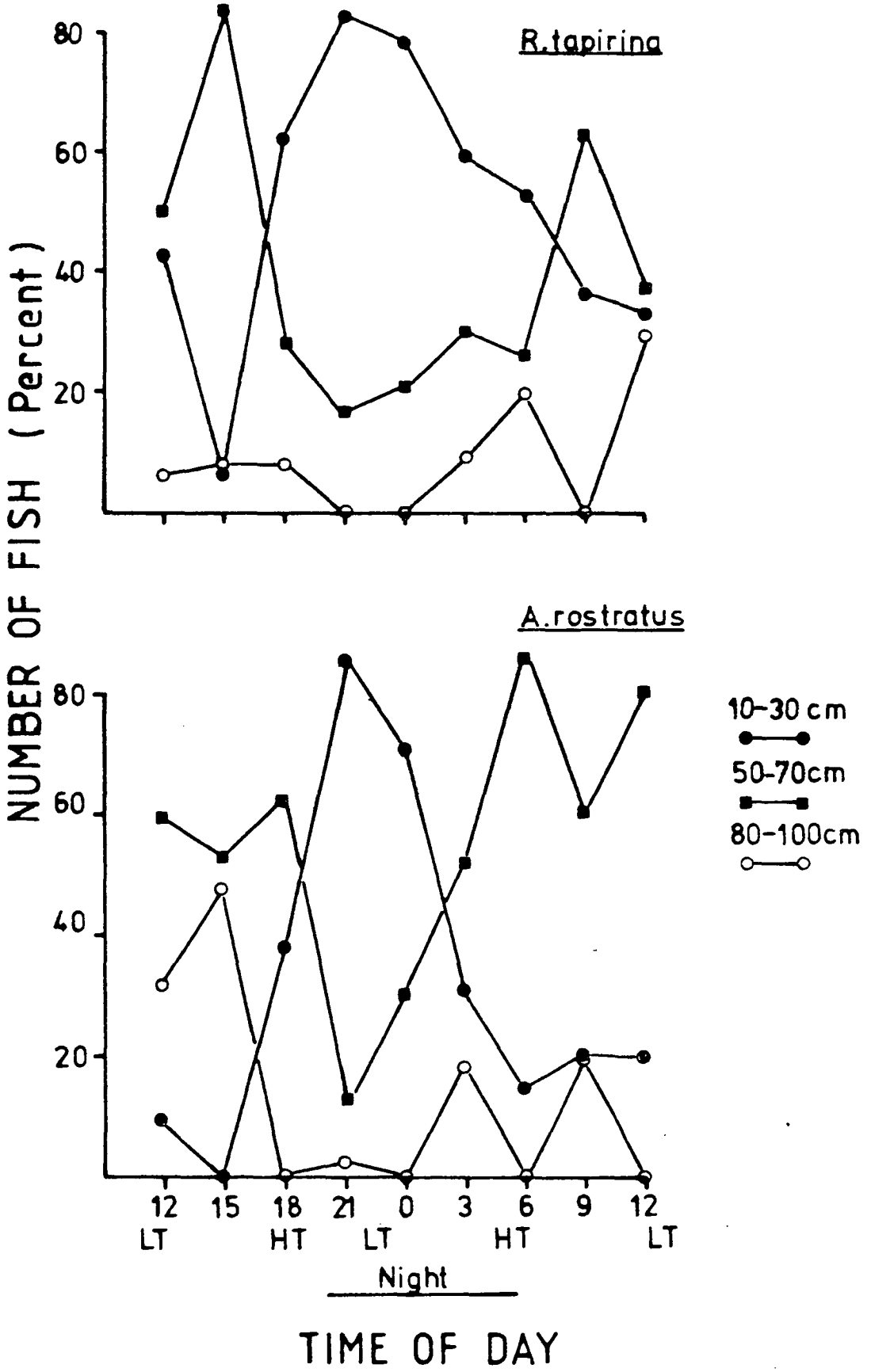


FIGURE 2.10 Percentage number of *R. tapirina* and *A. rostratus* juveniles caught at each depth (10-30 cm, 50-70 cm, 80-100 cm) every 3 h for 27 h at site D1
LT = low tide, HT = high tide

TABLE 2.7 Results of two-way ANOVA comparing ℓn(x+1) transformed abundances of *R. tapirina* and *A. rostratus* juveniles, separately, at different depths between day and night at site D1.

R. tapirina

ANOVA Table

	SS	DF	MS	F	P
Depths	14.7153	2	7.3577	9.7691	P<0.01
Times	0.7443	1	0.7443	0.9883	P>0.05
Depth x time	2.7558	2	1.3779	1.8295	P>0.05
Error	15.8163	21	0.7532		
Total	33.4963	26	1.2883		

SNK Test

Depth (cm)	90-110	50-70	10-30

A. rostratus

ANOVA Table

	SS	DF	MS	F	P
Depths	7.3445	2	3.6723	5.3538	P<0.05
Time	2.1334	1	2.1334	3.1103	P>0.05
Depth x time	4.6578	2	2.3289	3.3953	P>0.05
Error	14.4043	21	0.6859		
Total	28.1325	26	1.0820		

SNK Test

Depth (cm)	90-110	10-30	50-70

(iv) Distributions Within an Estuarine Lagoon and a Marine Inlet

Abundances of *R. tapirina* juveniles in the estuarine lagoon were highest in most months on the extensive, unvegetated sandflats (stations F3b and F3c) and were slightly less on the partially vegetated sandflats at station F3d (Figure 2.11). Few fish were caught at the entrance to the lagoon except in September. By contrast, densities of *A. rostratus* juveniles were highest in most months at station F3a, and were generally low at stations F3b, F3c and F3d. No *A. lituratus* juveniles were caught in the lagoon.

Abundances of *R. tapirina* only were compared using ANOVA; the numbers of *A. rostratus* caught were too low for statistical analysis. Abundances differed significantly between stations and between sites but the station x season interaction was not significant (Table 2.8). Abundances at stations F3b, F3c and F3d were similar and significantly higher than at station F3a. They were significantly greater at all sites in July - September and October - December than in January - March and April - June of 1981.

TABLE 2.8 Results of two-way ANOVA comparing ℓn(x+1) transformed mean abundances of *R. tapirina* at different stations of site F3 in different seasons; (seasons: 1 = January 81 - March 1981, 2 = April 81 - June 1981, 3 = July 81 - September 1981, 4 = October 81 - December 1981)

	R. tapirina				
ANOVA Table	SS	DF	MS	F	P
Station	37.1260	3	12.3753	8.0265	P<0.001
Season	28.8664	3	7.6221	4.9436	P<0.01
Station x season	8.1825	9	0.9092	0.5897	P>0.05
Error	49.3377	32	1.5418		
Total	117.5126	47	2.5003		

SNK Test

Station	a	b	c	d
Season	2	1	3	4

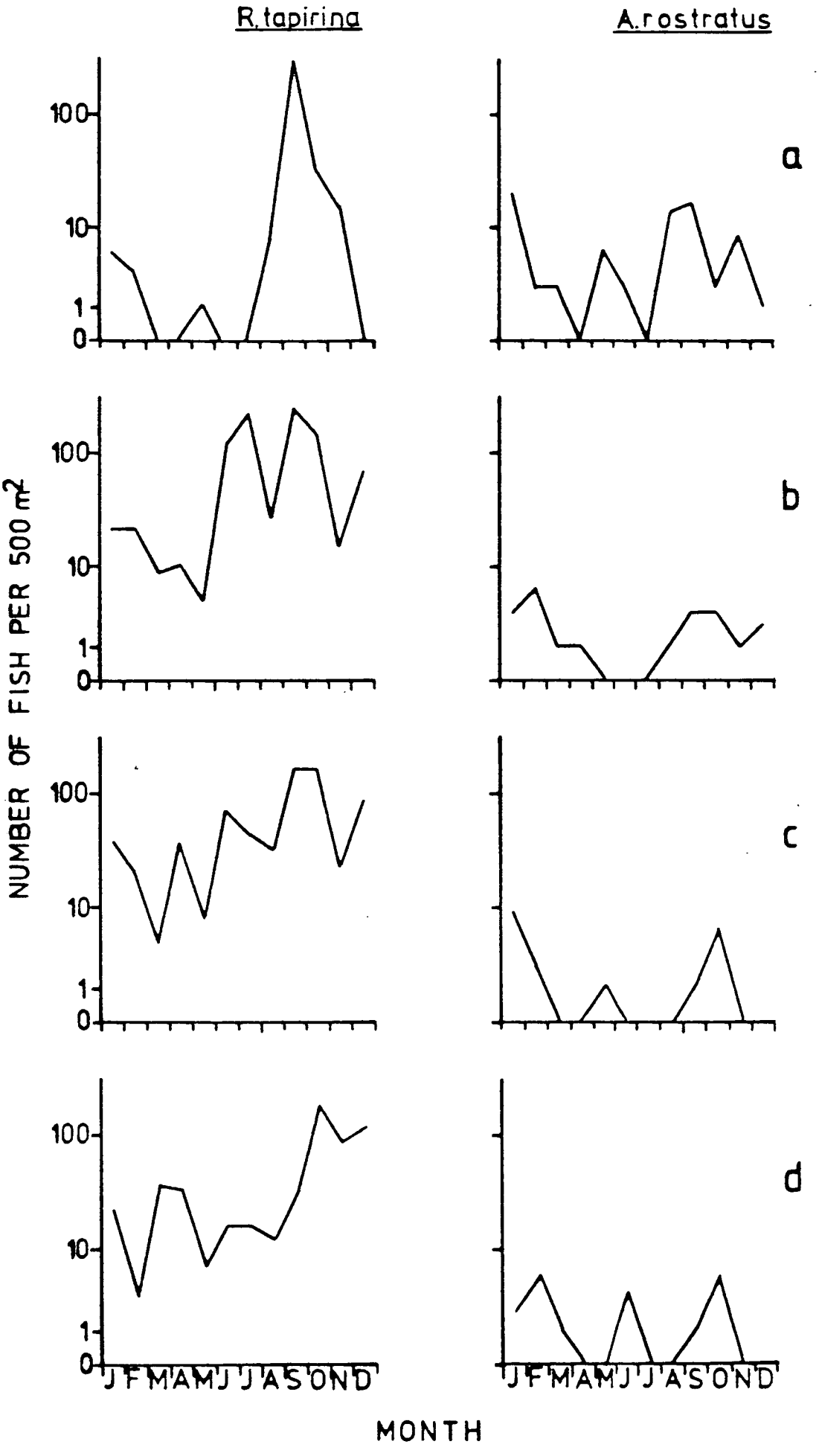


FIGURE 2.11 Monthly abundances of *R. tapirina* and *A. rostratus* juveniles at four stations a,b,c, and d of site F3; depth 50-70 cm

Distributions of flounder at the three stations of site F4, a marine inlet, are presented as mean monthly abundances for seven months at station F4a and 12 months at stations F4b and F4c (Table 2.9).

TABLE 2.9 Mean monthly abundances $\bar{X}$ (number of fish per 500 m^2) of *R. tapirina*, *A. rostratus* and *A. lituratus* at stations a, b and c of site F4.

Species	Stations					
	a		b		c	
	$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.
R. tapirina	0.14	±0.38	22.54	±11.49	60.68	±93.03
A, rostratus	0.14	±0.38	5.68	±2.38	0	
A. lituratus	1.29	±1.38	0		0	

A. lituratus were caught only at station F4a, a semi-exposed beach. *A. rostratus* juveniles were most abundant at station F4b and were never caught on the shallow sandflats of station F4c. *R. tapirina*, overall, occurred in the highest densities at station F4c, although the high standard deviation shows that densities varied greatly between months at this station. The mean monthly abundance at station F4b was lower but difference between months was not as great as at station F4c.

(v) Seasonal Abundances of Juvenile Flounder in Deeper Water

The numbers of juvenile *R. tapirina* and *A. rostratus* caught in deeper water (1.5 - 4 m) were much lower than in shallow water, especially at site D1 (Figure 2.12). As few fish were caught, the data were not analysed statistically. The numbers of each species caught per area fished are given in Appendix 4.

R. tapirina juveniles occurred in higher densities at the Frederick Henry Bay sites than in the Derwent River; densities of *A. rostratus* were lowest at site D1. In most months, *R. tapirina* were more abundant at the Frederick Henry Bay sites than *A. rostratus*, but were less abundant than *A. rostratus* at site D2a. Overall, the densities of *R. tapirina* were highest in October - December 1980, March - April 1981 and November 1981; *A. rostratus* were most abundant in March - May 1981.

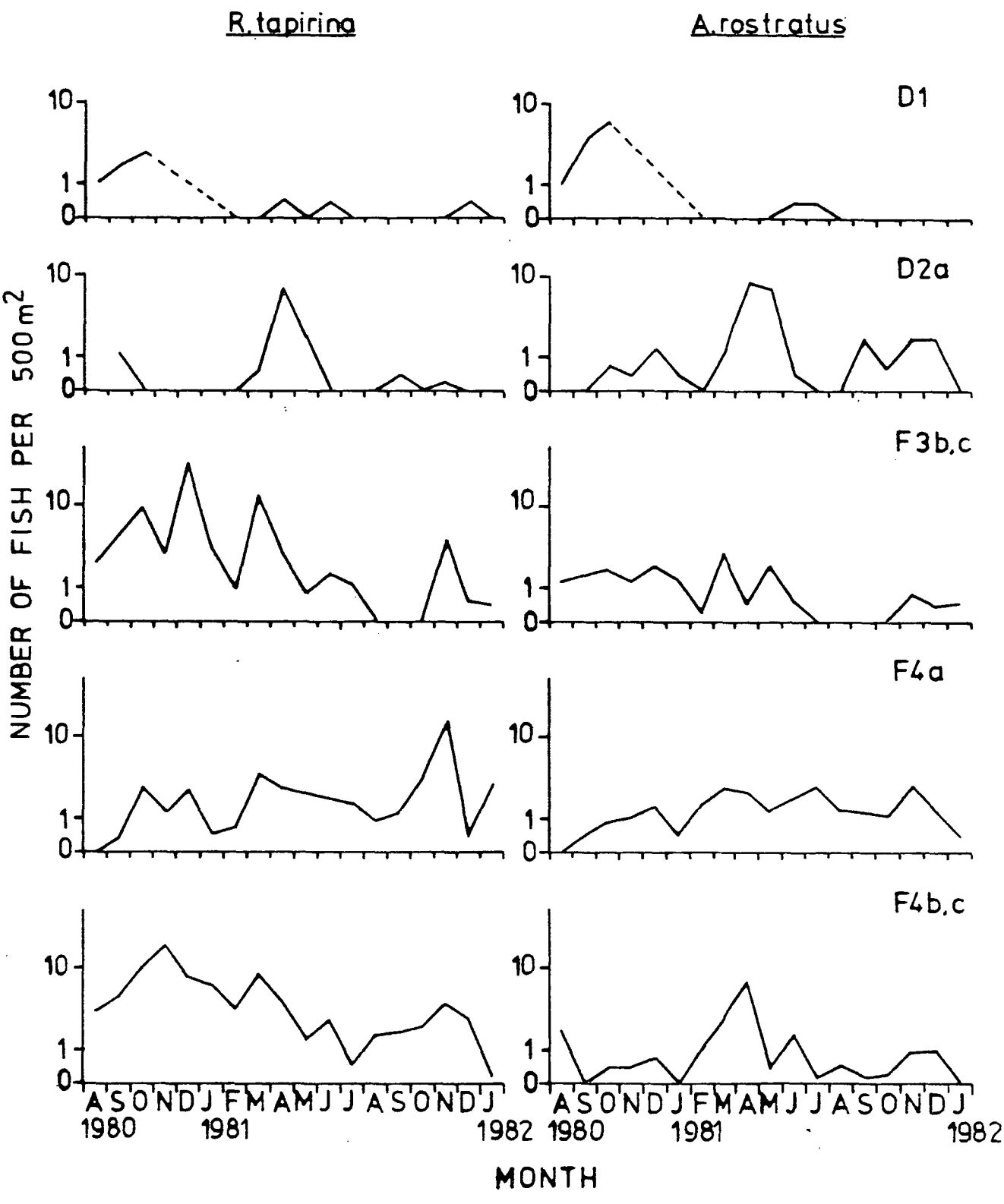


FIGURE 2.12 Monthly abundances of *R. tapirina* and *A. rostratus* juveniles in 1.5-4.0 m depth at sites D1, D2a, F3b,c, F4a and F4b,c

Length frequency histograms for *R. tapirina* (Figure 2.13) and for *A. rostratus* (Figure 2.14) show that although the smallest fish of either species caught in the beam trawl were 2-3 cm in length, the majority were from 5 to 15 cm. By contrast most fish caught in the push-net were 1-4 cm in length. Smaller juveniles are apparently more abundant in shallow water and larger juveniles in deeper water. The push-net, however, was probably less efficient at catching larger juveniles than the beam trawl.

Length frequency histograms for *R. tapirina* do not show clear trends in monthly abundances or length increments of the population as few fish were caught at sites D1 and D2a, at site F3b,c greater numbers of fish were caught only in three months and at site F4a the few fish caught each month generally varied in length. Densities of *R. tapirina* were considerably higher at site F4b,c but a progressive monthly increase in the mean length of the population was apparent only in February to May, 1981.

Length frequency histograms for *A. rostratus* also do not show any clear patterns. Abundances were low in most months at each site and the lengths of fish caught varied. From the few fish caught, a progressive monthly increase in length from 6 cm in February to 12 cm in November, 1981 at site F4a is suggested.

The estimation of growth rates from the progressive monthly increase in mean length was not possible for juveniles of *R. tapirina* or *A. lituratus* in either shallow or deeper water. The length frequency histograms showed little evidence of monthly increases in length of the populations as recruitment to shallow water occurred in many months and juveniles were not consistently abundant in deeper water due possibly to emigration of larger juveniles from the nursery grounds.

A. lituratus juveniles were caught in low numbers at depths of 1.5 - 4 m at the semi-exposed sites, D2a and F4a, only (Figure 2.15). The length frequency histograms show a progressive monthly increase in length from 7-9 cm to 12-14 cm during March to December, 1981 at site D2a but not at site F4a. The smallest fish caught were 3.5 cm TL which suggests that juveniles move into the shore zone at a later stage of development than *R. tapirina* or *A. rostratus*.

FIGURE 2.13 Length frequency histograms of *R. tapirina* juveniles caught in the beam trawl in 1.5-4.0 m depth at sites D1, D2a, F3b,c F4a and F4b,c from August 1980 to January 1982

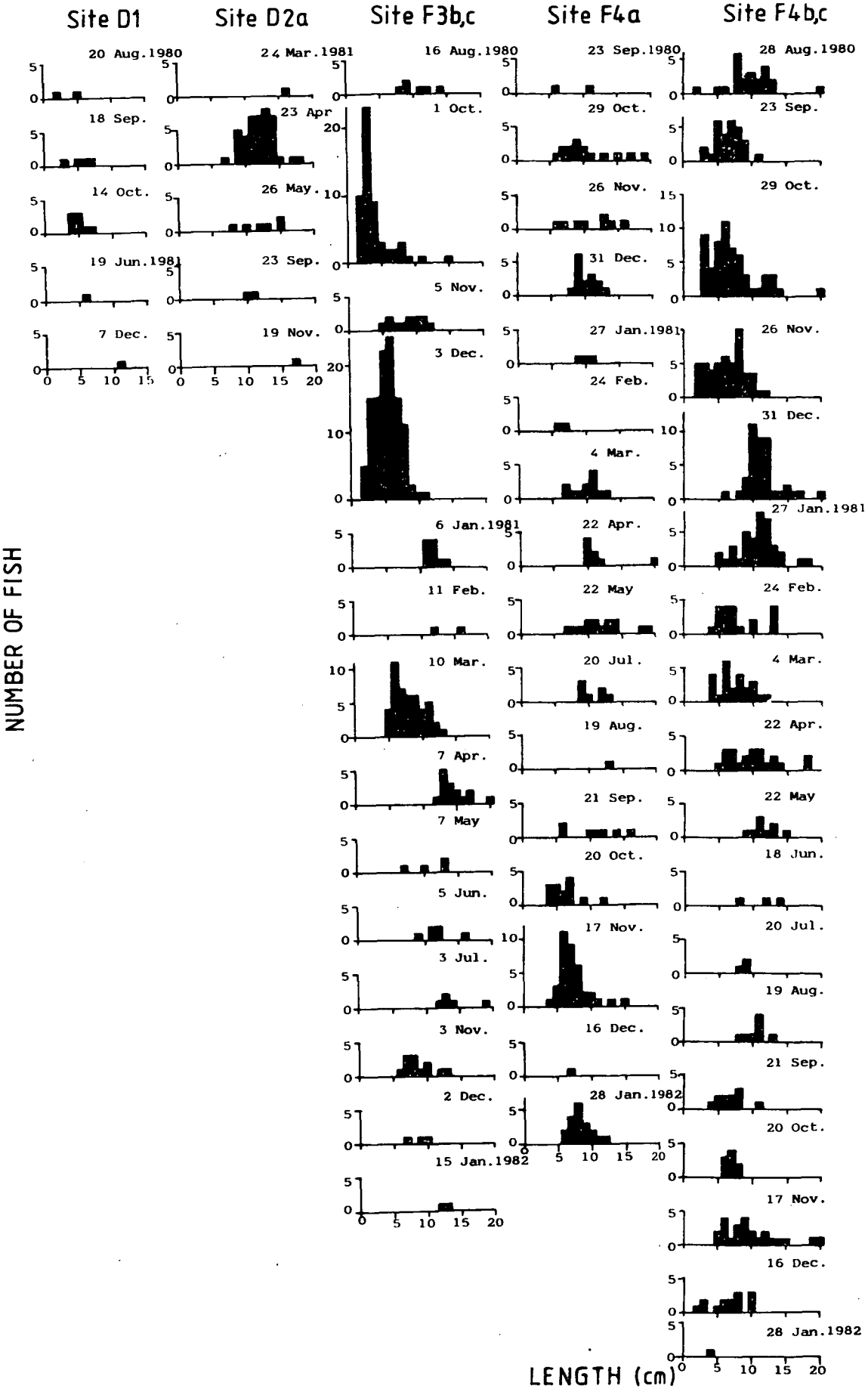
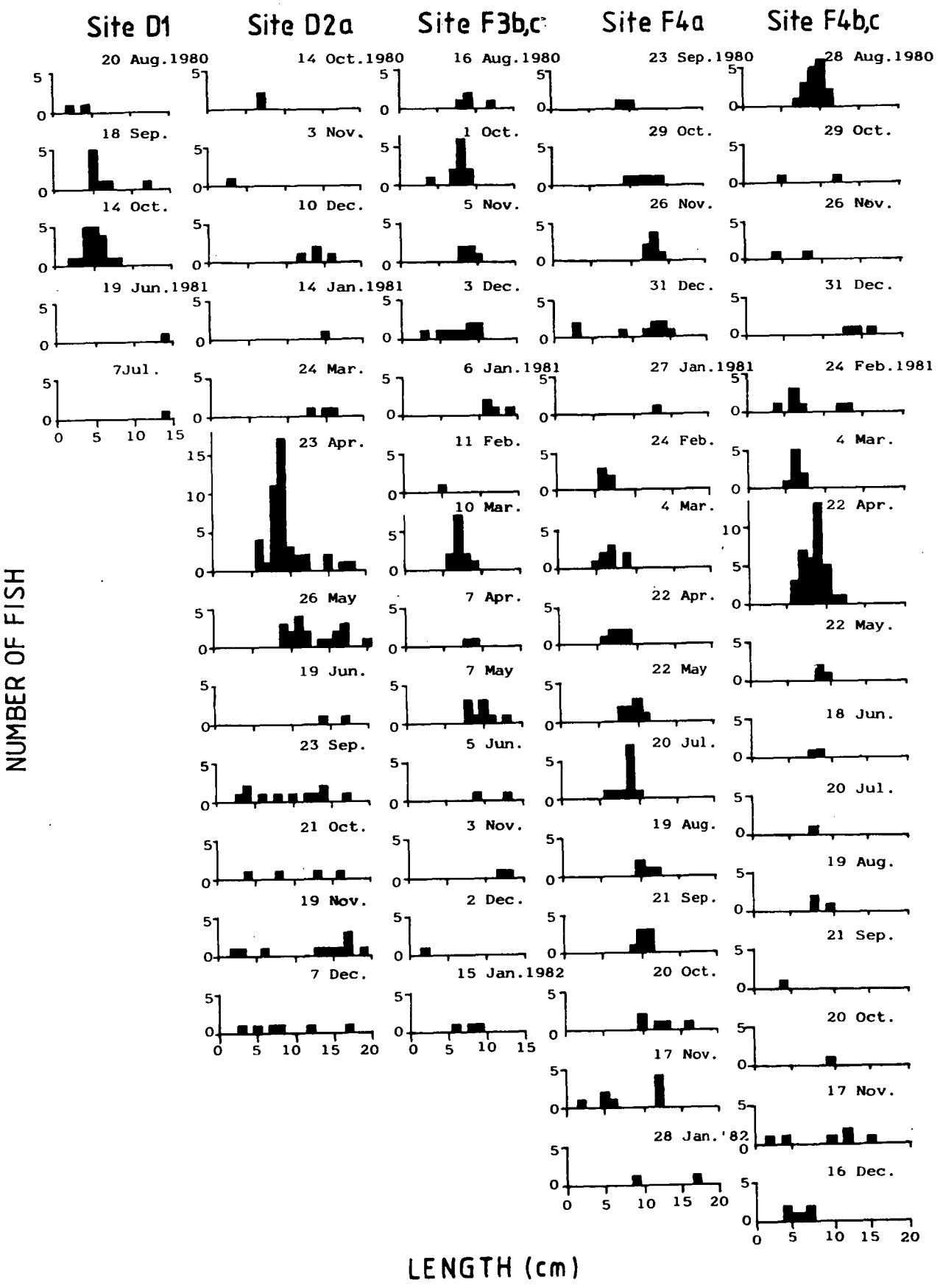

Site D1
Site D2a
Site F3b,c
Site F4a
Site F4b,c
NUMBER OF FISH
LENGTH (cm)
20 Aug.1980
18 Sep.
14 Oct.
19 Jun.1981
7 Dec.
24 Mar.1981
23 Apr
26 May.
23 Sep.
19 Nov.
16 Aug.1980
1 Oct.
5 Nov.
3 Dec.
6 Jan.1981
11 Feb.
10 Mar.
7 Apr.
7 May
5 Jun.
3 Jul.
3 Nov.
2 Dec.
15 Jan.1982
23 Sep.1980
29 Oct.
26 Nov.
31 Dec.
27 Jan.1981
24 Feb.
4 Mar.
22 Apr.
22 May
20 Jul.
19 Aug.
21 Sep.
20 Oct.
17 Nov.
16 Dec.
28 Jan.1982
28 Aug.1980
23 Sep.
29 Oct.
26 Nov.
31 Dec.
27 Jan.1981
24 Feb.
4 Mar.
22 Apr.
22 May
18 Jun.
20 Jul.
19 Aug.
21 Sep.
20 Oct.
17 Nov.
16 Dec.
28 Jan.1982

FIGURE 2.14 Length frequency histograms of *A. rostratus* juveniles caught in the beam trawl in 1.5-4.0 m depth at sites D1, D2a, F3b,c F4a and F4b,c from August 1980 to January 1982


Site D1
Site D2a
Site F3b,c
Site F4a
Site F4b,c
20 Aug.1980
14 Oct.1980
16 Aug.1980
23 Sep.1980
28 Aug.1980
18 Sep.
3 Nov.
1 Oct.
29 Oct.
29 Oct.
14 Oct.
10 Dec.
5 Nov.
26 Nov.
26 Nov.
19 Jun.1981
14 Jan.1981
3 Dec.
31 Dec.
31 Dec.
7Jul.
24 Mar.
6 Jan.1981
27 Jan.1981
24 Feb.1981
23 Apr.
11 Feb.
24 Feb.
4 Mar.
10 Mar.
4 Mar.
22 Apr.
26 May
7 Apr.
22 Apr.
22 May.
19 Jun.
7 May
22 May
18 Jun.
23 Sep.
5 Jun.
20 Jul.
20 Jul.
21 Oct.
3 Nov.
19 Aug.
19 Aug.
19 Nov.
2 Dec.
21 Sep.
21 Sep.
7 Dec.
15 Jan.1982
20 Oct.
20 Oct.
17 Nov.
17 Nov.
28 Jan.'82
16 Dec.
NUMBER OF FISH
LENGTH (cm)

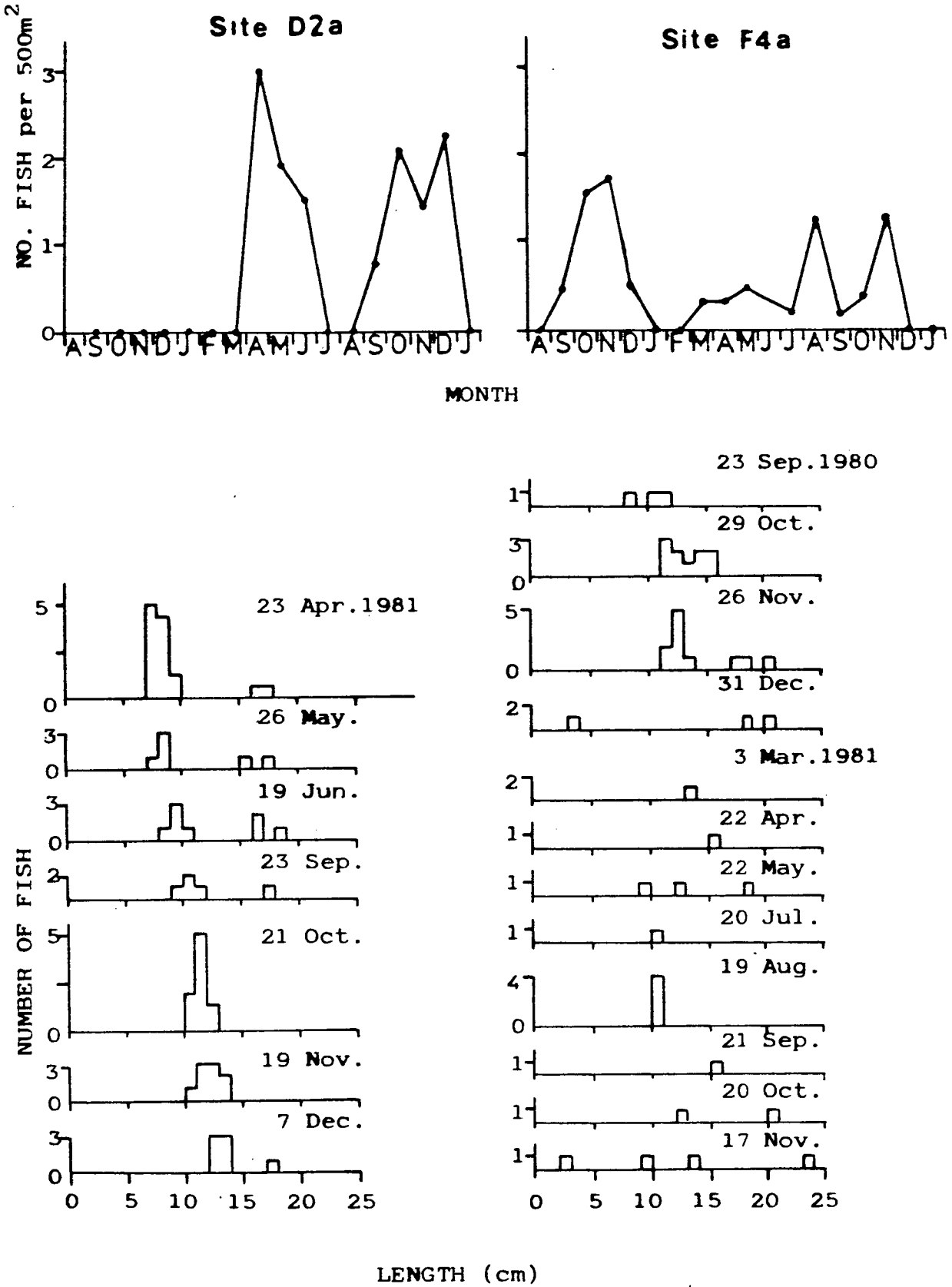


FIGURE 2.15 Monthly abundances and length frequency histograms of *A. lituratus* juveniles caught in the beam trawl in 1.5-4 m depth at sites D2a and F4a from August 1980 to January 1982

2.4 DISCUSSION

The abundances of *R. tapirina* larvae in the plankton were low compared with the numbers of newly-metamorphosed juveniles caught in shallow water. The larvae were possibly widespread over the area or the samples taken may not have been representative of the region because of patchy distributions of larvae. Patchiness of larval fish distributions frequently occur (Steele, 1976). Larvae occurred in the plankton from May to November which indicates that spawning occurs from late autumn to spring. At surface water temperatures of 8.5 - 16.5°C during these months, the time from hatching to metamorphosis and movement inshore would be 2-3 months (Chapter 6). This agrees with the high densities of juveniles found inshore from late winter to early summer. In Otago Harbour, New Zealand, *R. tapirina* larvae were abundant in the plankton for a shorter period of time and later in the year, August to November. Peak abundances of juveniles inshore also occurred later in summer (Roper, 1979). As both larvae and juveniles were collected using nets of larger mesh size than in the present study, only well-developed larvae and juveniles of greater mean length were caught. Peaks in abundance would thus be expected to occur later. Also the mean monthly water temperatures at the New Zealand site ranged between 6.4 and 16.0°C; it is probable that spawning is delayed until the water temperatures increase in spring.

The higher numbers of *R. tapirina* larvae caught at the entrances to the Derwent Estuary and Frederick Henry Bay and their generally smaller size than at other sites in each area suggests that spawning occurs in deeper, coastal waters. Roper (1979) also suggested that most juvenile *R. tapirina* originated from off-shore waters. The very low numbers of *A. rostratus* larvae in the plankton implies that either the larvae do not move through these areas to the nursery grounds or they occur too low in the water column to be sampled by the net.

The larvae of both *R. tapirina* and *A. rostratus* appear to settle in water deeper than 100 cm and then move into shore along the bottom. Abundances were highest at 100 cm depth in months of peak recruitment. The numbers of metamorphosing larvae were also greatest at this depth. Turbot larvae, *Scophthalmus maximus* (Jones, 1973a) and plaice larvae, *Pleuronectes platessa* (Gibson, 1973; Lockwood, 1974b) have also been

observed to settle and metamorphose in deeper water before moving inshore. Gibson (1973) suggests that newly-metamorphosed plaice actively select for a particular depth, or factors associated with depth, according to their length; *R. tapirina* and *A. rostratus* juveniles may also behave in this way.

Juveniles of the two species were sympatric in shallow water at the four sites. However, *R. tapirina* were obviously more abundant than *A. rostratus* at all sites. The abundances of juveniles inshore depends on several factors including numbers of spawning adults, their fecundity, survival of eggs and larvae and the number of larvae which find suitable nursery areas. As fewer *A. rostratus* adults are caught locally (unpublished data, Tasmanian Fisheries Development Authority), the lower densities of *A. rostratus* juveniles than *R. tapirina* probably reflects the smaller size of the spawning stock.

Differences in abundances amongst sites of *R. tapirina* juveniles inshore were not independent of differences between seasons. Seasonal densities at the Derwent River sites apparently differed from those at the Frederick Henry Bay sites. *A. rostratus* juveniles, however, were more abundant at the Derwent River than Frederick Henry Bay sites, over all seasons. There are several possible explanations for these differences in abundances between sites for *A. rostratus* and between sites and seasons for *R. tapirina* including proximity of sites to spawning grounds and/or favourable water movements in the area, or selection by juveniles for particular environmental variables. Abundances of *R. tapirina* may have differed because current flows to, or environmental parameters of, the Derwent River sites were more favourable in November 1981 - January 1982 than in the same months of 1980-81, but did not differ between the two years at the Frederick Henry Bay sites. It is also possible that recruitment to the two areas originated from different spawning stocks. Differences in environmental parameters at the four sites and habitat selection by *A. rostratus* are probably important in determining their distributions, as discussed later. However, the lower abundances of *A. rostratus* at the Frederick Henry Bay sites may be partly due to sampling on the large sandflats rather than at the entrances of the inlet and the lagoon.

It is difficult to compare densities of *R. tapirina* and *A. rostratus* with those of juveniles of other species of flatfish because of the different sampling methods and gear used. Also, many authors do not give all the information required to determine numbers of fish per unit area. However, rough comparisons can be made with 0-group plaice which were caught in 2 m or 4 m beam trawls with mesh of similar size to that used in the present study. To enable comparisons, densities per unit area are used. The maximum monthly densities over 0-1 m depth at the four sites ranged from 0.11 to 1.38 fish per m^2 for *R. tapirina* and 0.02 to 0.16 fish m^{-2} for *A. rostratus*. The maximum mean density of *R. tapirina* over all sites was 0.51 fish m^{-2} and of *A. rostratus* 0.06 fish m^{-2}. These densities are not corrected for gear efficiency and are almost certainly underestimated as the smallest juveniles were observed to escape through the mesh.

Kuipers (1977) sampled the plaice population in the western Wadden Sea by a series of tows at four sites. After correcting for gear efficiency, he estimated a maximum density of 0.5 0-group plaice per m^2 at any one site and 0.22 fish m^{-2} for all sites. In Britain population densities of 0-group plaice were estimated from the average number of fish caught in several tows made from the shoreline to the outer limit of the area occupied by plaice. These densities were corrected for gear efficiency. The maximum mean population densities of 0-group plaice observed by Lockwood (1981) were 0.02, 1.17, 0.27 and 0.15 fish per m^2 for the years 1968-69 and 1972-73, and by Steele and Edwards (1970) 0.72, 0.33, 0.29 and 0.15 fish per m^2 for 1965-68.

Thus, the uncorrected densities of *R. tapirina* observed in this study were within the range of densities (corrected for gear efficiency) of 0-group plaice whereas the uncorrected densities of *A. rostratus* were generally lower than those of plaice. It would not be appropriate, however, to extrapolate the efficiencies of the nets used to catch plaice to the net used in the present study because of differences in the biology of plaice and flounder. Also plaice net efficiencies varied between studies from 15 to 100% (Kuipers, 1975a).

Major recruitment and highest abundances of *R. tapirina* and *A. rostratus* juveniles at each site generally occurred at the same time of year, from late winter to early summer. Few newly-metamorphosed

juveniles of either species were caught in other months except for a large recruitment of *A. rostratus* in April at site D1. There is thus no evidence for temporal habitat partitioning by juveniles of the two species.

Evidence for partitioning the spatial resources of the habitat, however, was found from abundances of the two species at different stations of the estuarine lagoon and the marine inlet, and from densities at different depths. *A. rostratus* were most abundant at the mouth of the lagoon and towards the entrance of the marine inlet whereas densities of *R. tapirina* were highest on the large sheltered sandflats. The higher abundance of *A. rostratus* juveniles at the mouths of estuaries was also evident in the Derwent River. They were most abundant at site D2b which is 9 km closer to the mouth than site D1. Although depth distributions varied during the year, overall *A. rostratus* juveniles were most abundant in 50-110 cm depth whilst *R. tapirina* were widespread over 0-100 cm depth. The absence of *A. rostratus* on the extensive shallow sandflats of station F4c may be partly due to a preference for deeper water. Over 24 h both species were caught more frequently at the shallowest depth (10-30 cm) than in the monthly samples. *R. tapirina*, however, were relatively more abundant at this depth for a longer period of time than *A. rostratus*. They also occurred less frequently at 90-110 cm in comparison to other depths, than *A. rostratus*.

The partitioning of the habitat by the two species on a spatial level is probably linked with preferences for substrate type, particularly by *A. rostratus*. At the Derwent River sites where *A. rostratus* were most abundant, the percentage of medium-sized sand grains was considerably higher than at the Frederick Henry Bay sites. Also, within the estuarine lagoon, the percentage of very fine sand and silt was least at the mouth.

Ranges in temperature and salinity did not, however, appear to be important in determining the distributions of the two species. They were both tolerant of widely fluctuating temperature and salinity regimes, in particular at site D2b.

A. lituratus juveniles were caught in low numbers on semi-exposed beaches only. They were thus separated spatially from the major populations

of *R. tapirina* and *A. rostratus*. Also, as no newly-metamorphosed juveniles of *A. lituratus* were caught, they must occupy a different habitat to those of *R. tapirina* and *A. rostratus*.

The distributions of the three species observed in this study generally agree with the results of Last (1983). He found that *A. lituratus* occurred on semi-exposed and exposed beaches only whilst *A. rostratus* and *R. tapirina* occupied a wide range of habitat types. *A. rostratus* were most abundant at the mouths of estuaries and *R. tapirina* on sheltered sandflats. However, he also observed that *R. tapirina* occurred frequently at the mouths of estuaries.

Distributions and abundances of juveniles of other flatfish in shallow coastal waters have also been found to be influenced by environmental factors. For example, Gibson (1973) and Riley *et al.* (1981) found that when several species of flatfish occurred together, each species occupied a distinct depth zone thus reducing overlaps in distribution. They suggested that the depth distribution is selected and maintained by each species. In New Zealand, *R. tapirina* and *P. latus* also showed differences in depth distribution (Roper, 1979). These differences in preferred depth probably reduce competition for food. Gibson (1973) and Riley *et al.* (1981) also suggest that depth distribution is linked with other environmental factors such as preferred temperature, salinity, turbulence level or substrate type.

After the peaks in abundance of 0-group *A. rostratus* and *R. tapirina* in shallow water, the monthly rates of decline in numbers of the two species were similar to those recorded for other species of flatfish. Plaice (*Pleuronectes platessa*) juveniles decreased at a rate of 30-50% per month (Riley and Corlett, 1966; Macer, 1967; Edwards and Steele, 1968; Kuipers, 1977), turbot (*Scophthalmus maximus*) at 52% (Jones, 1973a), dabs (*Limanda limanda*) at 44% (Macer, 1967), *R. tapirina* at 37-48% and *R. plebia* at 35-48% (Roper, 1979). However, in years of large settlements of plaice inshore, the rates of decrease were much higher (Steele and Edwards, 1970; Lockwood, 1981).

No direct evidence of predation by other fish on juvenile flounder was obtained. However, this is possibly a major cause of the decline in numbers. Metamorphosing flounder have been found seasonally in

large quantities in the guts of salmon *Arripus trutta* and hardyheads, *Atherinosoma presbyteroides* and *A. microstoma* (P. Last, personal communication). The former two species occur in high numbers at site D1 (Last, 1983) and to a lesser extent at the other sites. The flathead *Platycephalus bassensis* has also been observed to consume small quantities of *R. tapirina* (Brown, 1977). Riley and Corlett (1966), Macer (1967) and Edwards and Steele (1968) attributed predation by other fish on O-group plaice as a major cause of mortality. The amount of predation on juvenile flounder by shore birds is unknown although local fishermen have reported that shags, *Phalacrocorax* spp. feed on flounder in the shallows. Serventy *et al.* (1971) list fish as the major food of several species of shags, but do not specify flounder. In New Zealand, juvenile flounder are a major component of the diet of shags (Roper, 1979).

Movements of juvenile flounder into deeper water may also be partially responsible for the decline in numbers. Emigration from the nursery ground must occur as adults of both species are caught in deeper, coastal waters. However, the age at which emigration occurs is not known, although juveniles of both species were caught in Danish-seine nets at depths of 5-10 m. Roper (1979) suggested that most *R. tapirina* emigrate to deeper offshore waters near the end of their first year. Adult *R. tapirina* and *A. rostratus* are also found in shallow water; they possibly returned at a later stage or are a residual population that did not emigrate. An increase in preferred depth with increase in age has been observed in other species of flatfish, e.g. plaice and dabs (Gibson, 1973; Lockwood, 1974b), turbot (Jones, 1973a) and English sole, *Paraphrys vetulus* (Toole, 1980). These movements may be to avoid low sea temperatures inshore in winter (Gibson, 1973; Jones, 1973a) or a change to different feeding grounds (Gibson, 1973; Lockwood, 1974b).

However, the low abundances of larger *R. tapirina* and *A. rostratus* juveniles in deeper water on semi-exposed beaches or in the channels adjacent to push-net sampling sites implies that these areas do not support large populations of juvenile flounder. *R. tapirina* juveniles were most abundant at the Frederick Henry Bay sites; these areas adjacent to large sandflats may provide a more favourable environment than at the Derwent River sites. Seasonal trends in abundances of both species in deeper water were not obvious except for possibly a peak in autumn.

This suggests that juveniles may move into deeper water in winter to avoid low temperatures inshore. The seasonal changes in densities of juveniles in deeper water thus differed to those in the intertidal region. The higher numbers of *A. rostratus* relative to *R. tapirina*, caught in the beam trawl than in the push-net indicates that either the areas sampled were more suited to *A. rostratus* or that the emigration and/or mortality rates of *R. tapirina* were higher.

In summary, although juvenile *R. tapirina* and *A. rostratus* are widely distributed from semi-exposed beaches to upper reaches of estuaries, they appear to partially partition the habitat on a spatial level but not temporally. *A. lituratus* juveniles, which only occurred on semi-exposed beaches, were separated spatially from the major populations of the other two species.

CHAPTER 3

ABIOTIC FACTORS INFLUENCING HABITAT SELECTION AND DISTRIBUTIONS OF LARVAL AND JUVENILE FLOUNDER

3.1 INTRODUCTION

Field studies on the distribution of planktonic larvae and the reproductive biology of adult *R. tapirina* and *A. rostratus* suggested that the major spawning grounds of both species are in deeper, coastal waters (Chapters 2 and 5). After passing through planktonic embyronic and larval stages, newly-metamorphosed juveniles of the two species occur together in high densities on estuarine sandflats during late winter to early summer. Although both species are widespread over the sandflats, differences in distributions have been observed (Chapter 2).

These field results prompted certain questions. How do larvae move to and select estuarine sandflats as nursery grounds? Which environmental parameters are important in determining the distributions of the two species on the nursery grounds?

Previous workers have suggested that flatfish larvae congregate at the nursery grounds by utilizing preferences for particular environmental conditions, such as shallow water (Gibson, 1973; Lockwood, 1974b; Toole, 1980; Riley *et al.*, 1981), optimal feeding conditions (Lockwood, 1974b; Creutzberg *et al.*, 1978) or low salinities (Tsuruta, 1978; Riley *et al.*, 1981). Larvae generally depend on water movements rather than active swimming for transportation to these grounds (see Ketchen, 1956; Simpson, 1959; Smith, 1973; Cushing, 1975; Markle, 1975; Skud, 1977). Within nursery areas, juvenile flatfish have been found to respond to a wide range of interacting environmental factors, including depth, substrate type, degree of exposure, tidal scour, temperature and salinity (Gibson, 1973; Roper, 1979; Riley *et al.*, 1981; Burchmore, 1982). These findings have usually been based on field studies.

In order to determine which abiotic factors influence habitat selection and distributions of larval and juvenile *R. tapirina* and

A. rostratus, a series of laboratory experiments was conducted. The factors considered to be of potential importance, and therefore investigated in this study, included current velocity, light and position in the water column because *R. tapirina* larvae were most abundant and *A. rostratus* larvae rarely caught amongst surface water plankton. Substrate, temperature and salinity preferences were also examined because the two flounder species were most abundant in different areas of the estuary and at different depths, which correspond with gradual changes in these conditions.

3.2 METHODS

3.2.1 Experimental Animals and Experimental Conditions

Wild 0-group *R. tapirina* and *A. rostratus* of length 1.2 - 3.0 cm were caught in the push-net at sites D1 and D2b (Chapter 2) from September 1982 to January 1983. They were kept in holding tanks at 13°C temperature, 32-36‰ salinity and 12 h light:12 h dark for 3-14 days before experimentation. Juveniles used more than once were left in the holding tanks for at least three days between experiments. Cultured larvae and juveniles of both species were obtained from rearing experiments detailed in Chapter 6. They were maintained in static seawater until after metamorphosis at either ambient room or seawater temperature, salinity 32.6 - 34.5‰ and a natural light regime. The developmental stages of larvae are described in Chapter 6. In the present Chapter, stage 5-M refers to larvae which have settled on the bottom and are undergoing metamorphosis and those which are less than five days post-metamorphosis.

All experiments were conducted separately for each species and were repeated at least once. Environmental conditions, other than those being examined, were kept constant throughout each experiment. When necessary, fish were first acclimatised to the experimental temperature over at least 4 h.

3.2.2 Current Velocity

The effect of water current velocity on the behaviour of larvae of different developmental stages was examined using an apparatus similar to

that of Bishai (1960). This consisted of an experimental tube, 100 cm long and 2.5 cm in diameter, with T-pieces at either end. One branch of each T-piece received inflowing water from a constant-level header tank and the other was connected to an overflow tube. The direction of water flow was controlled by stopcocks on the inflow and outflow tubes and could be reversed when required. The flow rate in the experimental tube was altered by opening or closing a stopcock positioned below the header tank.

The mean current velocity ($\bar{V}$ cm sec^{-1}) in the tube was measured using the equation

$$\bar{V} = \frac{q}{\pi r^2}$$

where q is the volume of water passing along the tube in one second and r is the radius of the tube. The velocity (V_1 cm sec^{-1}) at a point b from the centre of the tube was calculated using

$$V_1 = \frac{2q}{\pi}\left[\frac{1}{r^2} - \left(\frac{b}{r^2}\right)^2\right] \quad \text{(Ryland, 1963)}$$

Larvae were added individually to the experimental tube and left for 15 min to acclimatize to the experimental conditions before being exposed to a flow of current. The behaviour of larvae at each current velocity was recorded for 15 min or for 5 traverses of the experimental tube if they were displaced along the tube. The current velocity was increased in stages until the larvae were rapidly swept away. The reactions of from five to ten larvae of each developmental stage to different current velocities were observed. Occasionally larvae did not react to the current and were swept away; these results were discarded.

3.2.3 Light

The responses of larvae of different stages of development to light were investigated. Between 20 and 30 larvae of each developmental stage were placed in a vertically-mounted glass tube filled with seawater. The tube was 150 cm long, 2.4 cm in diameter and was marked off into 30 sections. The positions of larvae in the tube were recorded every 30 min for 2 h when a light source was directed at the top of the tube, or the bottom, or in total darkness. Larvae in total darkness were observed quickly using a dull light. The light source was a 25 W light bulb and the

light intensity, measured using a Licor Quantum/Radiometer/Photometer, ranged from 67-0 μE $m^{-2}sec^{-1}$ from the top to the bottom of the tube when the light was at the top, and 0.1-59 μE $m^{-2}sec^{-1}$ when the light was at the bottom.

3.2.4 Salinity

The preferred salinities of metamorphosing and metamorphosed juveniles of both species were examined using a salinity gradient apparatus modified after that of Hansen (1972). This apparatus, which is described in Appendix 5, regularly provided salinity gradients of 0-33‰. The observation chamber contained 1.7 cm depth of water and was marked off into 12 equal sections. Between 20 and 30 fish were placed in the middle sections and their positions and corresponding salinities were monitored at 30 min intervals for 4 h. Control experiments with seawater entering both inlet reservoirs were conducted.

3.2.5 Substrate

Preferred substrate types of wild *R. tapirina* and *A. rostratus* juveniles were investigated by monitoring their positions in a choice situation of four substrates. The floor of each quarter (96 x 73 mm) of a 20 ℓ tank was covered with sand of different particle sizes to a depth of 10 mm. The particle sizes of sand in different quarters were

2.0 - 0.5 mm	coarse sand
0.5 - 0.25 mm	medium sand
0.25 - 0.125 mm	fine sand
<0.125 mm	very find sand and silt.

The tank contained aerated seawater of depth 60 - 80 cm and was illuminated from above by dull red light. Between 20 and 36 juveniles, fed prior to each experiment, were evenly distributed over the four substrates. The number of fish on each substrate type was recorded at approximately 6 h intervals for 48 h. Each experiment was repeated with the four substrates rearranged so that different substrates were diagonally opposed. Control experiments were conducted with sand of particle size 0.5 - 0.125 mm evenly distributed over the floor of the tank.

3.2.6 Temperature

Temperature preferences of wild-caught juveniles were investigated by observing their positions in a temperature gradient. This gradient was set up in a galvanized-iron experimental chamber (length 120 cm, width 15.5 cm) by circulating heated or cooled water through reservoirs at each end of the chamber. Glass baffles across the chamber and 2 cm above the bottom divided it into 12 sections and reduced the vertical temperature gradient. The chamber contained aerated seawater of salinity 34‰ to a depth of 3-4 cm. The temperature of each section close to the bottom was measured to 0.1°C with a mercury thermometer. A temperature difference of 7-10°C was regularly obtained between the two ends of the gradient and this was maintained for up to 24 h. Maximum temperature variation in each section of the chamber during an experiment was 2-3°C. At the commencement of each experiment 20 fish were placed in the section with temperature closest to that of the holding tanks (13°C). The fish were left overnight (8-12 h) to gravitate towards their final preferred temperature (Fry, 1947). During the next day their positions in the gradient and corresponding temperatures were recorded every 0.5 - 1 h for up to 8 h. Control experiments were conducted under the same conditions except that the heating and cooling units were not operating; the temperature of the experimental chamber was uniformly 14°C.

3.2.7 Statistical Analysis

The results of replicate experiments on distributions of fish in salinity, temperature and substrate preference tests were pooled and relative frequency distributions were determined. The null hypothesis that the absolute frequency distribution of experimental fish was independent of the distribution of control fish was tested using Chi-square analysis. To avoid bias in the Chi-square value, due to low numbers in some sections of the temperature or salinity gradient, the total number of observations in sections 1-3, 4-6, 7-9 and 10-12 of the gradient were pooled before computing the Chi-square value.

3.3 RESULTS

3.3.1 Current Velocity

Current velocities in the experimental tube were much higher at the centre than near the sides. For example, at a mean current velocity of 2 cm sec^{-1}, the velocity was 4 cm sec^{-1} at the centre and 0.61 cm sec^{-1} at 1 mm from the sides. Larvae of both species spent much of their time near the sides of the tube. They swam against the current for only a few seconds at a time, regardless of flow rate. At the lower current velocities against which larvae could swim in the centre, the fish generally alternated between remaining stationary near the sides and short bursts of active swimming followed by drifting passively with the current. In this way they either maintained their position, or were slowly displaced along the tube. As the current velocity increased stronger swimming was required to move against the current, and the fish spent less time stationary near the sides and were more readily displaced along the tube.

The swimming ability of each developmental stage of larvae was examined by measuring the current velocity which larvae could just swim against in short bursts of activity. As larvae usually swam against the current in between the sides and the centre, the mean current velocity ($\bar{V}$) was used. The maximum current velocity at which larvae could remain stationary near the sides of the tube was also recorded. This velocity (V_1) was calculated at 1 mm from the side of the tube.

Average current velocities $\bar{V}$ and V_1 withstood by larvae of both species increased with development of larvae (Table 3.1). They were not significantly different (t-test, $P>0.05$) between *R. tapirina* and *A. rostratus* larvae at similar stages of development before metamorphosis. Metamorphosing and metamorphosed *A. rostratus* larvae, however, could swim against significantly stronger current velocities than *R. tapirina* larvae. Displaced *A. rostratus* larvae were able to re-establish contact with the sides of the tube more readily and remain stationary at higher current velocities than *R. tapirina*.

TABLE 3.1 Mean current velocity ($\bar{V}$) which larvae could just move against in short bursts of rapid swimming and maximum current velocity (V_1) at which larvae could remain stationary near the side of the tube. V_1 was calculated at 1 mm from the side of the tube. Current velocities are averaged for 4-8 larvae at each stage of development.

Rhombosolea tapirina			*Ammotretis rostratus*		
Developmental Stage	$\bar{V}$ (cm sec^{-1})	V_1 (cm sec^{-1})	Developmental Stage	$\bar{V}$ (cm sec^{-1})	V_1 (cm sec^{-1})
2a	1.1	0.5			
2b	1.8	0.8	2b-3b	1.5	0.8
3b4b	2.2	1.0	4b	2.3	0.9
5-M	5.2	2.5	5-M	7.4	>2.8

3.3.2 Light

Regardless of the experimental light regime, *R. tapirina* larvae at stage 2a were significantly more abundant in the bottom 25 cm of the tube than the top 25 cm (t-test, $P<0.05$) (Figure 3.1). The numbers at the surface increased at stage 2b and by stage 3b4b were significantly higher at the surface than the bottom (t-test, $P<0.05$). After settling on the bottom (stage 5-M) the number of larvae at the surface decreased, and juveniles metamorphosed for 2-3 weeks were significantly more abundant at the bottom (t-test, $P<0.05$). *A. rostratus* larvae, however, were significantly more abundant at the bottom of the tube than the top at all three developmental stages (t-test, $P<0.05$), irrespective of the light regime employed.

Thus, larvae of both species apparently had a stronger preference for position in the water column than light intensity. The results suggest, however, that stage 2a and 2b *R. tapirina* larvae avoided light whereas stage 5-M larvae appeared to be attracted to it.

3.3.3 Salinity

Cultured, metamorphosing *R. tapirina* were distributed across the salinity gradient. This distribution, however, was significantly different

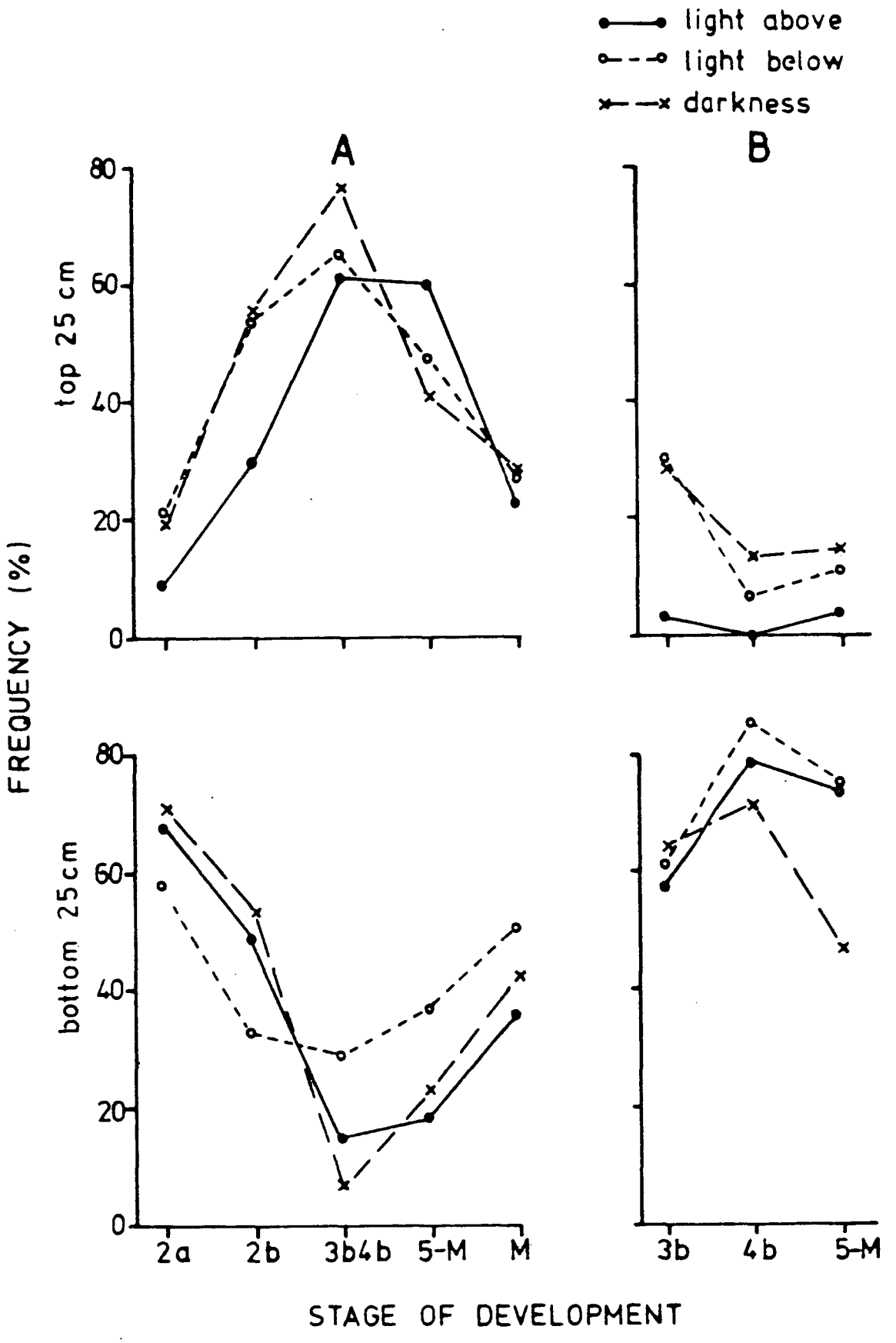


FIGURE 3.1 Relative frequency distribution of *R. tapirina* (A) and *A. rostratus* (B) larvae at different stages of development in the top 25 cm and bottom 25 cm of a vertical column at three light regimes

to that of control fish (χ^2 = 11.71, P<0.01). They were slightly more abundant and differed the most from control fish at 31-32‰ (Figure 3.2a). After 12-14 h from the start of the experiment, the distribution of fish in the gradient was similar to that occurring during the first four hours (χ^2 = 6.50, P>0.05), although more fish were observed at each end of the gradient (Figure 3.2b). Stage 5-M larvae showed a bimodal distribution in the salinity gradient which was significantly different from that of the control fish (χ^2 = 101.95, P<0.001); 30% of the fish were in 0-5‰ and 35% in 32-34‰ (Figure 3.2c). At the end of the experiment, the mean length of fish (9.6 mm) in sections 1-3 of the observation chamber (0-10‰) was significantly greater than the mean length (7.7 mm) in sections 10-12 (30-34‰) (t-test, P<0.001).

Juveniles which had been metamorphosed for approximately 2 weeks and 7 weeks had similar distributions in the salinity gradient (χ^2 = 7.34, P>0.05) and the results were combined. They congregated at the freshwater end of the gradient with 61% of the fish in 0-5‰ (Figure 3.2d); this distribution was still apparent 24-29 h after starting the experiment (χ^2 = 1.51, P>0.05) (Figure 3.2e).

Cultured stage 5-M *A. rostratus* were observed in the salinity gradient most frequently in 19-24‰ with most other fish at salinities >24‰ (Figure 3.3a). This distribution, however, was not significantly different to the control (χ^2 = 7.72, P>0.05). At the end of the experiment, the mean length of fish (11.6 cm) in sections 1-6 of the observation chamber (0-27‰) was significantly greater than the mean length of fish (9.7 mm) in sections 7-12 (25-33‰) (t-test, 0.005<P<0.01). *A. rostratus* juveniles, approximately three weeks after metamorphosis, preferred low salinities with 51% of the fish in 0-6‰; this distribution was significantly different to the control (χ^2 = 153.60, P<0.001) (Figure 3.3b).

Wild-caught *R. tapirina* juveniles rapidly moved towards freshwater when placed in the salinity gradient; 58% of the fish were recorded in 0-2‰ (Figure 3.4a). This distribution was significantly different to that of control fish (χ^2 = 46.85, P<0.001). Similarly, wild *A. rostratus* juveniles were observed most frequently in low salinities; 75% of the fish were in 0-4‰ (Figure 3.4b). However, control fish (1) of Figure 3.4b also showed a strong preference for section 1 of the observation chamber. Water entered the mixing compartment of section 1 at the bottom only but

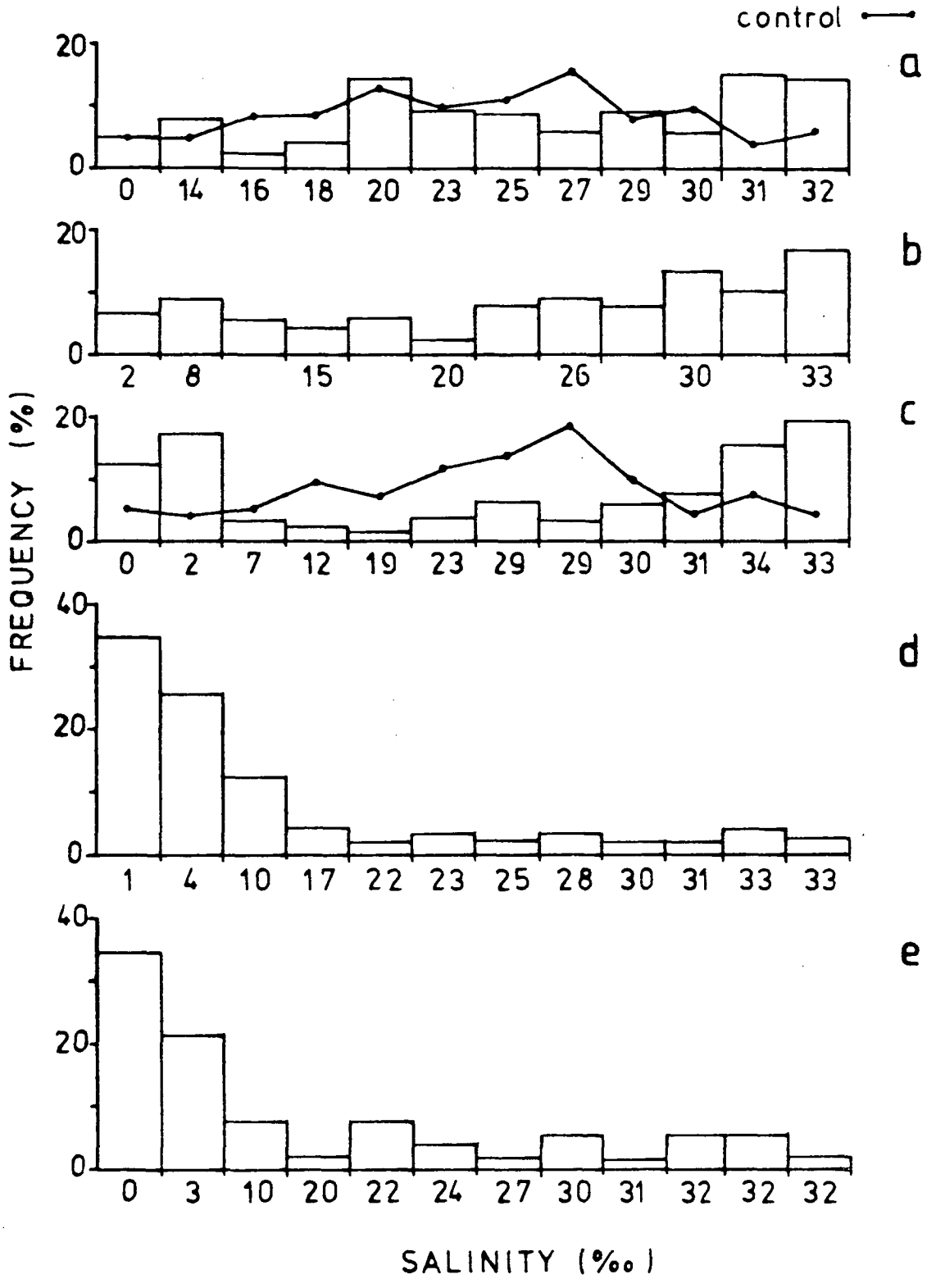


FIGURE 3.2 Relative frequency distribution of cultured *R. tapirina* larvae and juveniles in a salinity gradient
a - larvae at stage 5
b - 12-14 h after starting experiment a
c - larvae at stage 5-M
d - juveniles metamorphosed for approximately 2 and 7 weeks
e - 24-29 h after starting experiment d

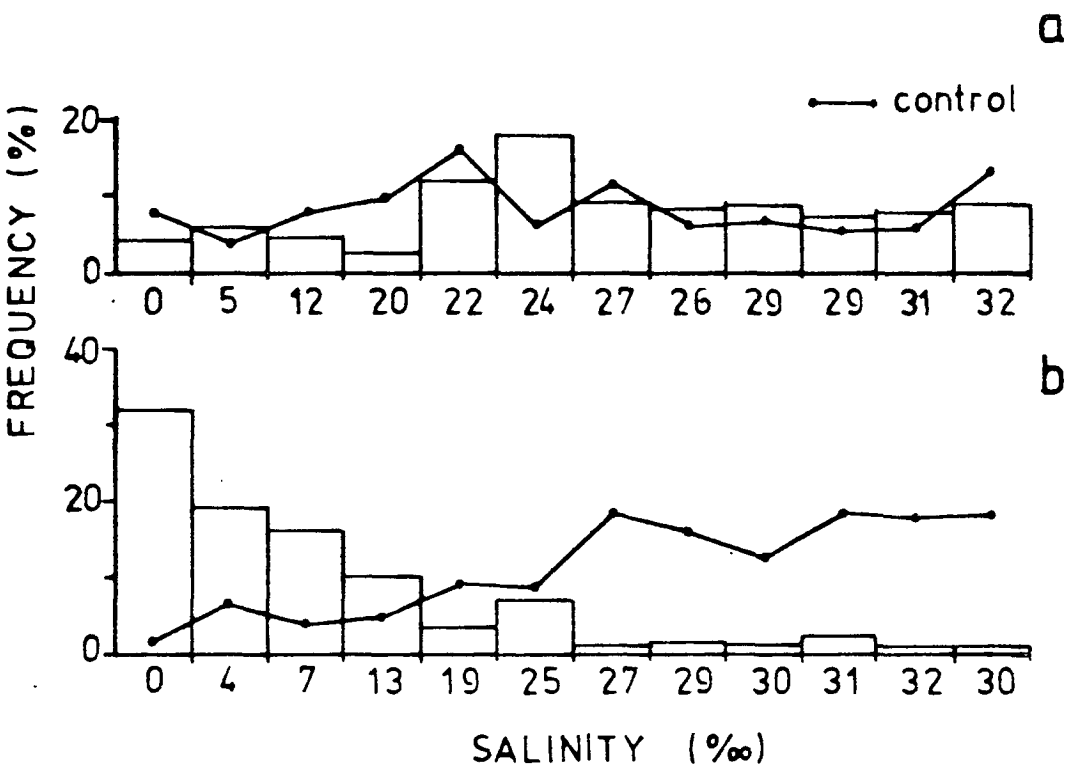


FIGURE 3.3 Relative frequency distribution of cultured *A. rostratus* larvae and juveniles in a salinity gradient
a - larvae at stage 5-M
b - juveniles metamorphosed for approximately 3 weeks

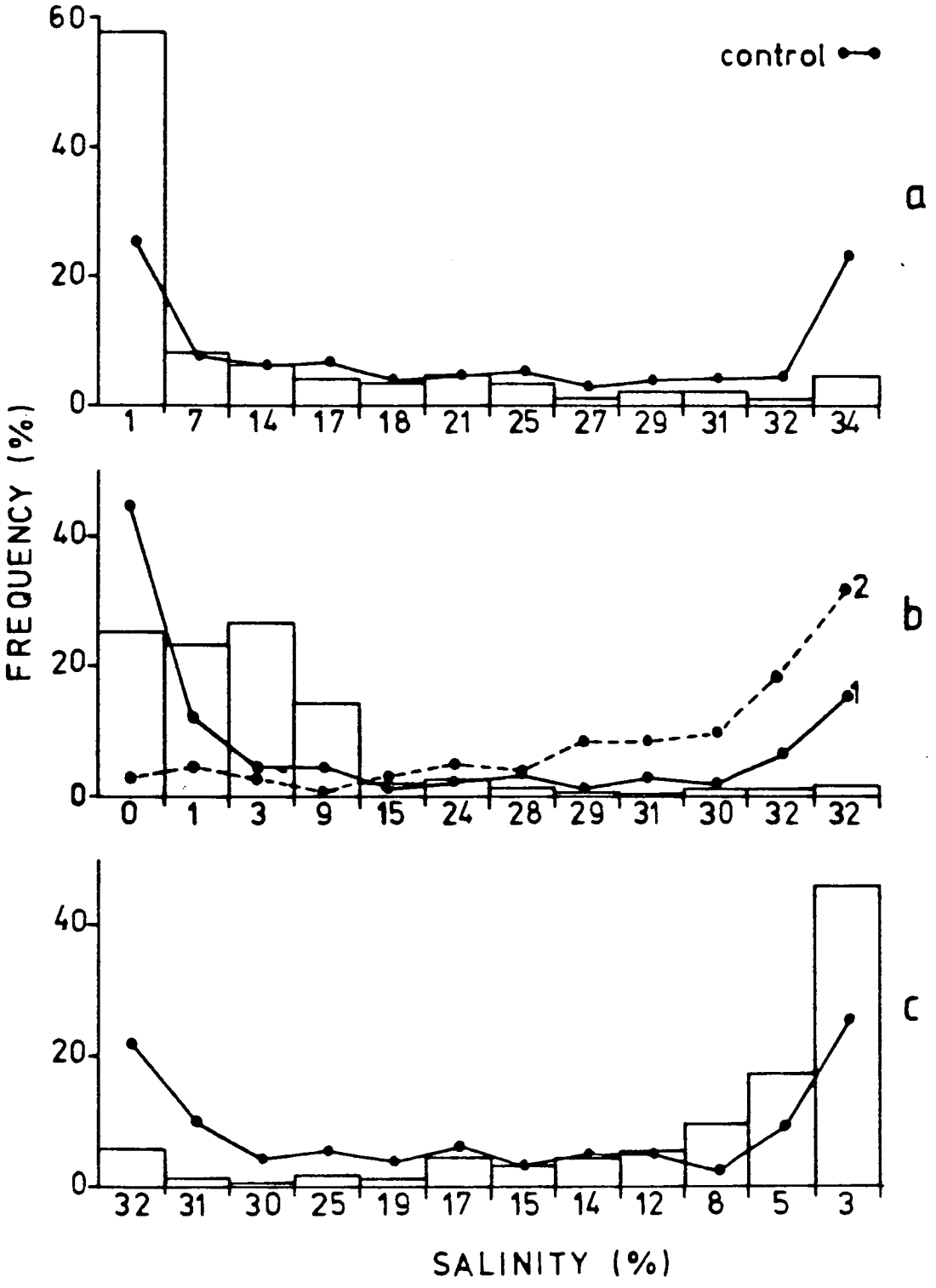


FIGURE 3.4 Relative frequency distribution of wild-caught *R. tapirina* and *A. rostratus* in a salinity gradient
a - *R. tapirina*
b - *A. rostratus*; control 1 ___, control 2 ----
c - *A. rostratus*, gradient reversed
(see text for explanation of b and c)

dropped from a height of 12 cm into the other mixing compartments, thus causing turbulence. Only when the inlet reservoirs were turned around, so that water entered mixing compartment 12 from the bottom only, did fish move from section 1 to section 12 (Figure 3.4b, control 2). Control *A. rostratus* thus appeared to prefer the least turbulent section of the observation chamber. The experiment was repeated with the maximum flow of freshwater dropping from a height, causing turbulence at section 12 and seawater at section 1 with least turbulence. The fish again selected low salinities, with 63% occurring in 0-6‰ (Figure 3.4c). *A. rostratus* juveniles thus have a stronger preference for low salinities than low levels of turbulence. The distribution of control fish was re-examined with the tubing removed; they showed an equal end-of-tank bias and the distribution was significantly different to that of fish in the salinity gradient ($\chi^2 = 60.72$, $P<0.001$; Figure 3.4c).

3.3.4 Substrate

Both wild *R. tapirina* and *A. rostratus* juveniles showed a preference for fine sand of particle size 0.25 - 0.125 mm (Figure 3.5). However, the distributions of the two species on the four substrate types were significantly different ($\chi^2 = 43.40$, $P<0.001$). *R. tapirina* were considerably more abundant on the fine and very fine (<0.125 mm) sand, but less abundant on the coarse sand (2.0 - 0.5 mm), than *A. rostratus*. Also, *R. tapirina* juveniles showed a strong preference for fine sand whereas abundances of *A. rostratus* were similar on coarse, medium and fine sand.

The distributions of both species were significantly different to the controls (*R. tapirina* $\chi^2 = 53.03$, $P<0.001$; *A. rostratus* $\chi^2 = 17.20$, $P<0.001$).

3.3.5 Temperature

Wild-caught *R. tapirina* juveniles in a temperature gradient were most abundant at 11.3 - 15.0°C (56.0% of total observations). Wild *A. rostratus* juveniles, however, preferred higher temperatures with 47% of fish at 18.0 - 20.9°C (Figures 3.6a,3.6b). The distributions of both species in the temperature gradient were significantly different from the controls (*R. tapirina* $\chi^2 = 11.48$, $0.001<P<0.01$; *A. rostratus* $\chi^2 = 29.33$, $P<0.001$).

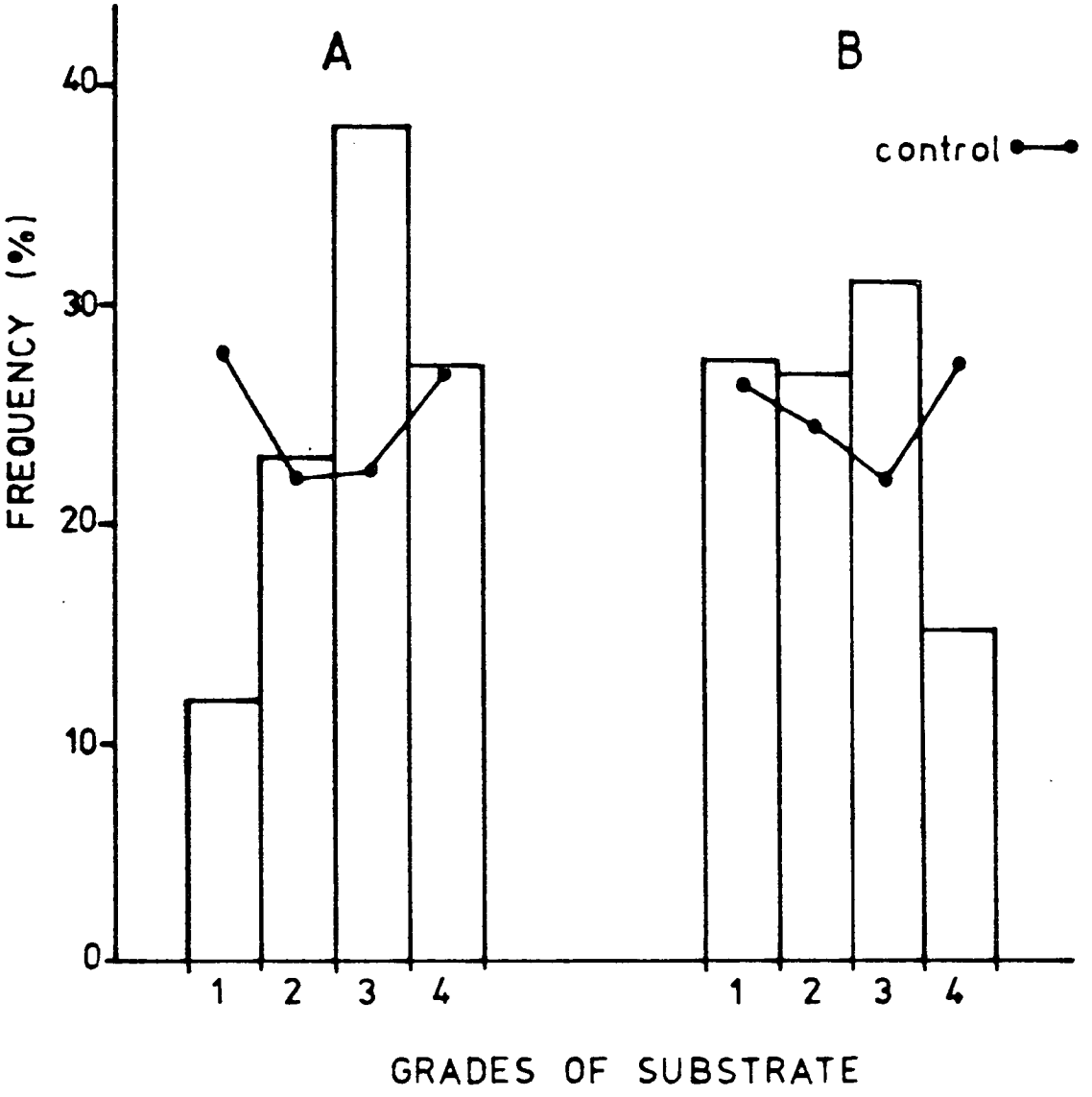


FIGURE 3.5 Relative frequency distribution of *R. tapirina* (A) and *A. rostratus* (B) on four substrate types of particle size:
(1) 2.0 - 0.5 mm
(2) 0.5 - 0.25 mm
(3) 0.25 - 0.125 m
(4) <0.125 mm

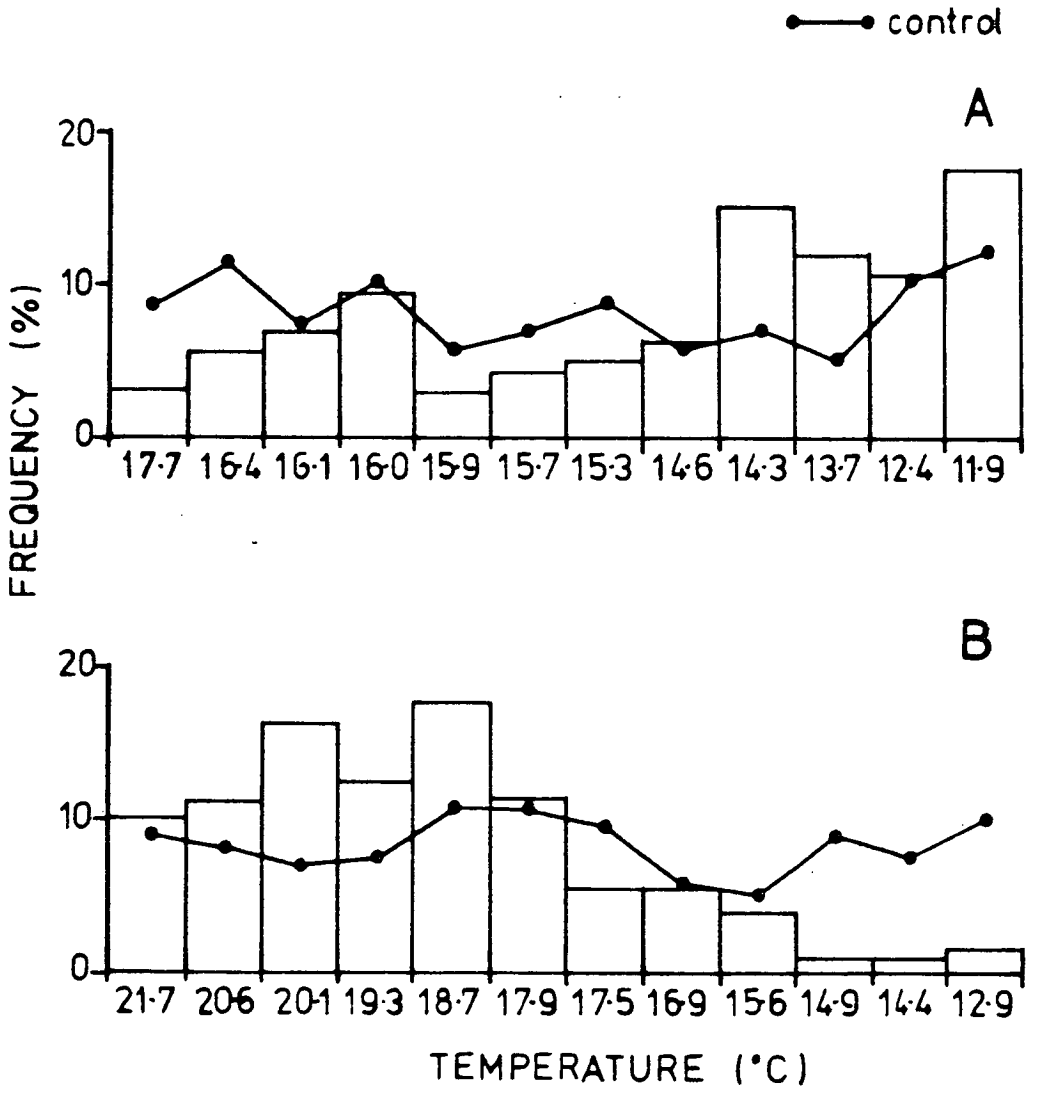


FIGURE 3.6 Relative frequency distribution of *R. tapirina* (A) and *A. rostratus* (B) juveniles in a temperature gradient

3.4 DISCUSSION

The larvae of both *R. tapirina* and *A. rostratus* were observed to be weak swimmers. They mostly drifted passively with the current and only swam against low current velocities in short bursts of activity. Once settled on the bottom they were able to maintain themselves against current velocities which were more than twice those withstood by planktonic larvae. Plaice, *Pleuronectes platessa*, larvae generally showed similar responses to current velocities but were able to swim against stronger currents than the two species in the present study (Arnold, 1969; Ryland, 1963). Plaice larvae, however, are larger in size at all stages of development. The results therefore suggest that *R. tapirina* and *A. rostratus* larvae are mainly dispersed by water currents or wind-induced surface water movements until they settle on the bottom. Other flatfish larvae are also thought to be transported mainly by water movements, e.g. turbot, *Scophthalmus maximus* (Riley *et al.*, 1981), plaice (Rauck, 1974) and lemon sole, *Paraphrys vetulus* (Ketchen, 1956).

The greater ability of *A. rostratus* to withstand higher current velocities than *R. tapirina* after settlement on the bottom is probably related to the larger size of *A. rostratus* at metamorphosis (Chapter 6). This is also in accordance with the greater abundances of *A. rostratus* juveniles than *R. tapirina*, at the mouth of the Pittwater estuary where the current flow was stronger than on the large sheltered sandflats (Chapter 2).

However, the responses to current velocities of metamorphosing larvae and juveniles, which have settled on the bottom, may be different in the natural environment to those in a glass tube. The fish can bury into a sandy substrate or seek protection behind sand ripples. Nevertheless, Arnold (1969) observed that buried 0-group plaice generally did not react to the current until the covering layer of sand had been eroded away. They then orientated upstream which was hydrodynamically advantageous in maintaining station on the sea-bed. As the morphology of *R. tapirina* and of *A. rostratus* are similar to that of plaice, they may behave in a similar manner.

R. tapirina larvae showed an ontogenetic change in their response to position occupied in the water column and possibly to light intensity. Conversely, *A. rostratus* larvae were most abundant at the bottom of the

tube at all developmental stages studied, regardless of light regime. These results suggest that *A. rostratus* larvae were selecting for depth or proximity to the substrate, which might explain their low numbers in the plankton tows (Chapter 2). The larvae of each species were most abundant at different levels of the water column at the three developmental stages studied. This separation of larvae by depth may reduce interspecific competition, particularly for food, in the natural environment. From these results additional experiments are suggested, e.g. monitoring the positions of larvae in a horizontal tube with light at one end, to investigate the responses of larvae to light without the influence of depth. However, only one batch of larvae was cultured in 1982 and further experimentation was not possible.

There was no evidence of vertical movements in response to different light regimes by larvae of either species at any one stage of development. Vertical migration, however, has been observed in *R. tapirina* in the field (Roper, 1979). Other species of flatfish also undergo vertical migration, although the results vary in the time and duration of migration between methods of study and between species. Late larval stages of *R. tapirina, R. plebia* and *Peltorhamphus latus* (Roper, 1979) and the stone flounder, *Kareius bicoloratus* (Tsurata, 1978) were more abundant near the surface at night than during the day. Creutzberg *et al.* (1978) caught higher numbers of late larval stages of plaice during the flood tide than the ebb, regardless of time of day. However, Blaxter (1973) and Gibson *et al.* (1978) found experimentally that plaice larvae moved to the surface at dusk and moved away at dawn in response to changing light intensities. The lack of evidence for vertical migration in the two species of flounder studied may be due to experimental conditions not reflecting the natural situation or because the cultured larvae had not been exposed to natural environmental variables such as tidal movements.

An ontogenetic change in preferred salinities from marine to almost freshwater conditions was observed in both *R. tapirina* and *A. rostratus* during metamorphosis. After metamorphosis they both preferred 0-6‰ salinity. This change in preferred salinities is probably important in drawing larvae into estuaries. However, salinity preferences after metamorphosis probably do not influence habitat partitioning by the two species. Evidence for similar changes in preferred salinities during the larval to juvenile stage of other species of flatfish have been found.

Stone flounder (Tsuruta, 1978) and flounder *Platichthys flesus* (Riley *et al.*, 1981) spawn in the open sea whereas 0-group juveniles are abundant in areas of low salinities in estuaries. Early post-larvae of summer flounder, *Paralichthys lethostigma* had maximum growth rates at 30‰ (Deubler, 1960) and more advanced post-larvae at 5-15‰ (Stickney and White, 1973). Also, plaice yolksac larvae tolerated salinities of 15-60‰ for one week but only 2.5-45‰ after metamorphosis (Holliday and Jones, 1967). They suggest that at metamorphosis, functional changes in the epidermis reduce the high salinity tolerance, and the development of a well-organized kidney increases the ability to survive at low salinities.

Young winter flounder, *Pseudopleuronectes americanus*, were observed in laboratory studies by Frame (1973) to consume 40-50% less oxygen when the salinity decreased from 30‰ to 20‰ or 10‰. This decrease in respiration represented a significant decrease in energy expenditure and apparently resulted from a reduced osmotic load as the external medium approached isotonicity. Frame (1973) suggested that this is a physiological reason why juvenile winter flounder are abundant in estuaries.

After settling on the bottom, the type of substrate available (which is related to the degree of exposure and turbulence) is probably important in determining the distributions of the two species. Although wild-caught fish of both species preferred fine sand, *A. rostratus* were more abundant than *R. tapirina* on coarse and medium sand. By contrast, *R. tapirina* showed a stronger preference for the finest sand. Roper (1979) also found that *R. tapirina* juveniles preferred fine sand and were not common on the coarser substrates. These substrate preferences of the two species agree with their distributions in estuaries (Chapter 2). *A. rostratus* were most abundant at the mouth of the estuary which had a slightly coarser substrate than on the large, sheltered sandflats where *R. tapirina* occurred in the highest numbers. Distributions of other species of flatfish have also been related to substrate types. The distributions of 0-group turbot were linked with shallow depths and exposed beaches of coarse sand by Gibson (1973) and Riley *et al.* (1981). Dover sole, *Solea solea* and plaice occupied nursery grounds which were characterised by shallow water protected from wave and tidal scour and a mud/sand substrate (Riley *et al.*, 1981).

The effect of turbulence on the distributions of juvenile *R. tapirina* and *A. rostratus* was not examined experimentally due to the practical difficulties of establishing a gradient of turbulence and associated tidal scour conditions which simulated the natural situation. However, the results of the salinity gradient experiments suggest that *A. rostratus* have a stronger preference for low turbulent conditions than *R. tapirina*. The highest abundances of *A. rostratus* in deeper water, therefore, may be related to a preference for low turbulent and tidal scour conditions. As mentioned above, the distributions of other species of flatfish have been found to be influenced by levels of turbulence.

In a temperature gradient, the higher preferred temperatures of *A. rostratus* compared to *R. tapirina* are not in accordance with their field distributions. *A. rostratus* were most abundant in deeper, cooler water during spring and summer. This possibly relates more to *A. rostratus* being at the southern end of its distributional range. Even so, Roper (1979) found that the critical thermal maximum for *R. tapirina* was 32.6°C which is higher than any field temperatures recorded during the present study. Thus temperature does not appear to be important in separating the distributions of the two species of flounder although it has been found to be important in other species of fish (e.g. Reynolds, 1977; Magnuson *et al.*, 1979).

These results thus suggest that larvae are dependent on water movements to transport them towards nursery grounds. During metamorphosis ontogenetic changes in preferred salinity, position in the water column in *R. tapirina* and possibly depth (Chapter 2), as well as a preference for fine sand, would play a role in guiding larvae towards settling on shallow estuarine sandflats. Once on the nursery grounds different swimming abilities and preferences for depth (Chapter 2), substrate type and possibly levels of turbulence by each species appear to play a partial role in determining the local distributions and partitioning of the habitat. These results agree with the ideas presented by Moore (1975) that the local distribution of a marine species is determined initially by ecological opportunity followed by behavioural habitat selection in conjunction with habitat availability.

The behavioural and physiological responses of other flatfish larvae and juveniles to environmental factors during immigration onto nursery

grounds are not well understood. As high mortalities occur at this stage, such information is important to the study of fish population dynamics. Tsuruta (1978) suggested from field studies that stone flounder develop the ability to perceive changes in salinity and light intensity, and become strongly dependent on substrate at metamorphosis. This results in the larvae being transported onto the nursery grounds by the night flood tide. Creutzberg *et al.* (1978) found evidence for the immigration of plaice larvae onto tidal sandflats by selective transport in tidal currents; the larvae move with the flood tide and settle on the bottom during the ebb where they are probably retained by favourable feeding conditions. They suggest that larvae move from the open sea to tidal inlets by transport in onshore residual currents along the bottom. Moreover, prolonged periods of pelagic swimming by larvae are probably induced in situations of an inadequate food supply and are reduced when larvae detect food substances transported from inshore waters. It is not known whether *R. tapirina* and *A. rostratus* larvae behave in a similar way but the results suggest that they may also move into estuaries by selective tidal transport. Both species are sensitive to changes in salinity at this time. It is also likely that the newly-metamorphosed juveniles are retained in shallow water by the favourable feeding conditions.

The distributions and abundances of other 0-group flatfish which coexist in shallow, coastal waters have been found to be influenced by many interacting environmental parameters. In particular, the juveniles of numerous species have distinct depth distributions which differ from species to species (Gibson, 1973; Pearcy, 1978; Roper, 1979; Riley *et al.*, 1981). These preferred depths are usually linked with other factors which variously affect distributions, e.g. substrate, degree of exposure, tidal scour, temperature, salinity (Gibson, 1973; Riley *et al.*, 1981). The differences in depth distributions of *R. tapirina* and *P. latus* were also related to differences in temperature and oxygen concentration tolerances of the two species (Roper, 1979). The distributions of two congeneric species of bothid flounders, however, were found by Burchmore (1982) to be governed mainly by substrate, and by Powell and Schwartz (1977) by both substrate and salinity.

CHAPTER 4

FEEDING OF JUVENILE FLATFISH

4.1 INTRODUCTION

The food and feeding habits of juveniles of many northern hemisphere species of flatfish have been studied, often in conjunction with a study of their population dynamics on the nursery grounds; for example, turbot, *Scophthalmus maximus* (Jones, 1973a); plaice, *Pleuronectes platessa* Thijssen *et al.*, 1974; Kuipers, 1975b, 1977); plaice and dabs, *Limanda limanda* (Macer, 1967; Edwards and Steele, 1968) and English sole, *Parophrys vetulus* (Toole, 1980). The feeding of plaice, in particular, has been well documented and, due to their high numbers, they are considered to be important secondary consumers of the intertidal ecosystem (Kuipers, 1977). These studies have shown that juvenile flatfish feed predominantly on benthic invertebrates. Their feeding activity, however, may vary between habitats. De Groot (1971) reviewed the interrelationships between types of food eaten, feeding behaviour, diurnal activity and morphology of the alimentary tract in flatfishes.

Several studies on the morphology and the feeding of flatfishes in Australasia have shown that the morphology of the alimentary tract and diets of *Rhombosolea tapirina* and *Ammotretis rostratus* are similar. They both have asymmetrical jaws, small teeth, moderate number of small gill rakers and an elongate and convoluted intestine (Norman, 1926). Also, neither species has a functional stomach or pyloric caecae (Grove and Campbell, 1979).

The diets of juveniles of the two species have been examined in areas where they do not occur together. Burchmore (1982) found that juvenile *A. rostratus* (<14 cm T.L.) ate predominantly crustaceans (amphipods) and polychaetes in Botany Bay, N.S.W. and *R. tapirina* were observed by Roper (1979) during one day in Otago Harbour, New Zealand, to consume harpacticoids, amphipods and polychaetes. In Great Swanport Bay, Tasmania the two species occurred sympatrically and larger juveniles of both species ate mainly amphipods and polychaetes (Last, 1983).

These findings raise the question of whether co-existing juveniles of *R. tapirina* and *A. rostratus* on the nursery grounds consume the same food organisms or partition the food resources of the habitat. This may be critical during periods of peak recruitment when newly-metamorphosed juveniles of the two species occur together in high densities on estuarine sandflats. The aim of this particular study, therefore, was to investigate the feeding patterns of *R. tapirina* and *A. rostratus* juveniles and to examine for partitioning of the food resources of the habitat. Feeding patterns of *A. lituratus* juveniles also were studied, but in less detail. The diets and feeding activity of newly-metamorphosed *R. tapirina* and *A. rostratus* each season in two areas, and at one site over 24 hours were compared; those of larger 0-group and I-group juveniles, including *A. lituratus*, were studied at one site over 24 hours.

4.2 METHODS

4.2.1 Sampling Procedure

The food organisms eaten by recently-metamorphosed juveniles of *R. tapirina* and *A. rostratus* (T.L. 1-3 cm) were investigated at site D1 (Nutgrove) and site F3b,c (Pittwater). The foregut contents of twenty fish of each species, or less depending on the numbers caught, which were sampled in the push-net at each site (see Chapter 2) in December 1980 (summer), March-April 1981 (autumn), July-August 1981 (winter) and October 1981 (spring) were examined. No small *A. rostratus* were caught during March-May 1981 (autumn) at site F3. The diets and diurnal feeding activity of newly-metamorphosed *R. tapirina* and *A. rostratus* were examined in fish caught every 3 h for 27 h at site D1 in January 1982 (see Chapter 2). The push-net was pushed for 100 m along the beach at three depths 20 - 30 cm, 50 - 70 cm and 90 - 110 cm. The foregut contents of up to ten fish of each species in each depth at every time interval were examined.

The food and feeding habits of larger 0- and I-group *R. tapirina*, *A. rostratus* and *A. lituratus* were studied over 24 h at site F4 (Cremorne) in March 1981. The beam trawl was towed for 15 min every 3 h for 24 h at a depth of 1.2 - 2.4 m at station F4a, a semi-exposed beach, and at

station F4b, a sheltered sandflat in the marine inlet. The foregut contents of ten fish of each species, or less depending on the numbers caught, were examined from each time interval at each station.

4.2.2 Gut Content Analysis

All fish caught were immediately anaesthetised in tricaine methanosulfonate solution to prevent regurgitation of food and then preserved. in 5% V/V formalin. In the laboratory they were weighed and the total length was measured. The contents of the first loop of the foregut were removed and sorted into taxonomic groups under a dissecting microscope. The percentage by volume of each major food category (e.g. amphipods, polychaetes) was estimated by visual inspection to the nearest 5% for dominant food types and to 1% for those which were not abundant. Further identification of prey animals was often difficult as many guts contained partially digested food segments, particularly of amphipods. Where possible, the relative abundances of dominant prey species of each food category were recorded.

After this assessment, the total volume of the foregut contents was determined using a method similar to that of Hellawell and Abel (1971). The foregut contents were squashed to a uniform depth between two glass squares which were placed in a vertically-mounted slide projector. The outline of the squashed gut contents was projected onto graph paper and the area of the squash image was determined by counting the number of squares that it occupied. The relationship between a known volume of water and the area it covered when projected was used to calibrate the volume of the squashed gut contents.

4.2.3 Data Presentation and Analysis

The relative importance of each taxonomic group in the diets of the three species was examined by calculating the percentage volume, i.e. the volume of each taxonomic group expressed as a percentage of the total volume of food in the foreguts. The percentage frequency of occurrence, i.e. the percentage number of foreguts containing each food type to the total number of foreguts containing food,was also calculated. This

measure is useful in characterizing diets but not in determining overlap (Wallace, 1981).

The quantity of food eaten at different times of the day or in different months by fish of different sizes was standardized by expressing the weight of foregut contents as a percentage of fish weight (% S.S.R.), after converting gut volume to weight assuming that the specific gravity of gut contents equalled unity.

Intraspecific and interspecific differences in gut contents were estimated using the Schoener (1970) diet overlap index

$$\alpha = 1 - 0.5\left(\sum_{i=1}^{n} |Pxi - Pyi|\right)$$

where Pxi and Pyi are proportions of food category i in the diet of species x and y, or one species at sites x and y or in seasons x and y, respectively. This index is considered to be an adequate measure of dietary overlap in the absence of resource-availability data (Hulbert, 1978; Wallace, 1981). Percentage volume of food organisms only were used to calculate the index; the percentage frequency of occurrence is not suitable because it is not a proportional measure of diet. Overlap is accepted as being biologically significant when the index is greater than 0.6.

4.3 RESULTS

The food organisms found in the foreguts of the *R. tapirina* and *A. rostratus* juveniles, identified to the lowest possible taxonomic level, are given in Table 4.1. All food types were eaten by both species except for Isopoda by *R. tapirina*, and Leptostraca by *A. rostratus*, only. The organisms eaten by *A. lituratus* juveniles were identified only to the major food category as few fish contained food in their guts and always only small quantities of well digested prey segments.

4.3.1 Seasonal Feeding Patterns of Newly-metamorphosed *R. tapirina* and *A. rostratus*

The percentage volume of food organisms in the foreguts of newly-

TABLE 4.1 Food organisms found in the foreguts of juvenile *R. tapirina* and *A. rostratus*

Amphipoda	
Paracorophium excavatum	(Corophiidae)
Corophium sp.	(Corophiidae)
Limnoporeia yarrague	(Phoxocephalidae)
Metaphoxus tuckatuck	(Phoxocephalidae)
Podoceropsis sp.	(Corophiidae)
Guernia sp.	(Dexaminidae)
Paradexamine sp.	(Dexaminidae)
Exoediceros sp.	(Oedicerotidae)
Primitive Oedicerotidae	(undescribed genera)
Eusiridae	(not identified further)
Other unidentified amphipods	
Harpacticoida	
Dactylopodia sp.?	(Thalestridae)
Polychaeta	
Harmothoe sp.	(Polynoidae)
Phyllodoce sp. 1	(Phyllodocidae)
Phyllodoce sp. 2	(Phyllodocidae)
Eusyllis sp.?	(Syllidae)
Armandia sp.	(Opheliidae)
Other unidentified polychaetes	
Mysida	
Leptomysini?	(Mysinae)
Cumacea	
Cyclaspis caprella	(Bodotriidae)
Cyclaspis australis	(Bodotriidae)
Anchistylis waitei	(Diastylidae)
Leptostraca	(minor occurrence in foreguts,
Calanoida	not identified further)
Ostracoda	
Isopoda	
Nemertea	

metamorphosed juveniles of *R. tapirina* and *A. rostratus* at sites D1 and F3 in different seasons (Figure 4.1) indicates that the diet of each species generally varied seasonally and between sites. Diet overlap indices for each species were significantly different between the two sites in all seasons, except for *A. rostratus* in spring (Table 4.2). Both species consumed a greater quantity of polychaetes and amphipods at site D1 than site F3. Harpacticoids, as well as mysids for *A. rostratus*, were eaten more frequently at site F3. Overlaps in the diet of *R. tapirina* between seasons at site D1 were significant in autumn-winter when amphipods and harpacticoids were the major components of the diet, and in spring-summer due to the high consumption of polychaetes. At site F3 overlaps were significant in all seasons due to the high percentage volume of harpacticoids in the guts. The diet of *A. rostratus* in summer was significantly different from autumn and winter at site D1, and from winter and spring at site F3. The percentages of polychaetes and sand in the guts at site D1, and mysids at site F3, were highest in summer.

The diets of *R. tapirina* and *A. rostratus* overlapped significantly in autumn and winter at site D1 when both species consumed predominantly amphipods, and in winter at site F3 when harpacticoids were the major component of the diet of both species (Table 4.3). The overlaps in diets of the two species were not significant at either site, or for the two sites combined, when the results were pooled for all seasons (Table 4.4). Figure 4.1 and Table 4.4 show that although both species generally ate the same food organisms, the proportions of each food type differed between the two species. *R. tapirina* consumed a greater quantity of polychaetes and harpacticoids but fewer amphipods and mysids than *A. rostratus*.

TABLE 4.3 Schoener diet overlap indices between *R. tapirina* and *A. rostratus* (calculated using percentage volume of food organisms)

	Summer	Autumn	Winter	Spring	All Seasons
Site D1	0.57	0.75	0.62	0.39	0.56
Site F3	0.34	-	0.69	0.37	0.41
All sites	0.52	0.52	0.45	0.38	0.50

FIGURE 4.1 Cumulative percentage volume of food organisms in the foregut of newly-metamorphosed *R. tapirina* and *A. rostratus* at site D1 and site F3 in four seasons

n = number of fish examined, all contained food in the foregut

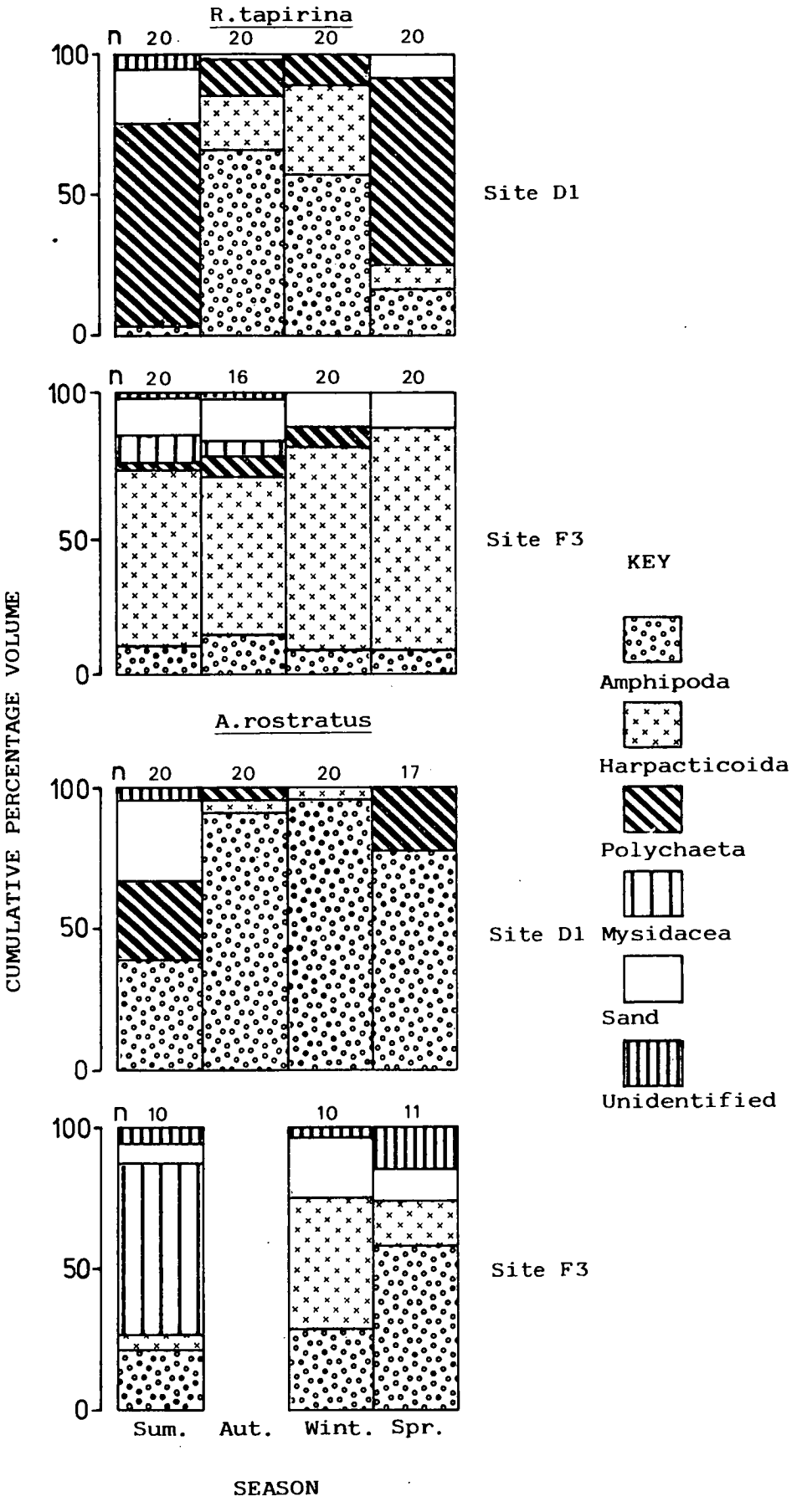

R.tapirina
n 20 20 20 20
100
50
0
Site D1
n 20 16 20 20
Site F3
A.rostratus
n 20 20 20 17
Site D1
n 10 10 11
Site F3
CUMULATIVE PERCENTAGE VOLUME
Sum. Aut. Wint. Spr.
SEASON
KEY
Amphipoda
Harpacticoida
Polychaeta
Mysidacea
Sand
Unidentified

TABLE 4.2 Schoener diet overlap indices (calculated using percentage volume of food organisms)

(a) Between sites D1 and F3 for each species

	Summer	Autumn	Winter	Spring	All Seasons
R. tapirina	0.21	0.42	0.48	0.27	0.38
A. rostratus	0.34	-	0.34	0.62	0.48

(b) Between seasons for each species

		Site D1				Site F3			
		S	A	W	SP	S	A	W	SP
R. tapirina	S	1	0.18	0.15	0.80	1	0.89	0.86	0.85
	A		1	0.88	0.38		1	0.84	0.78
	W			1	0.36			1	0.91
	SP				1				1

		S	A	W	SP	S	W	SP
A. rostratus	S	1	0.44	0.39	0.61	1	0.37	0.40
	A		1	0.95	0.83		1	0.60
	W			1	0.78			1
	SP				1			

S = Summer; A = Autumn; W = Winter; SP = Spring

TABLE 4.4 Percentage frequency of occurrence and percentage volume of food organisms in the foregut of *R. tapirina* and *A. rostratus* juveniles at site D1 and site F3. The results are pooled for all seasons.

Prey Taxa	*R. tapirina*				*A. rostratus*			
	Site D1		Site F3		Site D1		Site F3	
	Frequency (%)	Volume (%)	Frequency (%)	Volume (%)	Frequency (%)	Volume (%)	Frequency (%)	Volume (%)
Amphipoda	68.24	31.56	53.95	10.92	92.21	75.34	66.67	36.62
Harpacticoda	62.35	11.91	98.68	67.28	25.97	1.98	69.70	14.74
Polychaeta	62.35	45.51	14.47	4.04	38.96	12.84	9.01	0.08
Mysidacea			3.95	3.53			27.27	29.62
Sand	50.59	9.06	73.68	12.53	36.36	8.33	69.70	10.41
Digested material	18.82	1.68	21.05	1.69	12.99	1.32	36.36	8.49
Number examined	80		76		77		38	
Number empty	0		0		0		0	
Size range (mm)	10-28		11-29		11-29		12-27	

Schoener diet overlap index (percentage volume) between *R. tapirina* and *A. rostratus* at

Site D1 = 0.56

Site F3 = 0.41

Sites D1 + F3 = 0.50

The seasonal pattern of feeding of the two species was examined using the seasonal mean % SSR at the two sites (Figure 4.2). Feeding levels of *R. tapirina* were highest in summer at site D1, and in autumn at site F3. They were low in winter at site D1 and in summer at site F3. *A. rostratus* had the highest levels of feeding in autumn at site D1 but no fish were caught in this season at site F3; feeding levels at both sites were low in spring. The % SSR's of *R. tapirina* only, were compared by ANOVA (as described in Chapter 2). They were not compared for *A. rostratus* because the numbers of fish caught at site F3 were much lower than at site D1. The site x season interaction was significant and further testing showed that the % SSR's were significantly different between sites D1 and F3 in summer only (Table 4.5). At site D1 the % SSR's were significantly higher in summer than winter and at site F3 were higher in autumn and spring than summer.

TABLE 4.5 Results of two-way ANOVA comparing ℓn(x+1) transformed % SSR's of *R. tapirina* at sites D1 and F3 in each season

ANOVA Table

	SS	DF	MS	F	P
Site	0.0015	1	0.0015	0.0123	P>0.05
Season	1.0035	3	0.3345	2.7699	P<0.05
Site x season	1.8991	3	0.6330	5.2422	P<0.01
Error	17.8718	148	0.1208		
Total	20.7344	155	0.1338		

Site x season interaction

t-test of site D1 versus site F3

Summer	t = 3.3175, P<0.01
Autumn	t = 1.0259, P>0.05
Winter	t = 1.8159, P>0.05
Spring	t = 0.7009, P>0.05

S.N.K. test for seasons

Site D1	Winter	Spring	Autumn	Summer
Site F3	Summer	Winter	Spring	Autumn

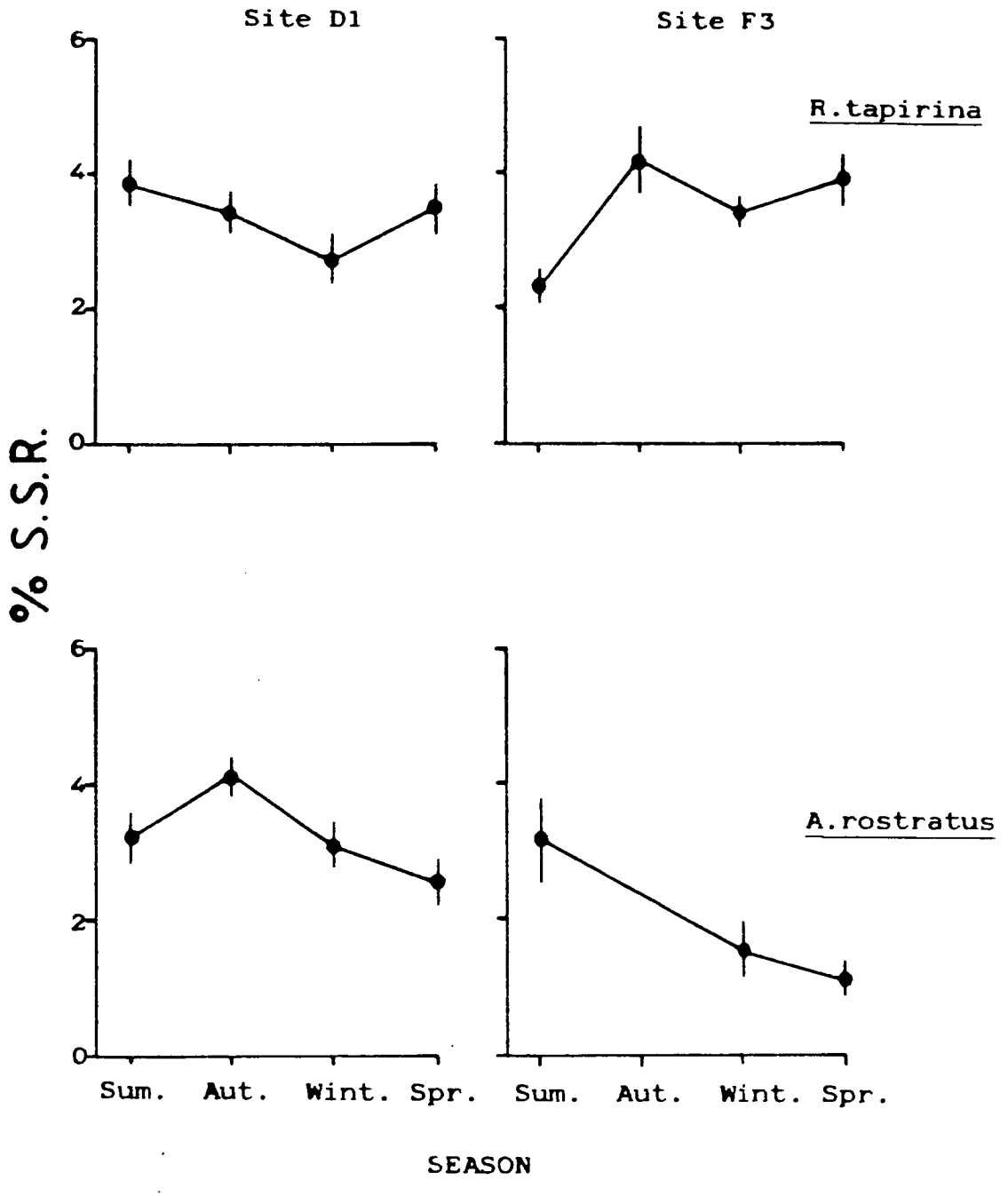


FIGURE 4.2 Seasonal pattern of feeding: mean % S.S.R. (± S.E.) of newly-metamorphosed *R. tapirina* and *A. rostratus* in each season at site D1 and site F3

4.3.2 Diel Feeding Patterns of Newly-Metamorphosed *R. tapirina* and *A. rostratus*

Newly-metamorphosed juveniles of both *R. tapirina* and *A. rostratus* at site D1 over 27 h ate predominantly amphipods, in particular species of corophiids (Figure 4.3, Table 4.6). The diet overlap index between the two species was significant. However, Table 4.6 shows that harpacticoids, polychaetes and sand particles occurred more frequently, by percentage volume and percentage frequency of occurrence, in the guts of *R. tapirina* than *A. rostratus*.

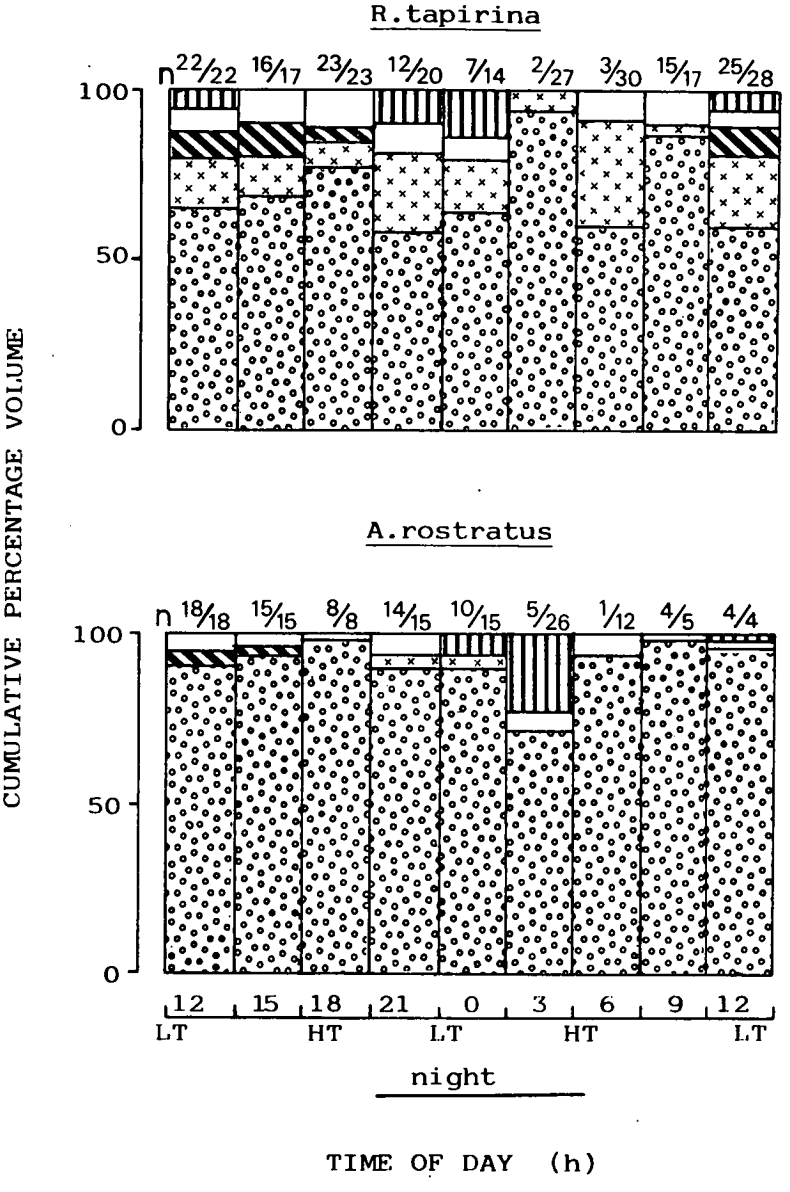
TABLE 4.6 Percentage frequency of occurrence and percentage volume of food organisms in foregut of *R. tapirina* and *A. rostratus* juveniles caught during 24 h sampling at Nutgrove (site D1)

Prey Taxa	*R. tapirina* Frequency (%)	*R. tapirina* Volume (%)	*A. rostratus* Frequency (%)	*A. rostratus* Volume (%)
Amphipoda	96.9	71.9	98.8	92.8
Harpacticoida	68.0	11.3	28.8	1.3
Polychaeta	10.2	6.1	5.0	1.7
Nemertea	1.6	0.1	2.5	0.1
Sand	71.1	7.9	38.8	2.3
Digested material	33.6	2.3	16.3	1.6
Other	3.1	0.4	2.5	0.2
Number examined	196		118	
Number empty	68		38	
Size range (mm)	10-40		17-40	

Schoener diet overlap index (percentage volume) between *R. tapirina* and *A. rostratus* = 0.791

Both species were predominantly daytime feeders. The % SSR of each species increased during the day and decreased during the night to lowest levels at dawn, and then increased during the next day (Figure 4.4). The percentage of fish with empty guts also increased during the night. *A. rostratus*, however, continued to feed for longer after nightfall than *R. tapirina* and both species had the greatest variation in % SSR values at this time.

FIGURE 4.3 Cumulative percentage volume of food organisms in the foregut of *R. tapirina* and *A. rostratus* every 3 h for 27 h at site D1 (Nutgrove)
The key to the different food types is given in Figure 4.1
n = number of fish with food/number of fish examined


R.tapirina
n 22/22 16/17 23/23 12/20 7/14 2/27 3/30 15/17 25/28
100
50
0
A.rostratus
n 18/18 15/15 8/8 14/15 10/15 5/26 1/12 4/5 4/4
100
50
0
12 15 18 21 0 3 6 9 12
LT HT LT HT LT
night
CUMULATIVE PERCENTAGE VOLUME
TIME OF DAY (h)

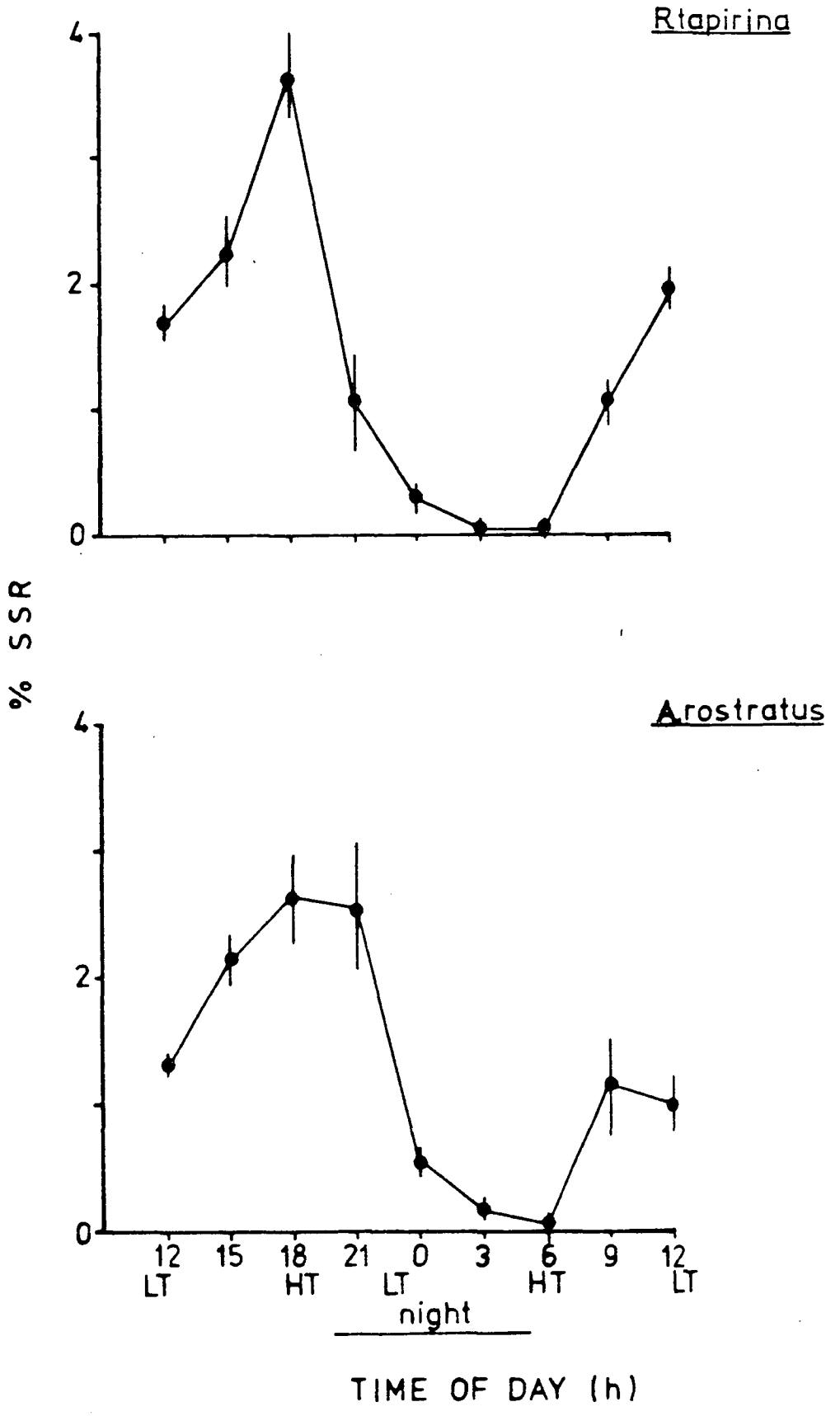


FIGURE 4.4 Diel pattern of feeding: mean % S.S.R. (± S.E.) of newly-metamorphosed *R. tapirina* and *A. rostratus* every 3 h for 27 h at site D1 (Nutgrove)
LT = low tide, HT = high tide

Relative abundances of newly-metamorphosed juveniles of *R. tapirina* and *A. rostratus* at different depths over 24 h indicate that both species move up and down the shore with the tide but are more concentrated in shallow water (10 - 30 cm) at night than during the day (see Figure 2.10). These movements into shallow water, however, are apparently not related to feeding as little or no food was consumed during the night. Both species were feeding predominantly at the same depth of 50 - 70 cm as they were both relatively more abundant at this depth during the day.

4.3.3 Diets and Diel Feeding Activity of Larger 0-group and 1-group Flounder

At site F4, Cremorne, amphipods were the major component of the diet of all three species with mysids, cumaceans and polychaetes of minor importance for *R. tapirina* and *A. rostratus* and cumaceans for *A. lituratus* (Figure 4.5, Table 4.7). The food categories eaten by *R. tapirina* and *A. rostratus* did not differ between day and night time. The index of overlap in diet between these two species was significant. However, when the amphipods were divided into families, clear differences in the diets of the two species were apparent (Table 4.8). Corophiids and oedicerotids were dominant more frequently in the diet of *R. tapirina* than *A. rostratus*. Conversely phoxocephalids and eusirids were consumed more often by *A. rostratus*.

The feeding activity over 24 h was similar for *R. tapirina* and *A. rostratus* (Figure 4.6). The % SSR of both species, pooled for the two stations, was highest during the daytime high and ebb tides, although the greatest variation in % SSR's also occurred at these times. They were lowest after low tide during the day. During the night small quantities of food were eaten by both species irrespective of the stage of the tide. The ratio of fish with food in the foregut to the total number of fish examined was generally higher at night than during the day for both species (Figure 4.5). However, the fullness of the foregut varied during the day from empty to full whereas most fish caught at night had eaten only small quantities of food. *A. lituratus* juveniles contained comparatively low volumes of food in the foregut during the night only.

FIGURE 4.5 Cumulative percentage volume of food organisms in the foregut of larger 0-group and I-group *R. tapirina*, *A. rostratus* and *A. lituratus* every 3 h for 24 h at site F4 (Cremorne)
n = number of fish with food/number of fish examined
LT = low tide, HT = high tide

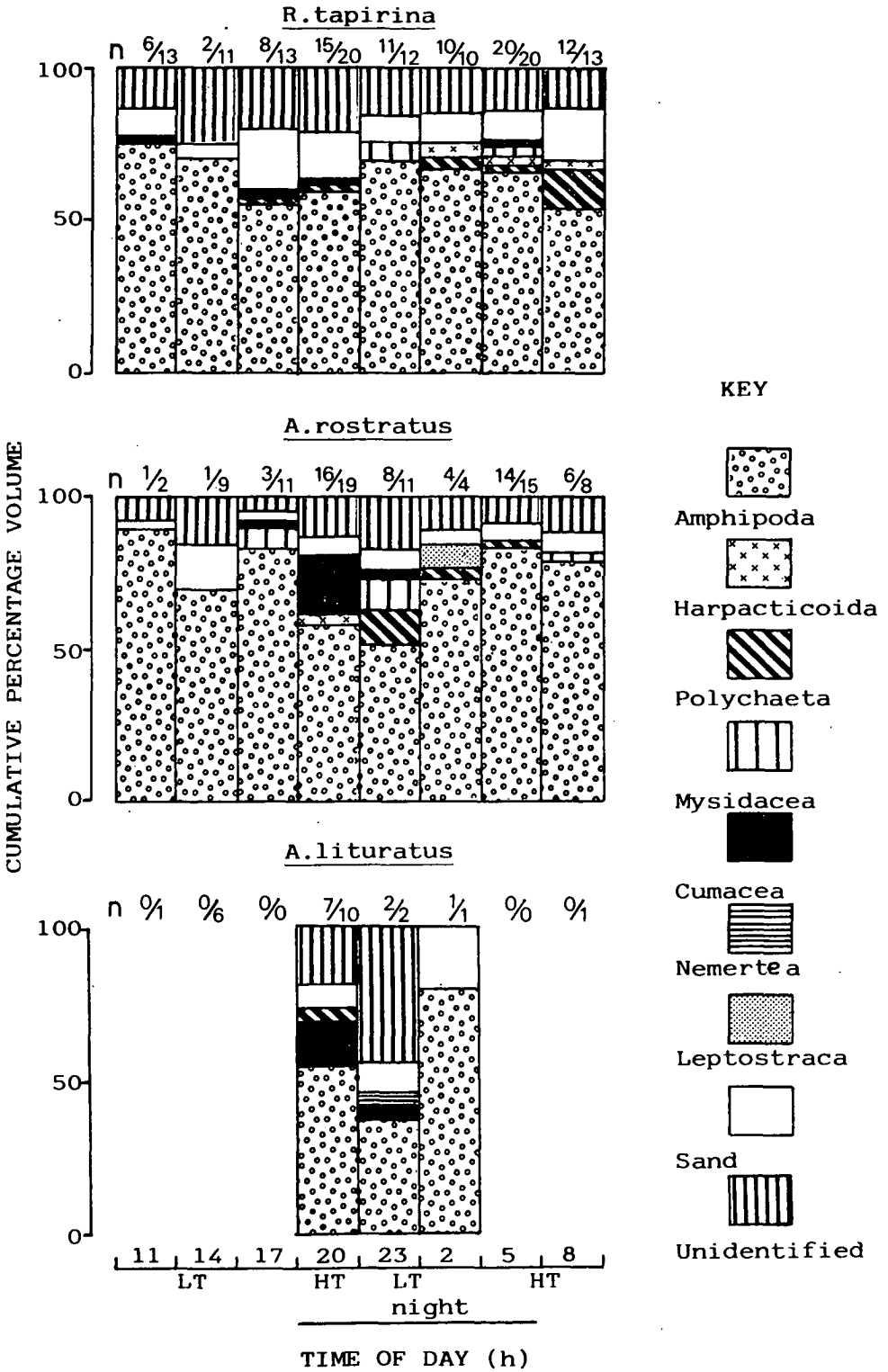

R.tapirina
n 6/13 2/11 8/13 15/20 11/12 10/10 20/20 12/13
100
50
0
A.rostratus
n 1/2 1/9 3/11 16/19 8/11 4/4 14/15 6/8
100
50
0
A.lituratus
n 0/1 0/6 0/0 7/10 2/2 1/1 0/0 0/1
100
50
0
CUMULATIVE PERCENTAGE VOLUME
11 14 17 20 23 2 5 8
LT HT LT HT
night
TIME OF DAY (h)
KEY
Amphipoda
Harpacticoida
Polychaeta
Mysidacea
Cumacea
Nemertea
Leptostraca
Sand
Unidentified

TABLE 4.7 Percentage frequency of occurrence and percentage volume of food organisms in the foregut of *R. tapirina*, *A. rostratus* and *A. liturata* juveniles over 24 h at site F4a,b

Prey Taxa	*R. tapirina*		*A. rostratus*		*A. lituratus*	
	Frequency (%)	Volume (%)	Frequency (%)	Volume (%)	Frequency (%)	VVolume (%)
Amphipoda	98.8	64.5	100.0	70.8	90.0	52.3
Polychaeta	17.9	3.5	16.7	2.0	20.0	3.7
Mysidacea	29.8	1.8	14.8	1.8		
Cumacea	41.7	1.8	24.1	4.7	50.0	12.1
Ostracoda	14.3	0.6	7.4	0.1		
Nemertea	10.7	0.3	1.9	0.1	20.0	0.9
Isopoda	9.5	0.6				
Leptostraca			1.9	2.3		
Sand	95.2	12.4	94.4	5.9	80.0	8.8
Digested material	96.4	13.9	85.2	12.1	60.0	22.1
Other	4.8	0.6	1.9	0.2	10.0	0.1
Number examined	114		79		21	
Number empty	30		25		11	
Size range (mm)	44-130		44-134		71-152	

Schoener Diet Overlap index between *R. tapirina* and *A. rostratus*

= 0.886 (percentage volume)

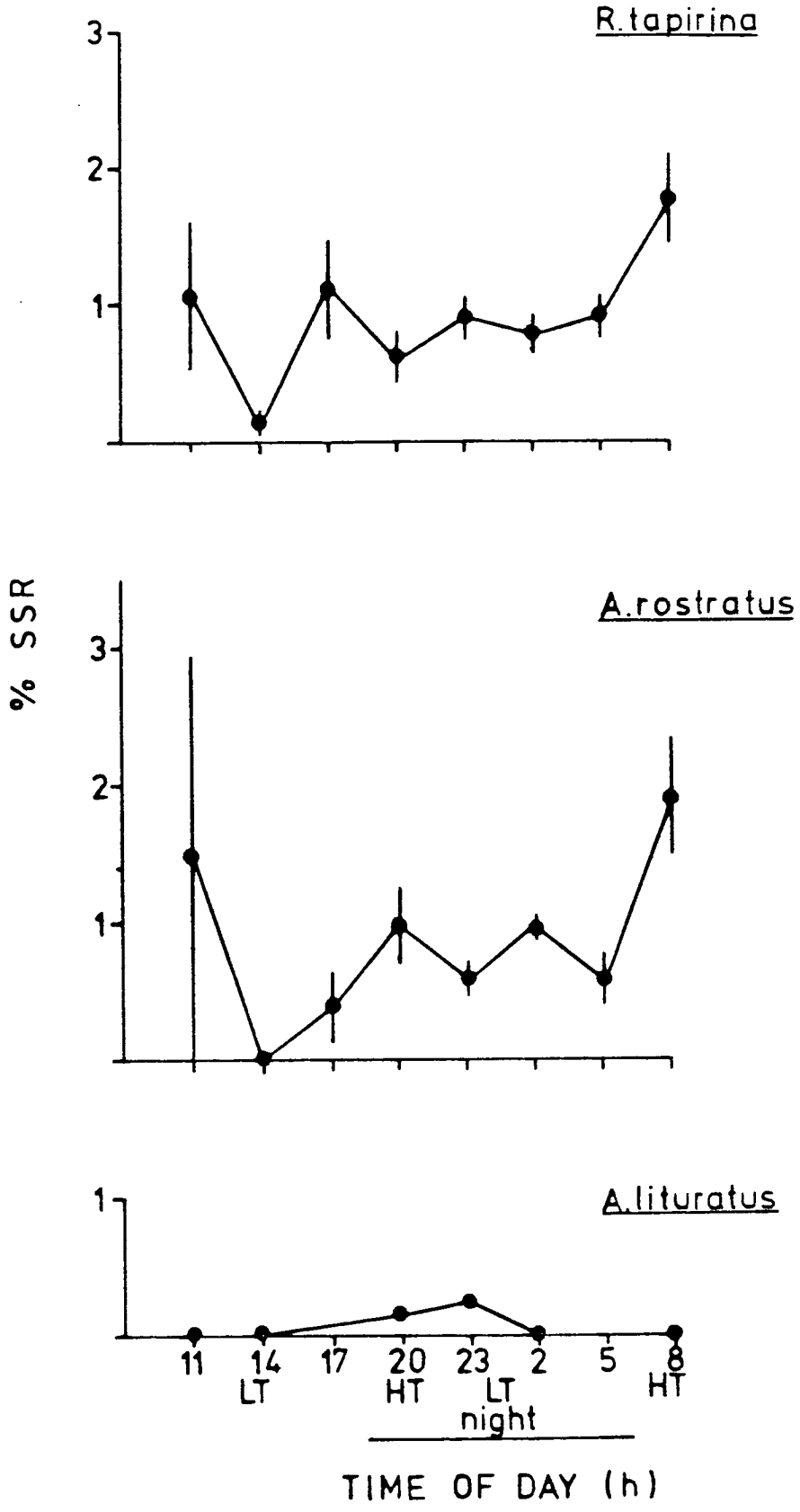


FIGURE 4.6 Diel pattern of feeding over 24 h: mean % S.S.R. (± S.E.) of larger 0-group and I-group *R. tapirina*, *A. rostratus* and *A. lituratus* at site F4 (Cremorne)
LT = low tide, HT = high tide

TABLE 4.8 Percentage frequency of occurrence of the most abundant families of Amphipods in the guts of *R. tapirina* and *A. rostratus*

Amphipoda	Percentage Frequency	
	R. tapirina	*A. rostratus*
Corophiidae	64.1	37.1
Phoxocephalidae	22.5	49.6
Dexaminidae	3.0	1.4
Eusiridae	0	11.9
Oedicerotidae	10.4	0

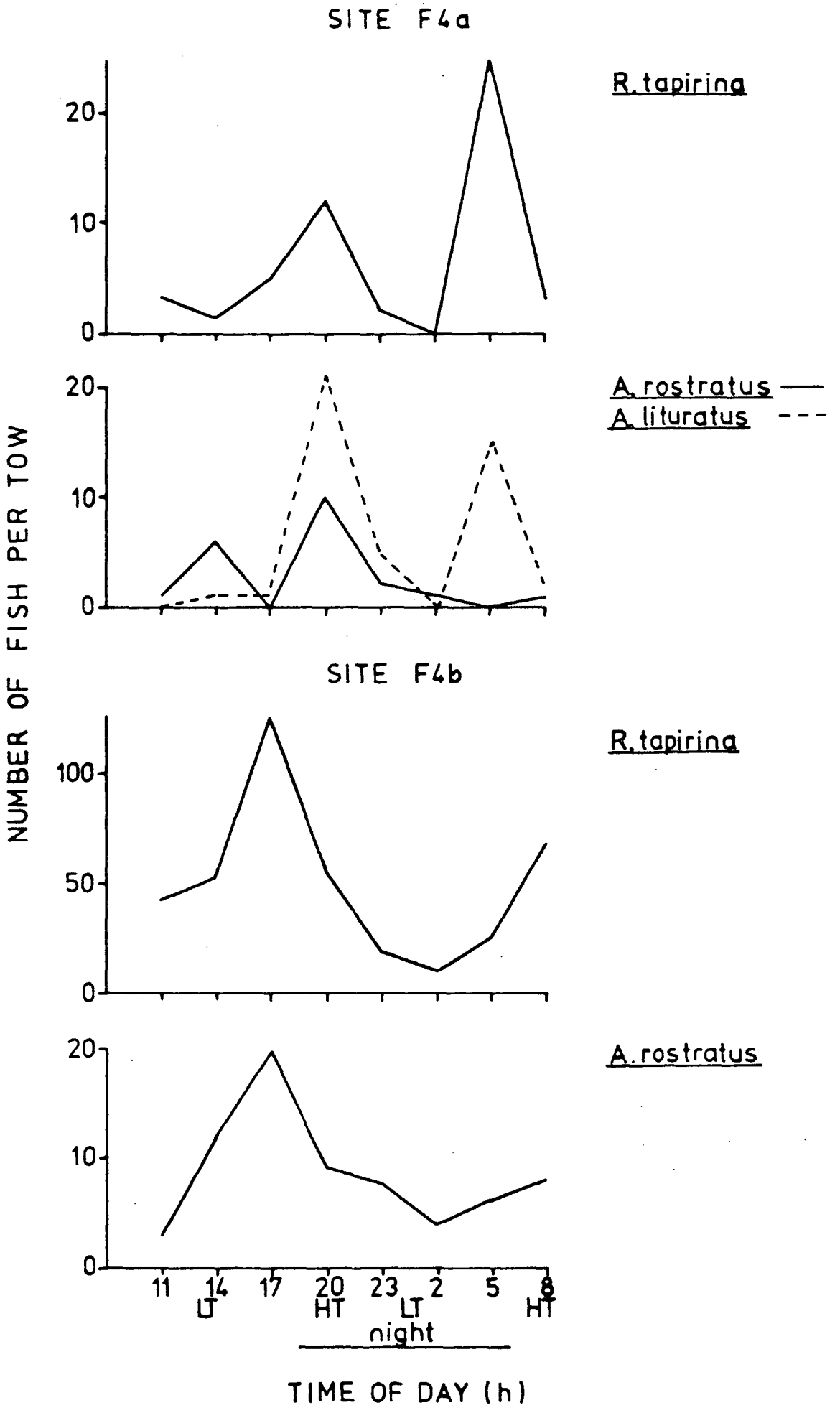
The variation in abundances over 24 h of *R. tapirina* and *A. rostratus* juveniles were similar at each station. However, the times of peak abundances differed between the two stations for both species (Figure 4.7). At station F4a they were most abundant during the high and flood tides at night; few fish were caught during the day or around low tide at night. At station F4b a peak in abundance occurred during the daytime flood tide for both species. *R. tapirina* juveniles were generally least abundant during the night whilst the numbers of *A. rostratus* were lowest during the day and nightime low tides. *A. lituratus* juveniles at station F4a did not show any trends in abundance; highest numbers were caught after the daytime low tide and high tide at night.

4.4 DISCUSSION

Newly-metamorphosed juveniles of both *R. tapirina* and *A. rostratus* were observed to be daytime feeders and consumed crustaceans, especially amphipods,and polychaetes. The quantities of each prey type eaten varied seasonally and between sites which implies plasticity in the feeding habits of both species. However, as the abundances of prey species in flounder habitat are not known, the change in diet could not be related directly to the availability of prey.

Although newly-metamorphosed juveniles of both species ate the same food organisms, they appear to partially partition the food resource by consuming different proportions of each food type. The greater number of

FIGURE 4.7 Number of larger O-group and I-group *R. tapirina*, *A. rostratus* and *A. lituratus* caught per 15 min tow at site F4a and site F4b
LT = low tide, HT = high tide


SITE F4a
R. tapirina
20
10
0
A. rostratus
A. lituratus
20
10
0
NUMBER OF FISH PER TOW
SITE F4b
R. tapirina
100
50
0
A. rostratus
20
10
0
11
14
17
20
23
2
5
8
LT
HT
LT
HT
night
TIME OF DAY (h)

the smaller harpacticoids eaten by *R. tapirina* probably relates to their differences in size at metamorphoses; *R. tapirina* are 8.5-9.5 mm in length and *A. rostratus* 10.5-11.5 mm (Chapter 6).

Larger O-group and I-group *R. tapirina* and *A. rostratus* were not segregated by major food categories eaten or by time of feeding. Amphipods were the major prey of both species; however, the proportions of each family of amphipod eaten differed between the two species of flounder. Thus, the larger juveniles of *A. tapirina* and *A. rostratus* partially partitioned the habitat on the food species level.

Although *A. lituratus* juveniles also ate mainly amphipods, the results suggest that their feeding habits are segregated from those of *A. rostratus* and *R. tapirina*. They apparently feed in areas other than those sampled as relatively low volumes of food were found in the gut, and they are probably night-time feeders.

The diets of juvenile *R. tapirina, A. rostratus* and *A. lituratus* observed in the present investigation are similar to those found in previous studies, regardless of whether the species occurred sympatrically or allopatrically. Last (1983) also found that the major components of the diet of *A. rostratus* and *R. tapirina* were amphipods and polychaetes, and of *A. lituratus*, amphipods and cumaceans. He observed that different prey species were taken by *R. tapirina* and *A. rostratus* at each area of the estuary. Although diets were similar at any one site, subtle differences in prey selection were apparent between the species. In particular, the relative proportions of each prey type eaten by *R. tapirina* and *A. rostratus* varied. In New Zealand O-group *R. tapirina* also consumed mainly harpacticoids, amphipods and polychaetes. They ate significantly less at night in terms of dry weight but not numbers of individuals as many small harpacticoids were eaten (Roper, 1979). The diet of small *A. rostratus* juveniles was observed by Burchmore (1982) to be dominated by amphipods and polychaetes.

De Groot (1971) classified the Pleuronectiformes into three groups - fish feeders, crustacean feeders and polychaete-mollusc feeders. Pleuronectids, however, had representatives in all three groups. He placed *R. tapirina* in Pleuronectid type II group (i.e. mainly crustacean feeders) based on the morphology of the alimentary tract and in Pleuronectid

type III (i.e. polychaete-mollusc feeders) by food organisms eaten. *A. rostratus* was classified by Burchmore (1982) as belonging to the Pleuronectid type III group. Thus, de Groot's (1971) classification, which is based on adult fish, is not appropriate for juveniles of these two species of flounder as they are polychaete-crustacean feeders, depending on locality or season. Similarly, juveniles of other species of flatfish feed mainly on polychaetes, small crustaceans and occasionally molluscs. Their diets may change also with season, locality or age, e.g. plaice (Macer, 1967; Edwards and Steele, 1968; Thijssen *et al.*, 1974; Kuipers, 1977), flounder (Moore and Moore, 1976; Summers, 1980), turbot (Jones, 1973a; dabs (Macer, 1967; Edwards and Steele, 1968), English sole (Toole, 1980).

Juveniles of some species of flatfish (e.g. plaice, dab, flounder) consume large quantities of regenerating parts of molluscs and polychaetes (Kuipers, 1973, 1975b; 1977; Macer, 1967; Edwards and Steele, 1968; de Vlas, 1979). About 36% of the total annual consumption of plaice and 12% for flounder in the Dutch Wadden Sea was regenerating parts of macrobenthic animals (de Vlas, 1979). This constituted a considerable flow of energy from the macrobenthos to flatfish without a concomitant mortality of prey. Edwards and Steele (1968) considered that this initial feeding on regenerating parts of polychaetes and molluscs was important in sustaining the flatfish populations as they were numerically much larger at this stage, than later. As *R. tapirina* and *A. rostratus* did not consume regenerating parts of prey and as they occurred in high densities for several months, food supplies may possibly have become limiting.

Partitioning of the food resources of the habitat by other co-existing species of flatfish have been observed. Juvenile plaice and dab ate similar food groups but differences in genera were apparent. Also differences in distributions of the two species probably help reduce over-exploitation of a common food supply; plaice have a wider distribution and are more abundant in shallower water than the dab population (Macer, 1967; Edwards and Steele, 1968). *R. tapirina* and *Peltorhamphus latus* ate similar food groups but the relative proportions eaten by each species varied. Also, *P. latus* consumed the same quantity of food by day as by night whereas *R. tapirina* ate significantly less at night (Roper, 1979). Roper considered that these differences in diet between the two species may have been due to their feeding in different areas or to the way in which they locate their prey.

The senses employed in feeding and the feeding activity of some species of flatfish appear to vary with locality. For example, juvenile plaice are mainly daytime feeders on open beaches (Edwards and Steele, 1968; Thijssen *et al.*, 1974) but on extensive sandflats where they retreat to tidal channels during low tide, a tidal rhythmicity in feeding is observed (Kuipers, 1973, 1975b,1977). De Groot (1971) observed experimentally that plaice may use visual or chemical stimuli in their search for food. Similarly, adult *R. plebia, R. leporina* and *Peltorhamphus novaezeelandia* are non-visual feeders on inactive benthis organisms in Wellington Harbour, probably due to the high turbidity, but feed on active prey in other areas of New Zealand (Livingston, 1981). She suggests that, as well as olfactory and visual sense organs, the external taste and acoustico-lateralis systems of these flatfish are important in feeding. As larger *R. tapirina* and *A. rostratus* juveniles were feeding both during the day and at night, which implies various methods of prey detection, they may also use different senses for feeding in different localities. It also appears that senses other than vision, such as olfaction, become more important in feeding with age as recently metamorphosed juveniles of both species fed only during the day.

The numbers of newly-metamorphosed juveniles of *R. tapirina* and *A. rostratus* in different depths over 27 h implies that both species have tidal-related movements, but the depths they occupy apparently vary mainly with the time of day. Roper (1979) also observed that most of the *R. tapirina* population moved onto the sandflats during high tide, both day and night, and that they possessed a pronounced circatidal rhythm of activity. This tidal activity rhythm, however, is not related to feeding rhythms as newly-metamorphosed juveniles of both *R. tapirina* and *A. rostratus* were observed to be daytime feeders. Activity independent of feeding rhythms has been observed in other species of flatfish. Juvenile plaice, for example, migrate into the intertidal zone during flood and high tide and are concentrated in shallower water at night. They feed, however, mostly during the day and do not show any evidence for a tidal effect on feeding (Gibson, 1973). Movements of *R. tapirina* and *A. rostratus* into shallower water at night are also not related to feeding. Gibson (1973) and Toole (1980) suggest that the nightly upshore movements of plaice and English sole, respectively, are to avoid predators. *R. tapirina* and *A. rostratus* may move into shallow water at night for similar reasons.

Abundances of larger juveniles of *R. tapirina* and *A. rostratus* at site F4 over 24 h are difficult to interpret. The differences in times of peak abundances of the two species between stations F4a and F4b may have occurred due to unrepresentative sampling or because they moved into water shallower than that sampled on the night-time high tide at the sheltered station F4b. However, at the semi-exposed station F4b they may not have moved so close into shore due to wave action. Both species possibly preferred deeper water during the day. Abundances of the two species at different times of the day may have been clarified by additional sampling, in particular with high and low tides occurring at different times of the day.

In summary, newly-metamorphosed juveniles of both *R. tapirina* and *A. rostratus* are daytime feeders and consume the same food organisms. They partially partition the food resources of the habitat by consuming different relative proportions of dietary constituents. The diets of larger 0- and I-group juveniles of the two species at one site over 24 h consisted predominantly of amphipods but different proportions of each family of amphipods were eaten by the two species of flounder. *A. liturratus* juveniles also ate mainly amphipods but their feeding habits appeared to be segregated from the other two species by both time and place of feeding.

CHAPTER 5

REPRODUCTIVE BIOLOGY OF *RHOMBOSOLEA TAPIRINA* AND *AMMOTRETIS ROSTRATUS*

5.1 INTRODUCTION

The occurrence of newly metamorphosed juveniles of *Rhombosolea tapirina* and *Ammotretis rostratus* in shallow water in most months of the year, with highest abundances generally from late winter to early spring, implies that both flounders have a prolonged spawning season. However, the patterns of egg maturation and release are not well understood for *R. tapirina* and are not known for *A. rostratus*. The objective of the study reported in this Chapter was to examine and compare the reproductive strategies of the two species. Gonad maturation, development of ova, sex ratios, length at first maturity and fecundity of the two species were investigated.

Aspects of the reproductive biology of *R. tapirina* including sex ratios, length at first maturity and development of gonads and ova were examined by Kurth (1957). His results are compared with those obtained in this study. The reproduction of closely related species of flounder, *Rhombosolea leporina* and *Rhombosolea plebia*, in New Zealand was investigated by Coleman (1972, 1973, 1974a) and Webb (1973).

There have been numerous studies on the reproductive biology of commercially important species of flatfish in the northern hemisphere. It is now generally accepted that recruitment to a fishery depends on the parent stock size and modulations in density independent and density dependent mortality in the pre-recruit stage (Cushing, 1975). Thus, for a commercially important species, a knowledge of reproduction is basic to the prediction of recruitment and hence to the assessment and management of a fishery. Examples of studies of flatfish reproduction are by Simpson (1959) and Bagenal (1966) on plaice, *Pleuronectes platessa*, Jones (1974) on turbot, *Scophthalmus maximus*, Htun-han (1978a,b) on dab, *Limanda limanda*, Bowering (1976, 1978) on witch flounder, *Glytocephalus cynoglossus* and Morse (1981) on summer flounder, *Paralichthys dentatus*.

5.2 METHODS

5.2.1 Sampling Procedure

Adult *R. tapirina* and *A. rostratus* were sampled irregularly in 1981 and 1982 from commercial catches taken by spearfishermen in shallow water and by Danish seine trawlers in 10-40 m. Spearfishing was conducted in Blackman Bay, Norfolk Bay and Marion Bay in most months of the year and Danish seining in Frederick Henry Bay, Storm Bay and Marion Bay irregularly during the winter months (Figure 5.1).

Total lengths (±1.0 mm), sex and maturity stage of up to 200 specimens were recorded at the site of capture or at seafood processing plants. Small quantities of flounder were purchased and total weight (±0.1 g), ovary weight (±0.01 g), gonad maturity and ova diameters were recorded in the laboratory. Maturing or mature ovaries were preserved in modified Gilson's fluid (Simpson, 1951) or 5% V/V formalin.

5.2.2 Maturity Stages of Gonads

Seven stages of ovarian development and four of testicular development, recognized in *R. tapirina* and *A. rostratus* by macroscopic examination of the gonads, are listed in Table 5.1. These stages were discernible only in subsampled fish when the gonads were removed and cut open in the laboratory. Most flounder examined, however, were from commercial catches and maturity stages had to be determined on whole fish without exsecting the gonads. A less-detailed classification of maturity stages (Table 5.2) was developed for field studies. Difficulty was encountered in separating immature from resting ovaries, and developing from partially spent ovaries. Stages I and II, and III and VI of Table 5.1 were therefore combined to give Stages A and B, respectively, of Table 5.2. Also, as very few running-ripe females were caught, these were grouped with mature fish. Similarly, only two stages were readily recognized in male fish - running and not running with milt.

Gonadosomatic indices (G.S.I.) were calculated for all subsampled female fish using

$$GSI = \frac{\text{weight of ovaries}}{\text{total fish weight-ovary weight}} \times 100$$

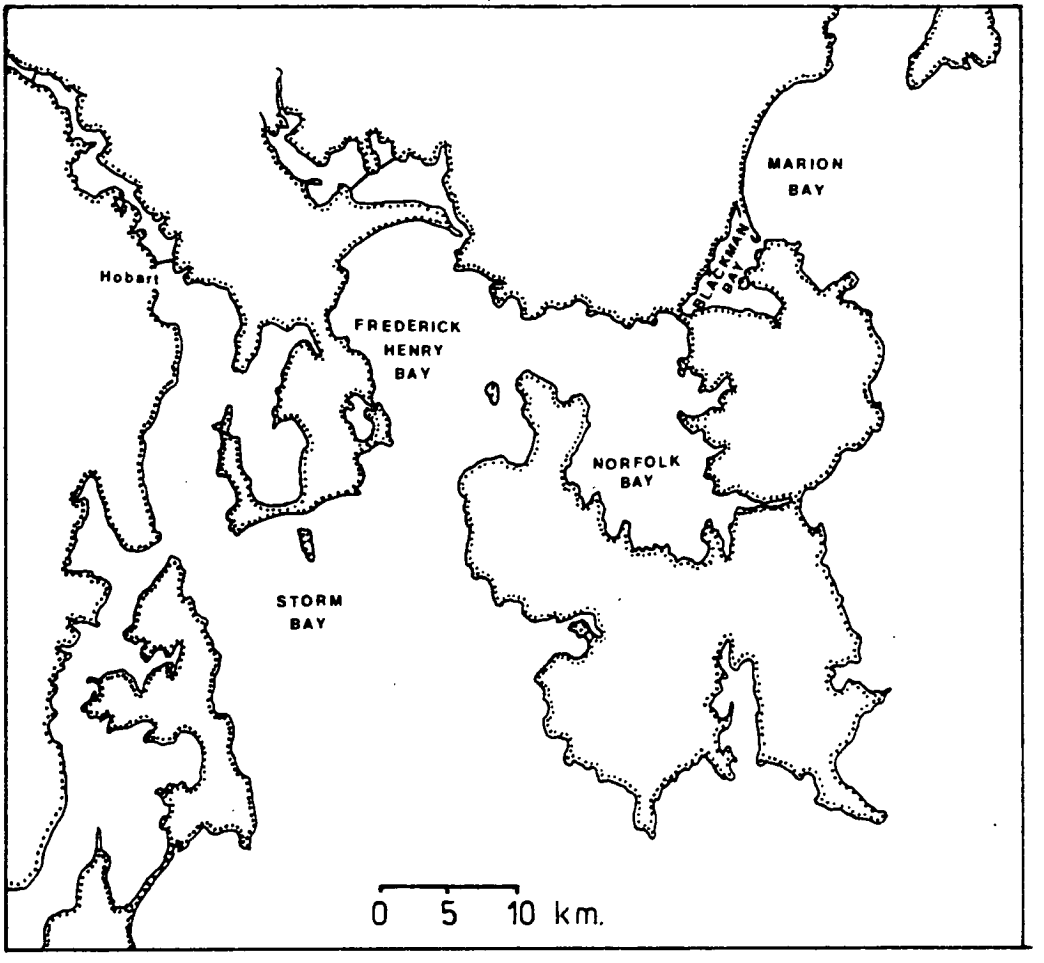


FIGURE 5.1 Map of southeastern Tasmania showing the bays which were fished for flounder.

TABLE 5.1 Classification of maturity stages of the gonads of *R. tapirina* and *A. rostratus*, modified after Macer (1974).

Maturity Stage		Description
Ovaries		
I	Immature	Ovaries small, translucent, grey-pink in colour; eggs not visible
II	Resting	Ovaries shrunken, semi-firm, no developing eggs
III	Developing	Ovaries larger, firm, opaque, yellow or pink; eggs visible and developing
IV	Mature	Ovaries enlarged and distended, yellow or orange in colour; opaque white eggs exuded under firm pressure
V	Running Ripe	Transparent eggs exuded under gentle pressure
VI	Partially spent	Ovaries still large but flaccid; large blood vessels; may be few transparent eggs remaining in the ovary
VII	Spent	Ovaries thin, flabby and bloodshot; frequently few transparent eggs present in a state of resorption At all stages of development, ovaries of *R. tapirina* were much larger, particularly in width, than those of *A. rostratus*.
Testes		
I	Immature	Testes small
II	Developing	Testes larger, no milt exuded under firm pressure
III	Mature - Running Ripe	Milt exuded under gentle pressure
IV	Spent	Testes flaccid, may exude little milt on cutting
		At all stages of development, testes of *R. tapirina* were blackish grey and larger than those of *A. rostratus* which were white in colour.

TABLE 5.2 Maturity stages recognized amongst undissected *R. tapirina* and *A. rostratus*

Maturity Stage		Description
Ovaries		
A	Immature - Resting	Ovaries small, posterior extension approximately half way to caudal peduncle
B	Developing - Partially Spent	Ovaries larger, firm, posterior extension approximately three quarters way to caudal peduncle
C	Mature	Ovaries enlarged and distended, bulging above body musculature, posterior extension almost to caudal peduncle
D	Spent	Ovary thin and flabby, still extends almost to caudal peduncle
Testes		
A	Immature - Spent - Resting	No milt exuded under firm pressure
B	Running Ripe	Milt exuded under gentle pressure

Mean GSI values were calculated for each month. The duration of the spawning season was estimated from the percentage frequency of maturity stages of gonads in the monthly samples and from mean monthly maturity indices (GSI's).

5.2.3 Ovarian Egg Diameters

The diameters of from 200 to 300 eggs were measured from unpreserved ovaries of most subsampled female fish. The ovaries, which were at various stages of maturity, were classified using Table 5.1. Eggs were taken randomly from each ovary as preliminary investigations demonstrated that mean egg diameters did not vary between anterior and posterior regions of the ovary (t-test, $P>0.05$).

5.2.4 Sex Ratios

The ratio of male to female fish of both species from different areas were calculated for each month of sampling and tested for significant differences from an expected 1:1 ratio using the Chi-square statistic. Sex ratios of *R. tapirina* in each season were compared using the contingency Chi-square test.

5.2.5 Length at First Maturity

The length at first maturity of male and female *R. tapirina*, separately, was investigated from the incidence of immature and mature fish in 1 cm length intervals and was estimated using probit analysis (Fleming, 1960; Pitt, 1966). This analysis determines the length (L50) at which 50% of the fish are mature (i.e. the median length). Sexual maturity in females was indicated by developing, mature or spent ovaries, (Stages B, C and D of Table 5.2), and in males by running-ripe testes (Stage B, Table 5.2). Only fish caught during the spawning season, i.e. May to October, were used in the analysis. As mentioned previously, the separation of immature from resting ovaries was not always possible. It is assumed for the analysis that fish with resting gonads did not occur during the spawning season. Only fish caught by trawling were investigated. The Danish seine nets had a cod-end of mesh size of 2.5 - 5.5 cm knot to knot, and juveniles, therefore, were included in the catch. Fish caught by spearing, however, were selected to be above the legal size

limit of 23 cm. Insufficient numbers of *A. rostratus* were caught during the main months of spawning for probit analysis.

5.2.6 Fecundity

Fecundity is defined as the number of ripening eggs in the female just prior to spawning. In a serial spawner it is the sum of all ripening eggs in all batches during the spawning season (Bagenal, 1978).

The fecundity of *R. tapirina* and *A. rostratus* was estimated only from late maturing or mature fish which apparently had not spawned during the current spawning season. A volumetric method of estimating fecundity was used. Eggs were removed from the preserved ovary, washed and sieved through a 1.00 mm sieve, which retained any ovarian tissue, onto a 0.15 mm mesh sieve for *R. tapirina* and a 0.2 mm mesh for *A. rostratus*. The eggs were diluted in 4 ℓ of water, stirred to ensure uniform distribution and four 1 mℓ aliquots extracted using a pipette. The number of eggs ≥0.15 mm diameter for *R. tapirina* and ≥0.2 mm for *A. rostratus* in each aliquot were counted and the mean number of eggs from the four subsamples was used to estimate the fecundity. These size ranges of eggs were used as microscopic examination showed that they contained accumulations of yolk whilst smaller eggs did not. The presence of yolk indicated that these eggs were developing and they were therefore assumed to be part of the current spawning season egg stock.

The consistency of the subsampling procedure was estimated from twenty replicate aliquots with replacement. This gave a coefficient of variation of 10.48%.

5.3 RESULTS

As data on adult flounder were obtained irregularly, the monthly results from two consecutive years were combined. Although some annual variation may occur, pooling the data gives a more complete picture of the reproductive cycle over twelve months.

5.3.1 Maturity Stages of Gonads

The percentage number of *R. tapirina* at each maturity stage varied

during the year (Figure 5.2). Over 50% of female fish caught each month from January to April were immature or resting. Maturing/partially spent females were most abundant in May; the relative numbers decreased in June, increased again in July and then slowly declined to November. The percentage of mature females was low from March to May and highest from June - October. No mature females were caught in November. The percentage of completely spent fish generally increased from April to November by which time nearly all fish were spent.

All *R. tapirina* males caught in January and February were immature or resting. The percentage of running-ripe males increased from March to May and from May to October almost all male fish were running ripe. The percentage declined in November although the majority were still ripe.

Mean monthly maturity indices (Figure 5.3), calculated for a small sample of female fish, followed a similar monthly pattern to the maturity stages of gonads. They were low in January to March, increased in April - May and were highest from June to October. The GSI values returned to a low level in November. The range of GSI's was highest during June - October, indicating non-synchronous maturation of females.

Stages of maturity of fish caught in different depths of water in several months were compared (Figure 5.4). However, the shortage of data due to the inconsistency of trawling by commercial fishermen makes it impossible to form any firm conclusions. In May the majority of fish caught by both spearing in <2 m depth and trawling in 5-10 m depth were maturing or partially spent (Stage B). However, the percentage of immature/resting females (Stage A) was higher, and mature fish (Stage C) lower, in shallow water than in 5-10 m depth. In July the majority of fish caught by spearing were still at Stage B but the percentage of Stage A fish had decreased. However, in 5-10 m depth the percentage of Stage B fish had decreased and mature and particularly spent fish increased relative to those caught in shallow water, or in 5-10 m depth in May. In 10-25 m depth in July the majority of fish were mature. In shallow water <2 m depth in August the greatest percentage of fish were at Stage B or mature whilst in 10-25 m depth the majority of fish were mature. By October spent fish were most abundant in both <2 m and 10-25 m depths. However, relatively more females were at Stage B and mature in shallow water than in 10-25 m depth. Thus, as mature fish were relatively more

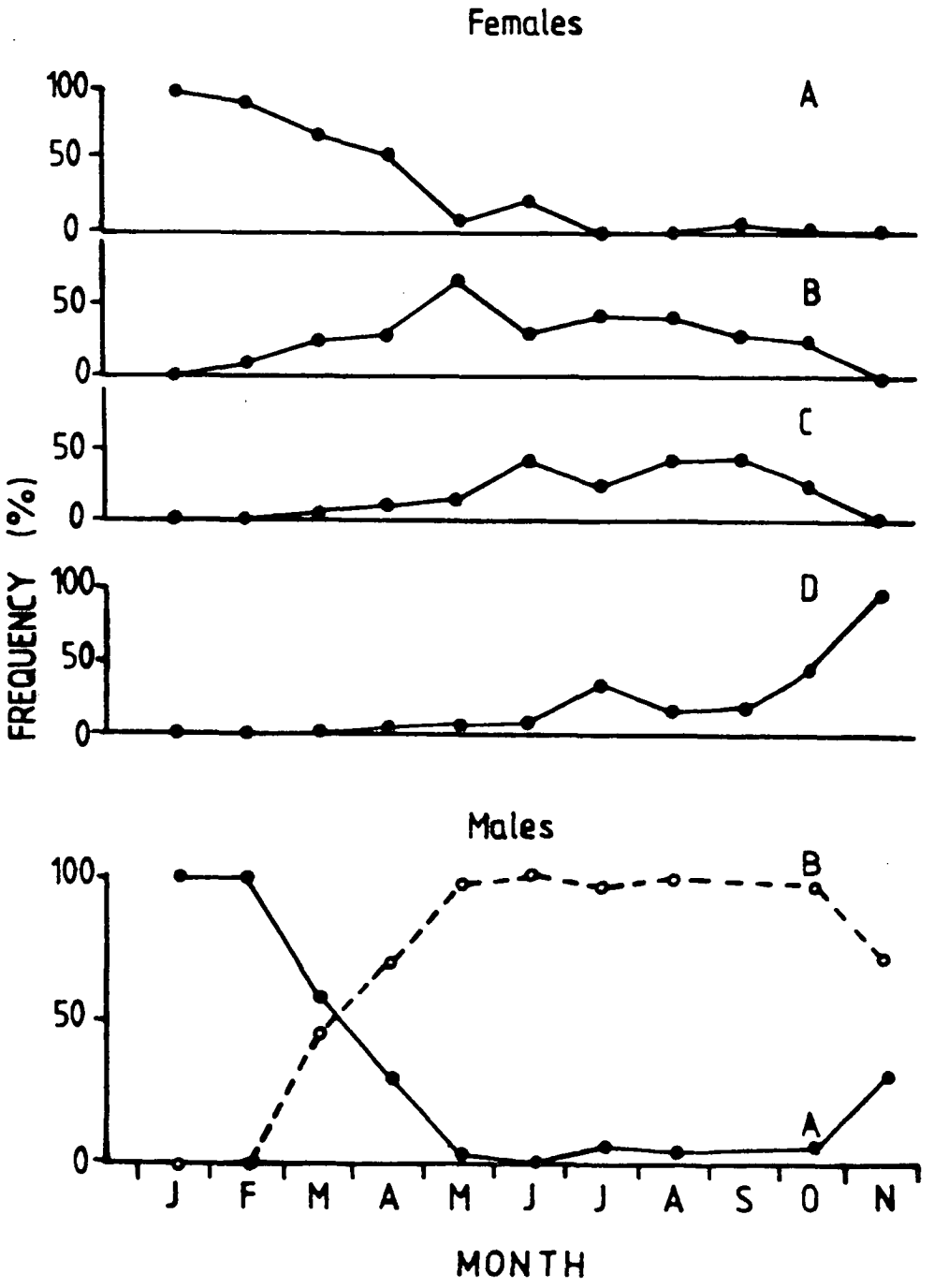


FIGURE 5.2 Percentage frequency of female and male *R. tapirina* at each maturity stage for 11 months of the year Maturity stages are described in Table 5.2

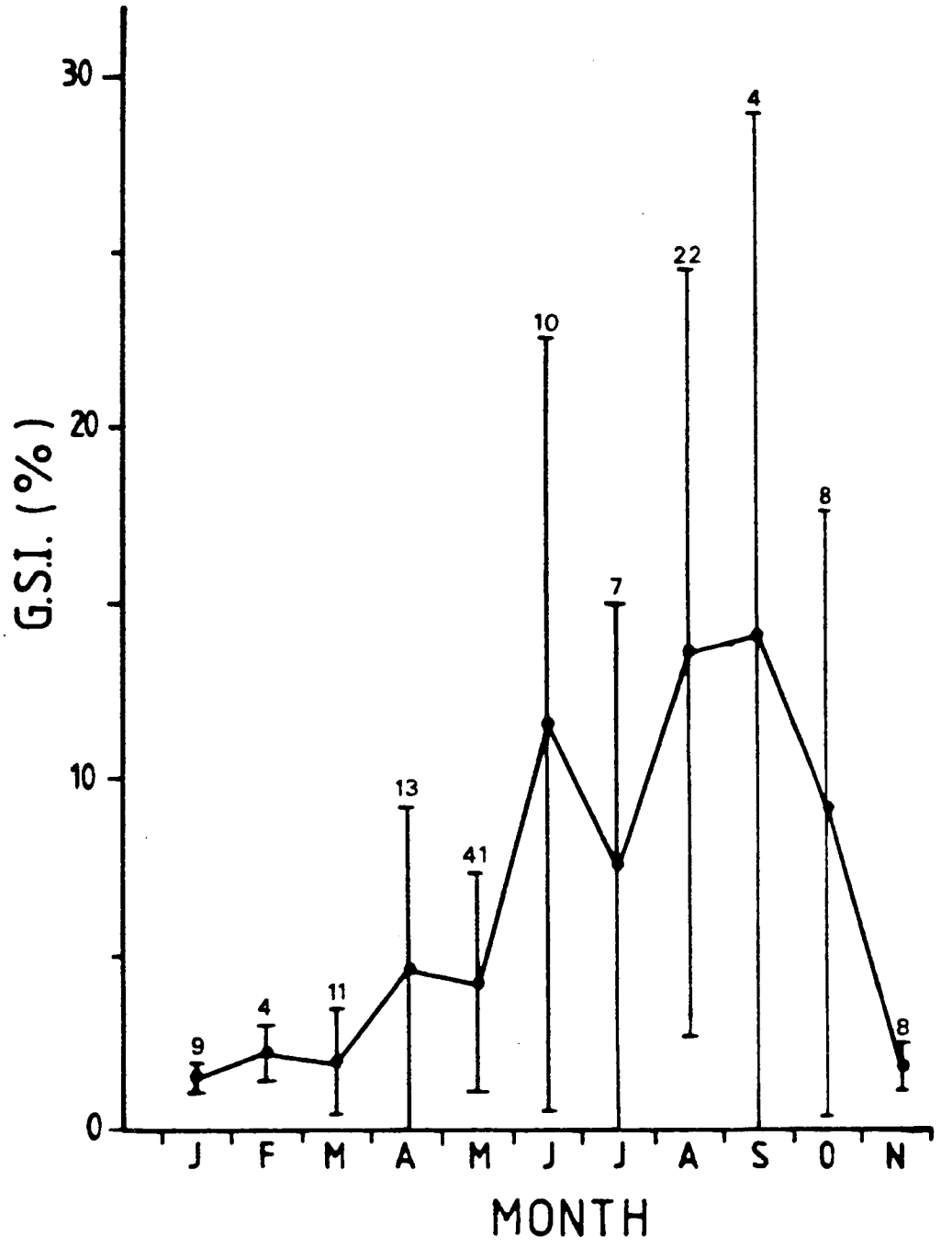


FIGURE 5.3 Mean monthly maturity indices - G.S.I. (± S.D.) of female *R. tapirina*
The number above each point indicates the number of fish examined in that month

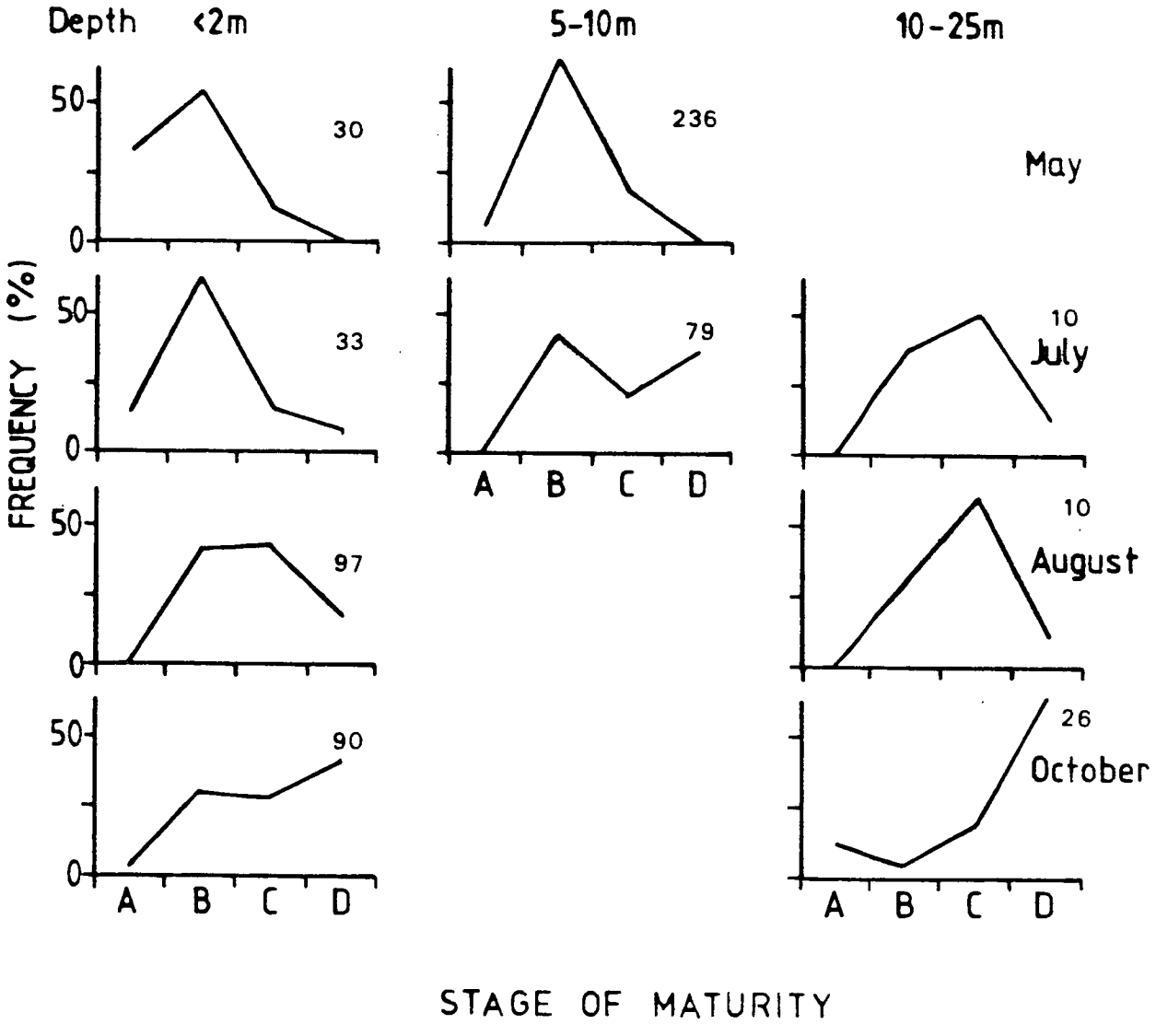


FIGURE 5.4 Percentage frequency of female *R. tapirina* at each maturity stage A-D (Table 5.2) in different depths (<2 m, 5-10 m, 10-25 m) in several months
The number on each graph indicates the number of fish examined.

abundant in deeper water than inshore in each month sampled, except in October at the end of the spawning season, it is suggested that spawning occurs predominantly in the deeper water.

Sufficient numbers of *A. rostratus* for comparison of maturity stages were caught in May and July only. In May 28% of the females caught were at Stage A, 68% were Stage B and only 4% were mature; 79% of the males caught were running-ripe. By July 36% of females were at Stage B, 58% were mature and 3% were spent; 100% of males were running-ripe. Of the eight females caught in August, four were mature.

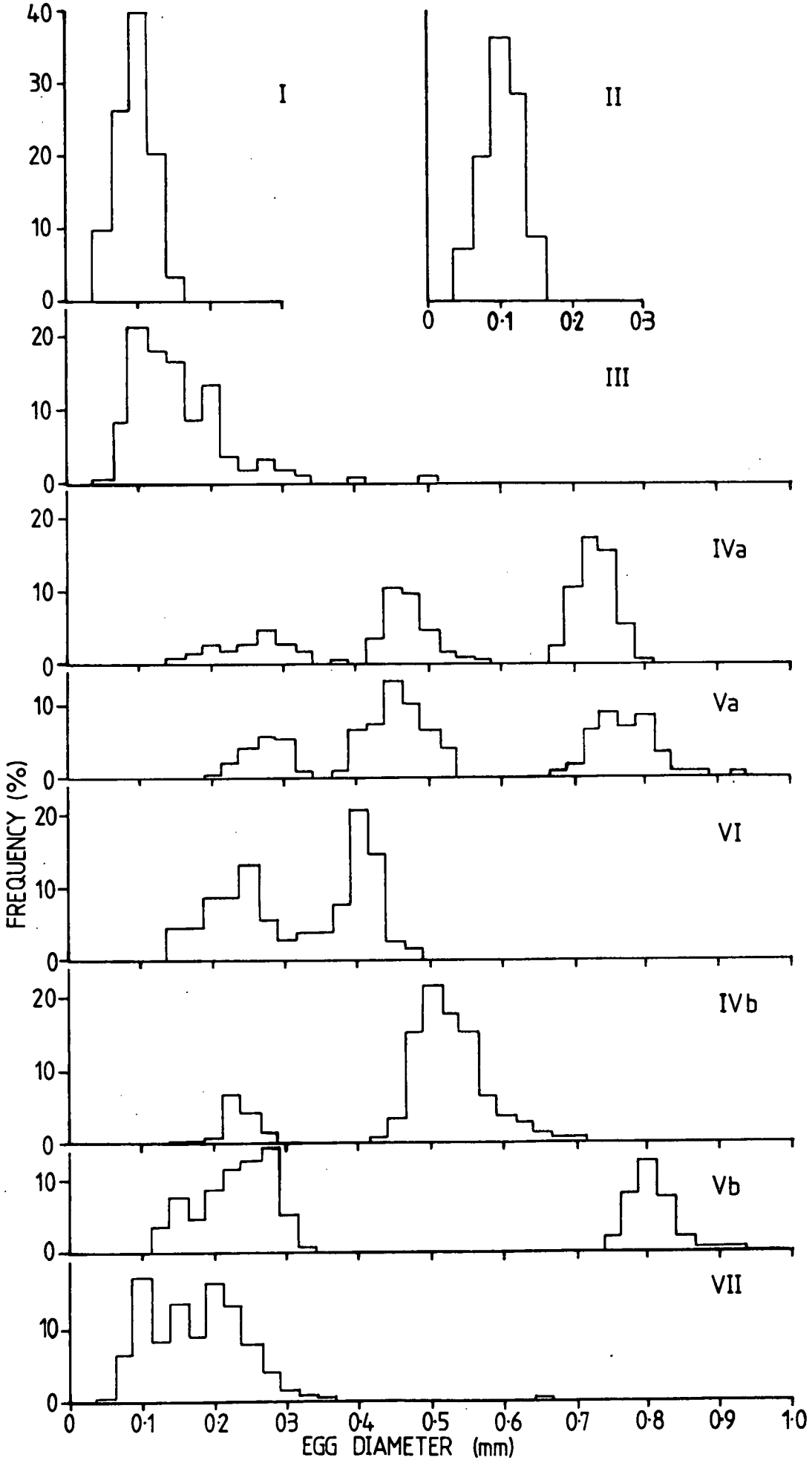
Few running-ripe females of either species were observed; presumably final maturation and spawning occurs quickly. The occurrence of mature females was therefore taken as indicative of spawning. *R. tapirina* thus have a prolonged spawning season with most spawnings occurring from June to October. A minor number of spawnings also probably occur as early as March. The length of the spawning season of *A. rostratus* could not be determined but spawning at least occurs during winter.

5.3.2 Ovarian Egg Diameters

The sequence of development of ova recognized in the ovaries of *R. tapirina* during a spawning season is shown in Figure 5.5. The nine stages of ova development are classified according to the maturity stage of the ovary, as described in Table 5.1. As not all fish were at the same maturity stage at any one time, the histograms were selected to show the representative sequence of events. Each histogram is representative of from two to four ovaries.

Immature ovaries (Stage I) contained transparent and yolkless primary oocytes of diameter 0.05 - 1.5 mm. During Stage III early maturing ova were evident from the additional smaller modes at 0.2 mm and 0.275 mm and a few ova of 0.4 mm and 0.5 mm diameter. These developing ova were granular and contained accumulations of yolk. In Stages IV to VI larger ova (generally >0.15 mm in diameter) only were measured so that their modes were more distinct. Primary oocytes, however, were relatively abundant. Mature ovaries (Stage IVa) contained three modes of larger ova at 0.275 mm, 0.45 mm and 0.725 mm diameter. These ova were granular and yellow or white in colour. The ova in running-ripe ovaries (Stage Va)

FIGURE 5.5 Egg diameter frequencies in *R. tapirina* ovaries of maturity stages I-VII. Maturity stages are described in Table 5.1. Primary oocytes were not counted in stages IV-VI.


I
II
III
IVa
Va
VI
IVb
Vb
VII
FREQUENCY (%)
EGG DIAMETER (mm)
0
0·1
0·2
0·3
0·4
0·5
0·6
0·7
0·8
0·9
1·0
10
20
30
40

were similar to those of Stage IVa except that the most advanced mode had increased slightly in size and ranged from 0.675 to 0.925 mm in diameter. Also, the larger eggs which ran freely from the ovary were transparent and contained oil droplets. These large eggs were fully developed as artifically fertilized eggs were of similar appearance and ranged in diameter from 0.73 - 0.87 mm (Chapter 6). Stage VI, the partially spent condition, had two modes at 0.25 mm and 0.4 mm. The more advanced mode continued to develop and by Stage IVb the two modes at 0.225 mm and 0.5 mm were completely separated. The second group of running-ripe ovaries, Stage Vb, contained two modes at 0.275 mm and 0.8 mm diameter; the latter mode contained transparent eggs. Completely spent ovaries, Stage VII, contained ova of diameter 0.05 - 0.35 mm and frequently a few large eggs which were in a state of degeneration and resorption. Ovaries then reverted to the resting Stage II and ova were of similar diameter to those in immature ovaries. Presumably ova of 0.15 - 0.35 mm diameter were resorbed.

Fewer *A. rostratus* were available for examination of ova diameters in ovaries of different maturity stages. The sequence of ova development is therefore not as clear. The six stages of ova development which were observed are shown in Figure 5.6 and are classified using the maturity stages of ovaries listed in Table 5.1. Early maturing ovaries (Stage III) contained a major mode of ova at 0.15 mm diameter and a smaller one at 0.25 mm. In late maturing ovaries, ova ranged in diameter from 0.125 mm to 0.8 mm but modes at 0.175 mm and 0.675 mm only were apparent. Primary oocytes were not measured in mature ovaries (Stage IV). Modes were not obvious in ova of diameter 0.175 - 0.75 mm in mature ovaries, but an advanced mode of 1.0 - 1.125 mm diameter was distinct as it was separated from the less-advanced ova. At the running-ripe Stage V the percentage of ova in the most advanced mode increased, and many ova were transparent and had oil droplets. Artifically fertilized eggs were of similar appearance and diameter 0.93-1.05 mm (Chapter 6) which implies that these larger transparent ova were fully developed. Stage VII, fully spent ovaries, contained ova of diameter 0.075 - 0.35 mm, mode 0.15 mm, and frequently a few large degenerating ova. In resting ovaries, Stage II, ova ranged in diameter from 0.05 mm to 0.2 mm; ova of diameter 0.225 - 0.35 mm were probably resorbed.

The egg development sequence of *R. tapirina* shows that they are

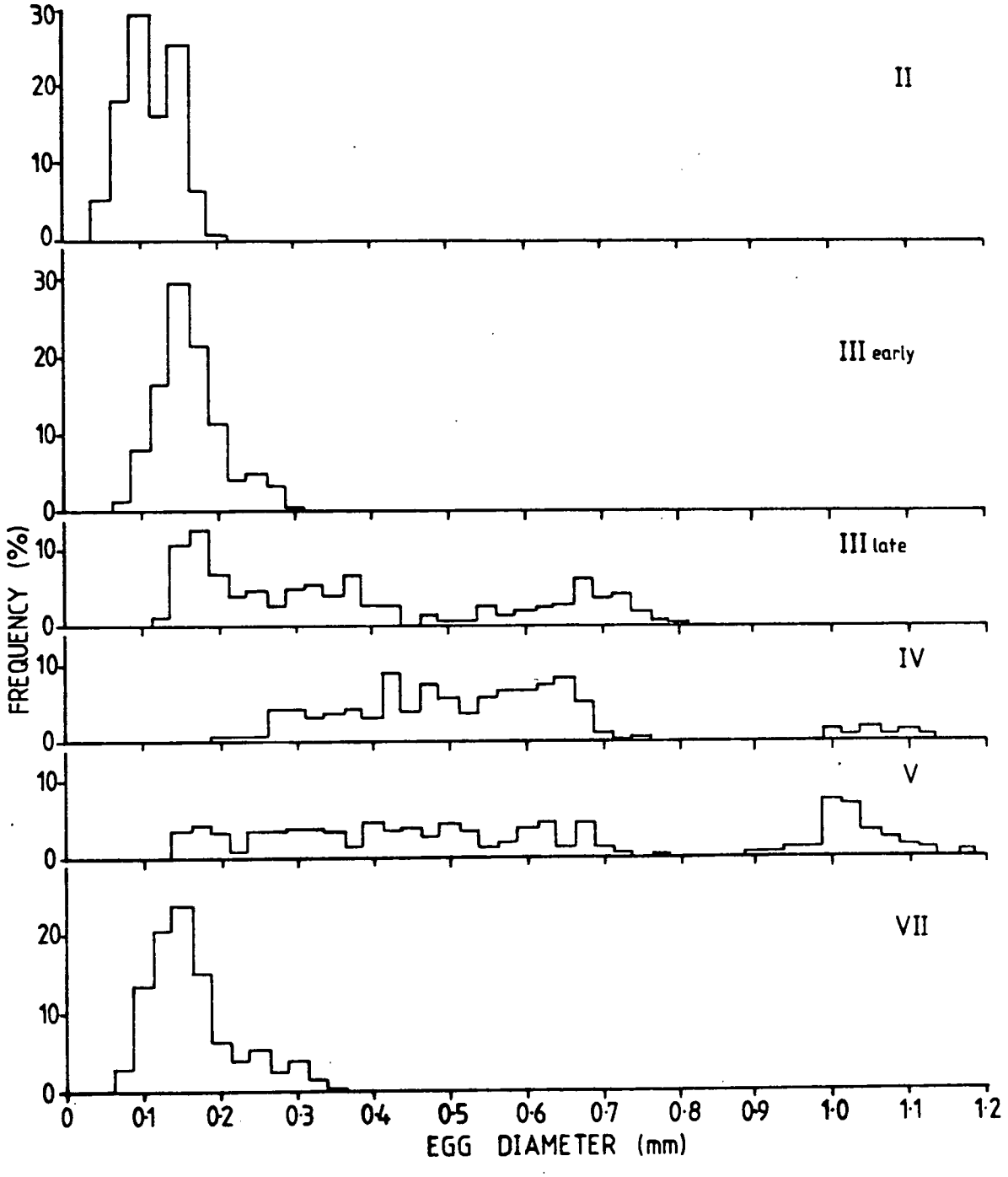


FIGURE 5.6 Egg diameter frequencies in *A. rostratus* ovaries of maturity stages II-VII. Maturity stages are described in Table 5.1. Primary oocytes were not counted in stage IV.

multiple spawners (Hickling and Rutenberg, 1936) with probably two, but possibly more, batches of eggs spawned every breeding season. The pattern of spawning in *A. rostratus* is not as clear due to an absence of multiple modes. They are also apparently serial spawners, possibly spawning more than twice each year.

5.3.3 Sex Ratios

Male *R. tapirina* were caught less frequently by spearfishing in shallow water than females. Chi-square analysis showed that the sex ratios were significantly different ($P<0.05$) from the expected 1:1 ratio in all months (Table 5.3). Although spearfishing is more selective for adult female than male flounder because a higher proportion of females are above the legal size limit of 23 cm, the differences in abundances of the sexes are sufficiently great to imply that they are due to factors other than just selective sampling. The percentage of males in the catch was higher during January to May and again in November than from June to October. The sex ratios in four seasons - January to March, April - June, July - September and October - November were compared using Chi-square analysis. They were significantly different in January - March, and in July - September, to all other seasons ($\chi^2 = 22.43$, 1 d.f., $P<0.001$; $\chi^2 = 14.51$, 1 d.f., $P<0.001$, respectively), but were not significantly different between April - June and October - November ($\chi^2 = 0.04$, 1 d.f.).

Females were also more abundant than males in 5-10 m depth in the two months when trawling was conducted at these depths and the sex ratios were significantly different. By contrast, males were more abundant than females and the sex ratios were significantly different in deeper water (10-25 m) in May, August and October; few fish were caught in July and the sex ratios were not significantly different.

Very few *A. rostratus* were caught in shallow water by spearing; more were caught in deeper water but the numbers were still low. Generally females were more abundant than males; the sex ratios in 5-10 m depth were significantly different in the two months sampled but not in 10-25 m depth in July.

TABLE 5.3 Sex ratios (male:female) of *R. tapirina* and *A. rostratus*
Levels of significance * 0.05<P<0.01, ** 0.001<P<0.01, *** P<0.001

		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
R. tapirina													
Spearing													
<2 m depth	n	18:116	18:36	6:20	10:84	6:33	.5:70	1:33	3:97	0:27	7:92	16:126	-
	ratio	1:6.4	1:2.0	1:3.3	1:8.4	1:5.5	1:14.0	1:33	1:32.3	-	1:13.1	1:7.9	-
	χ^2	71.67***	6.00*	7.54**	58.26***	18.69***	56.33***	29.12***	88.36***	27.00***	72.98***	85.21***	-
Trawling													
5-10 m depth	n					75:242		4:79					
	ratio					1:3.2		1:19.8					
	χ^2					87.98***		67.77***					
10-25 m depth	n					20:1		7:8	98:10		74:26		
	ratio					20:1		1:1.14	9.8:1		2.8:1		
	χ^2					17.19***		0.07	71.70***		23.04***		
A. rostratus													
Spearing													
<2 m depth	n	1:2	0	0	0:1	0	0:6	0	0:1	0	0	0:3	-
Trawling													
5-10 m depth	n					33:76		2:15					
	ratio					1:2.3		1:7.5					
	χ^2					16.96***		9.94**					
10-25 m depth	n					1:3		13:20	2:7		3:2		
	ratio							1:1.54	1:3.5		1.5:1		
	χ^2							1.49					

5.3.4 Length at First Maturity

The median length at which 50% of *R. tapirina* females were mature was 21.86 ± 0.36 cm and for males 19.04 ± 0.75 cm (Table 5.4). The smallest mature *A. rostratus* female caught was 20.5 cm and male 22.2 cm.

5.3.5 Fecundity

The fecundity of *R. tapirina* was estimated only from a small number of fish. Few fish in the subsamples had advanced ova but had not already spawned during the current breeding season. Fecundity ranged from 820,880 to 1,969,070 for lengths 24.7 cm to 34.3 cm and the fecundity per gram body weight ranged from 4343 to 5250. Relative fecundity when expressed as volume of eggs was from 1042 - 1260 $mm^3 g^{-1}$.

The relationship of fecundity and length only was examined to give an indication of the change in fecundity with size for the small sample of fish examined. A linear relationship between fecundity and length was apparent in Figure 5.7. This was expressed by the equation

$$F = -1053.65 + 85.85L$$

where F = fecundity in thousands of eggs and
L = length (cm).

The correlation coefficient r = 0.850 between F and L was significant ($P<0.001$, 12 d.f.). Analysis of variance showed that the slope of the regression line was significantly different from 0 ($F = 31.315$, 1 and 12 d.f., $P<0.001$). The regression accounted for 72.3% of the variation.

Fecundity estimates were possible for four *A. rostratus* only. They were 215,320 length 20.5 cm, 437,262 length 25.4 cm, 701,954 length 33.9 cm and 973,800 length 34.3 cm. The fecundity per gram body weight was from 1064 to 1810, and fecundity expressed as volume of eggs per gram body weight was from 571-972 $mm^3 g^{-1}$.

5.4 DISCUSSION

Maturity indices of females and maturity stages of both sexes of *R. tapirina* in different months indicate that they have a prolonged spawning season which occurs mainly from June to October. This is in agreement

TABLE 5.4 Length at first maturity of *R. tapirina*

Total Length (cm)	Females		Males	
	No.	% Mature	No.	% Mature
16	2	0	1	0
17	3	0	3	33.3
18	1	0	1	0
19	4	0	3	100
20	5	25	8	37.5
21	12	33.3	18	66.7
22	8	62.5	16	82.4
23	17	76.5	35	94.3
24	35	80.0	35	97.1
25	51	96.1	27	100
26	52	92.3	35	94.3
27	49	100	25	100
28	45	100	23	100
29	27	100	13	100
30	19	100	7	100
31	5	100	8	100
32	3	100		
33	5	100	1	100
34	4	100		
35	1	100		
36	2	100		
37	1	100		
L50	21.86 ± 0.36		19.04 ± 0.75	
95% confidence limits	20.84 - 22.55		16.44 - 20.28	
L95	25.50		24.06	
95% confidence limits	24.85 - 26.46		23.09 - 25.71	

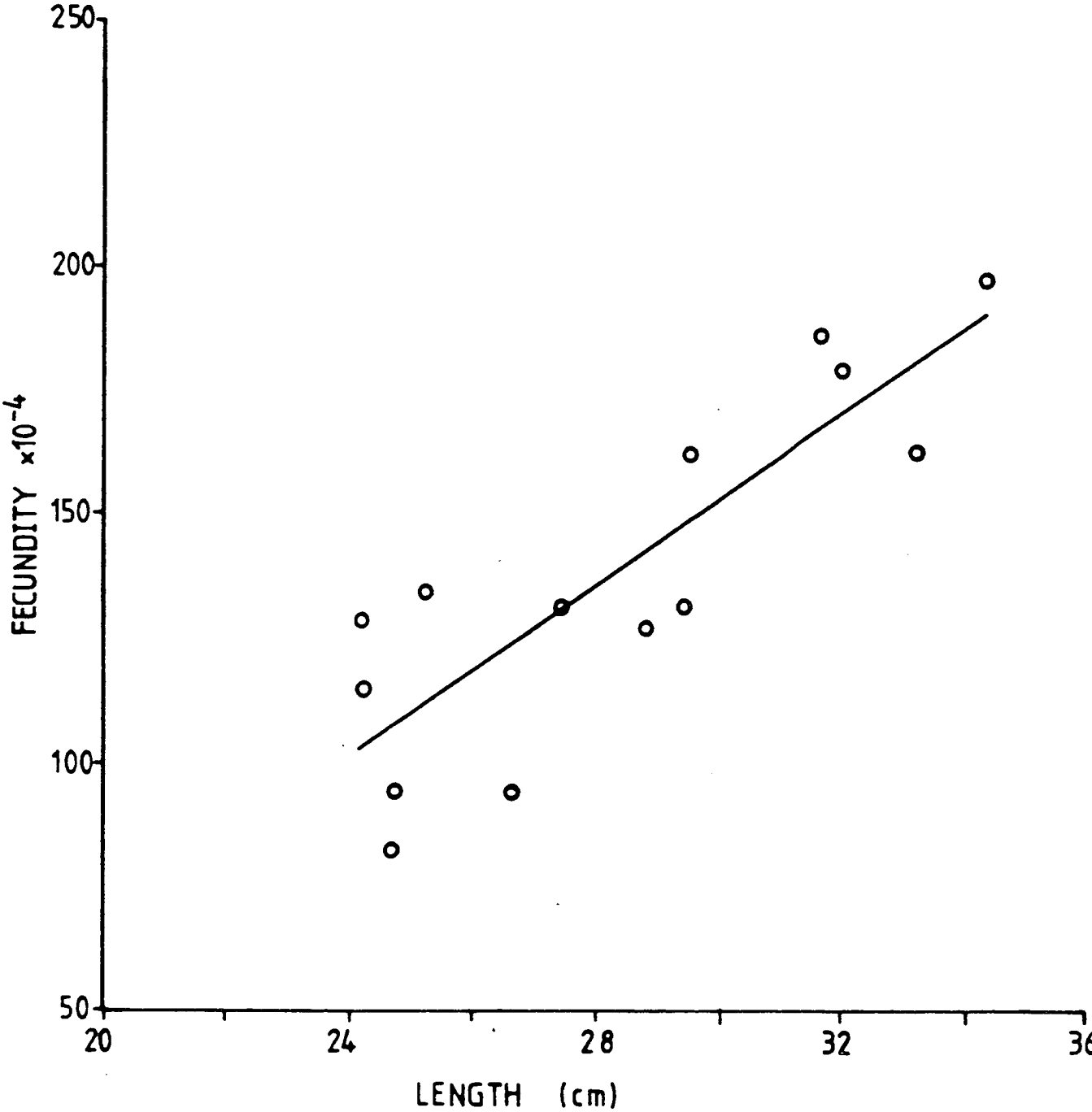


FIGURE 5.7 The relationship between fecundity and length in *R. tapirina*
The line was fitted by linear regression.

with the presence of larvae in the plankton from May to November and peak recruitment of newly-metamorphosed juveniles inshore during late winter to early summer (Chapter 2). Kurth (1957) also found from a study of egg diameter frequencies in different months that *R. tapirina* spawn during March to October. He suggests that temperature is an important factor in determining egg development and duration of the spawning season as eggs developed when the temperature was below 13°C.

The duration of the spawning season of *A. rostratus*, however, could not be determined because so few fish were caught; they at least spawn during winter. However, as they are apparently multiple spawners and as newly-metamorphosed juveniles occur inshore during many months of the year, they probably also have a prolonged spawning season.

The sequence of egg development in both *R. tapirina* and *A. rostratus* indicates that their prolonged spawning seasons are partially caused by serial spawning. Kurth (1957) observed a similar progressive development of the eggs of *R. tapirina* to that found in the present study but his interpretation of the sequence is confusing, probably because he did not record Stages VI, IVb and Vb.

Many other species of flatfish also spawn during winter or spring but few have prolonged spawning seasons or are serial spawners. For example, in the dab, *Limanda limanda*, only one batch of eggs develops synchronously each year and are spawned intermittently during a short spawning season in late winter to early spring (Htun-han, 1978). Similarly, plaice in the North Sea spawn predominantly during winter (Wimpenny, 1953) and have only one group of developing ova (Bagenal, 1966), and starry flounder, *Platichthys stellatus* have a definite and relatively short spawning season in winter (Orcult, 1950). *Rhombosolea plebia* in New Zealand have a long spawning season during winter and spring but apparently spawn only once each year as one group of maturing ova only were recognized in the ovaries (Coleman, 1973).

Serial spawning has been observed in *Paralichthys dentatus* (Morse, 1981) and *Psettodes erumei* (Ramanathan and Natarajan, 1979). These two species have at least two modes in the maturing egg diameter frequencies and a long spawning season of 5-6 months. Morse suggested from the ratio of the number of eggs in the most advanced mode to the total numbers of all

developing eggs that approximately six batches of eggs may be shed by each female *P. dentatus* in each season. The egg development sequence of these fish, however, was similar to that of *R. tapirina* except that only running-ripe eggs were separated by size from other developing eggs. This suggests that *P. dentatus* spawns two or three times each season depending on whether the least advanced group of developing eggs reach maturity. The Californian sand dab, *Citharichthys sordidus*, is probably also a multiple spawner as several size groups of ova were recognized in mature ovaries, but the spawning season appears to last for only three months (Arora, 1951).

The sex ratios of *R. tapirina* show that the major populations of males and females of this species are segregated in all months of the year. That these differences are not due to differential catchabilities of the sexes is shown by the results of trawling using the same gear in different depths; females were most abundant in 5-10 m depth and males in 10-25 m depth. Kurth (1957) also found that females dominated the catch in shallow water; he caught a maximum of 12.7% males in any one sample. The reasons for the differences in the distributions of the sexes are not known. Habitat preferences, including diet may differ between the sexes but these have not been examined.

The location of the spawning grounds of either species of flounder has not been determined. Kurth (1957) believed that *R. tapirina* spawn in shallow waters of estuaries and tidal rivers, and probably also in deeper offshore waters. He found no evidence for spawning migrations as abundances of adult *R. tapirina* inshore did not vary seasonally; this is supported by information from commercial fishermen. Kurth also concluded from tagging experiments that *R. tapirina* tend to remain within a restricted area. The number of fish recaptured was low and he suggested that high tagging mortality may have occurred. However, as almost no trawling was conducted at that time, tag returns were from spearing or beach-seining in shallow water only. Some fish may have moved into deeper water where they were not available for recapture.

The differences in the distributions of the sexes observed in this study imply that either males move into shallow water, or females into deeper water, for spawning. The sex ratios of flounder caught in shallow water, however, varied seasonally. The proportion of males, relative to

females, caught by spearing was lowest during the months of spawning and highest during the resting period. This indicates that males do not move inshore for spawning. Also a comparison of maturity stages of females caught in different depths in May, July and August showed that the highest percentage of mature females in any one month occurred in deeper water.

These results, in conjunction with the generally greater abundance of larvae and their small size nearer the mouths than further up estuaries, suggest that spawning occurs predominantly in deeper offshore waters. As the spawning season is prolonged, there may be regular movements of individual females between the inshore region and spawning grounds, thus masking an obvious spawning migration. However, as many mature females are caught in shallow water, they either do not move far or can remain in the mature condition for some time. A minor number of spawnings may also occur in shallow water.

Insufficient data were collected on *A. rostratus* to determine seasonal migrations or spawning grounds. However, they also possibly spawn in deeper water as they were caught more frequently by trawling than spearing in the few months that trawling was conducted. Also, a high percentage of the trawl catch in July consisted of mature fish. Lenanton (1974) classified *A. rostratus* as an estuarine-marine species on the basis of its field distribution, but observed that it was also capable of breeding within a system which had been closed to the sea for several years.

Many other species of flatfish make seasonal migrations from coastal and estuarine waters to offshore spawning grounds. For example, *Rhombosolea plebia* and *R. leporina* in New Zealand migrate to nearby deeper water during the spawning season and then back into shallow water after spawning (Coleman, 1973). The distributions of male and female *R. leporina* during winter and spring showed similarities to those of *R. tapirina*. Although females moved away from the inshore region, they were generally more abundant in shallower water than on the spawning grounds, even during the spawning season. Males, however, were concentrated on the spawning grounds at this time (Coleman, 1974a).

The majority of male and female *R. tapirina* were mature before they reached the minimum legal size limit of 23 cm. Nearly all fish would

therefore be capable of spawning before reaching the commercially exploitable size. Of the few *A. rostratus* caught, the majority of both males and females above the legal size limit of 23 cm were mature.

Kurth (1957), however, found that 60% of female *R. tapirina* were mature at 24 cm total length. He caught few males during the spawning season but all longer than 19 cm were mature. Kurth used the criterion of ova diameter of 0.35 - 0.8 mm during the spawning season to indicate maturity in females. The differences in size at maturity of female *R. tapirina* between this study and that of Kurth (1957) may be due, therefore, to the different methods of determining maturity or because the size at maturity possibly varies between year classes. Significant yearly and geographical variations in size and age at maturity have been observed in several temperate species of flatfish (Roff, 1982). Similarly, Morse (1981) found that the size at maturity of *Paralichthys dentatus* changed significantly during a six year study. Thus further work is required on the variation in size at maturity between year classes of *R. tapirina*, as well as on the total fishing effort in relation to population size, before the adequacy of the present size limit regulations in protecting spawning stocks of *R. tapirina* can be ascertained.

Fecundity in many species of fish increases in the same proportion as weight or at a power close to the cube of length (Bagenal, 1978). In *R. tapirina* this relationship was linear for the small sample size. Further samples are required to test this linear relationship. *R. tapirina* were considerably more fecund than the four *A. rostratus* examined. They are also apparently more fecund than many other species of flatfish including the closely related species, *R. plebia* and *R. leporina*, in New Zealand (Coleman, 1973). Jones (1974) and Roff (1982) compared the fecundities of ten and twelve species of flatfish, respectively, seven of which were common to both studies. They found large variations in fecundity between the species and Jones (1974) observed that the relative fecundity i.e. number of eggs per gram body weight also varied considerably. The interspecific variation, however, was much less when egg volume was taken into consideration. For example, the mean volume of eggs (mm^3) per mean body weight (g) of the ten species examined by Jones (1974) ranged from 535 to 922 $mm^3 g^{-1}$. Most of these species are thought to spawn only once each season. The relative fecundity, in terms of egg volume, estimated for *A. rostratus* is mostly within this range whilst that of *R. tapirina*

is slightly higher. The serial spawners *Paralichthys dentatus* (Morse, 1981) and *Psettodes erumei* (Ramanathan and Natarajan, 1979) also have relative fecundities which are mostly within this range.

The difficulties involved in determining the fecundity of multiple spawners has been discussed in some detail by Macer (1974) and Bagenal (1978). They both consider that a major problem is how to identify ova which are potentially capable of release in the current spawning season. In *R. tapirina* and *A. rostratus*, as well as in the horse mackerel, *Trachurus trachurus*, studied by Macer (1974), developing and resting (i.e. non-developing) ova are not clearly separated by size. The presence of yolk in ova is a commonly used criterion of egg development and hence spawning in the current season (Bagenal, 1978). This criterium, however, may not be appropriate for the two flounders studied because completely spent ovaries of both species contained some yolky ova which apparently were resorbed. The resorption of developing ova, therefore, could result in a substantial difference between fecundity and fertility, i.e. number of eggs shed. Macer (1974) also found that fecundity estimations were complicated by the resorption of ova both before and after spawning, although he was referring mainly to the degeneration of ova with advanced yolk formation.

Further work is required to show that additional oocytes are not recruited to the yolky stock in ovaries past Stage III or IVa, i.e. the stage at which fecundity is estimated. The proportion of ova which develop yolk but do not complete maturation and are ultimately resorbed also needs to be determined. This would indicate whether a more suitable criterium of egg development is required, in particular for fecundity and fertility to be similar.

In summary, *R. tapirina* and *A. rostratus* apparently have a similar reproductive strategy of a prolonged spawning season, serial spawning and comparatively high fecundity. They also mature for the first time at approximately the same length. As suggested by Morse (1981), this strategy tends to maximise reproductive potential and avoid catastrophe. The long spawning season reduces larval crowding and increases the chances of at least a proportion of eggs and larvae encountering favourable environmental conditions.

CHAPTER 6

DEVELOPMENT, GROWTH AND SURVIVAL OF EGGS AND LARVAE OF *RHOMBOSOLEA TAPIRINA* AND *AMMOTRETIS ROSTRATUS*

6.1 INTRODUCTION

During the past three decades considerable research has been conducted world-wide into the cultivation of marine fishes, in particular of commercially important species. The hatchery method of rearing flatfish eggs and larvae was developed by Shelbourne (1964, 1975) who successfully reared large numbers of plaice (*Pleuronectes platessa*). However, attention recently has centred on rearing more valuable species of flatfish such as the turbot (*Scophthalmus maximus*) and the Dover sole (*Solea solea*). The history of the marine hatchery movement has been described by Shelbourne (1964) and research on marine fish cultivation reviewed by Kinne (1977).

In Australia, however, there have been few studies on the cultivation of marine fishes or the identification of marine fish eggs and larvae. *Rhombosolea tapirina* is the only pleuronectid species found in Tasmanian waters in which the eggs and larvae have been studied; Kurth (1957) described mature, unfertilized ova and Roper (1979) the late larval stages caught in plankton tows in New Zealand.

Surprisingly, the culture of this species was probably first attempted early this century when Dannevig hatched and liberated 20 million flounder larvae into Gunnamatta Bay, N.S.W. in 1906 (Lockyer, 1915). Closely related species in New Zealand, the lemon sole (*Pelotretis flavilatus*) and sand flounder (*Rhombosolea plebia*) were cultured to the first feeding larvae stage by Rapson (1940) and Robertson and Raj (1971) respectively.

In this study, *R. tapirina* and *Ammotretis rostratus* were cultured for three reasons:

(1) to provide information on culture methods, and survival and growth rates of larvae, which may be used to develop the commercial cultivation of flounder;

(2) to provide descriptions of the developmental stages of eggs and larvae to complete life history studies and to assist identification of planktonic stages;

(3) to provide larvae and juveniles for experiments on environmental factors affecting habitat selection and habitat partitioning (Chapter 3).

6.2 METHODS

6.2.1 Broodstock

Mature flounder were collected either in Danish seine nets of commercial fishing vessels in 5-20 m depth in Frederick Henry Bay and Storm Bay or by beach seining in the River Derwent in depths of up to 1.5 m. Beach seining was preferred as mature females were less damaged. The broodstock were maintained for up to several months in a 4000 ℓ swimming pool with running seawater and aeration. They were fed every 2-3 days on live polychaetes (*Nereis* sp.) or chopped *Mytilus edulis* flesh.

6.2.2 Artificial Fertilization

Mature sperm were readily obtained by stripping ripe males. Eggs were stripped from ovaries which had hydrated and ovulated either naturally or after hormonal treatment. Only the latter method provided consistent quantities of mature eggs. Eggs spawned in the broodstock pond or stripped from mature females (the ovaries were swollen and eggs readily exuded by gentle pressure) could not be fertilized.

Human chorionic gonadotropin (HCG) was used to induce ovulation following methods similar to those of Smigielski (1975a, 1975b). 2000, 1000 or 500 iu kg^{-1} were dissolved in 1 mℓ of sterilized seawater and injected intramuscularly in mature and maturing flounder. Eggs were artificially fertilized according to the methods of Riley and Thacker (1969).

6.2.3 Larval Rearing

Eggs and larvae were mainly cultured in 250 ℓ fibreglass cylinders which contained either static seawater or flowing seawater entering at 20 ℓ h^{-1}; water flow was stopped for several hours when food was added.

Dead eggs and larvae were siphoned off the tank bottom and counted every 1-2 days. Approximately one-third of the total volume of static water tanks was replaced with fresh seawater every second day and, when necessary, this was first warmed to room temperature.

Rearing tanks were aerated continuously from the bottom and illuminated by natural daylight plus fluorescent tubes during the working day. Seawater of salinity 32.6 - 34.5‰ was filtered to 1 µm and exposed to sterilizing ultraviolet light; all equipment used was chemically sterilized in a weak sodium hypochlorite solution. Static water tanks were kept at either ambient seawater or room temperature.

Larvae were fed initially on the rotifer *Branchionus plicatilis*, followed by freshly-hatched *Artemia salina* nauplii. Rotifers were concentrated daily from one-quarter of the total volume of the rotifer cultures and added to rearing tanks. Microalgae, *Chlorella* sp., *Monochrysis lutheri* and *Phaeodactylum tricornutum* were added daily to the rotifer cultures. The methods used for the cultivation of algae, rotifers and *Artemia* are described in Appendix 6.

6.2.4 Stages of Development and Growth

Approximately 50 eggs were scanned at each examination to determine the average stage of development. After hatching, up to 70 larvae were sampled at increasing time intervals. Larvae were measured either directly after anaesthetization in tricaine methanosulfonate solution or from silhouette photographs (Neave and Batty, 1982). The larval length was measured from the tip of the lower mandible to the end of the caudal fin.

Larval development stages are based on those developed by Ryland (1966) for plaice, however in *R. tapirina* the migration of the left eye and flexion of the notochord occur together so Ryland's stages 3 and 4

are combined.

6.3 RESULTS

6.3.1 Cultivation of Flounder

(i) Hormonal Induction of Ovulation

Injections of 500 iu kg^{-1} HCG and stripping eggs 3-4 days later produced fertilizable eggs in both *R. tapirina* and *A. rostratus* (Table 6.1). Higher dosages were not successful. Other trials were conducted with groups of two maturing *R. tapirina* females which were injected with 500 iu kg^{-1} HCG once only and three times 2 days apart, or once with 1000 iu kg^{-1}, but none hydrated and ovulated.

(ii) Rhombosolea tapirina

In the 1981 rearing trials, 85% of stripped eggs were fertilized and almost 75% of these hatched. Approximately 24,000 larvae were fed *c.* 0.6 rotifers $m\ell^{-1}$ daily from day 5-12 but by day 13 all larvae were dead, presumably due to an inadequate food supply.

In 1982 about 85% of stripped eggs were fertilized and incubated in three tanks at different densities (Table 6.2). From the time of first feeding, larvae were reared in tanks 1 and 2 only at a reduced density which ensured that adequate food was available to support larval growth. Rotifers only were fed to larvae from day 5 to 20 at a mean daily concentration of 1.6 rotifers $m\ell^{-1}$. Between days 21-32 the concentration of rotifers was decreased, and *Artemia* nauplii increased to 0.5 - 1 nauplii $m\ell^{-1}$. This density of nauplii was maintained until after metamorphosis. The change in diet was achieved much faster in tank 2 than tank 1. At ambient seawater temperature (11.1 - 13.8°C) larvae metamorphosed after approximately 65 days at a mean length of 8.83 mm; at ambient room temperature from day 5 after hatching (12.7 - 17.7°C) metamorphosis occurred between days 44-53 (Table 6.3, Figure 6.1).

Survival rates from first-feeding to metamorphosis, determined by counting the number of dead larvae, were high in both tanks (Table 6.2).

TABLE 6.1 Percentage egg fertilization after treatment with human chorionic gonadotropin (HCG)

	HCG ($iu.kg^{-1}$)	No. Fish Treated	No. Fish Ovulated	Days to Stripping	% Eggs Fertilized	Comments
Rhombosolea tapirina	2,000	2	2	4	10	ovaries grossly swollen
	1,000	2	1	6	0	eggs probably overripe
Ammotretis rostratis	500	2	1	4	84	58-70% eggs hatched
	500	2	2	3	90	75% eggs hatched

TABLE 6.2 Density of eggs and larvae, culture systems used and survival rate to metamorphosis for *R. tapirina* in 1982

Tank	Type of System until 1st Feeding	Density eggs ℓ^{-1}	Hatching Success (%)	No. of Larvae from 1st Feeding[a]	Density Larvae ℓ^{-1}	Type of System from 1st Feeding	Survival Rate to Metamorphosis (%)
1	static water, ambient S.W. temp.	83	58	5,000	16	static water, ambient S.W. temp.	94
2	running seawater	137	-[b]	2,500	10	static water, room temp.	98
3	running seawater	69	70				

[a] Feeding larvae were reared in tanks 1 and 2 only.

[b] An unknown number of eggs were lost due to technical problems.

TABLE 6.3 Growth in length and stages of development of *R. tapirina* larvae at different temperatures in 1982

Tank 1 Ambient Seawater Temperature						Tank 2 Ambient Room Temperature					
Days from hatching	Sample size	Mean length (mm)	S.D.	Developmental stage[a]	Temperature (°C)	Days from hatching	Sample size	Mean length (mm)	S.D.	Developmental stage[a]	Temperature[b] (°C)
0	11	2.00	0.26	1a	11,9	0	11	2.00	0.26	1a	11.9
1	31	2.08	0.20	1b	11.7	1	31	2.03	0.20	1b	11.7
2	21	2.39	0.12	1b'	11.6	2	21	2.39	0.12	1b'	11.6
3	15	2.54	0.14	1c	11.6	3	15	2.54	0.14	1c	11.6
4	9	2.70	0.17	1c'	11.5	4	9	2.70	0.17	1c'	11.5
5	20	2.71	0.13	1d	11.4	5	14	2.73	0.14	1d	12.7
8	13	2.67	0.13	1d	11.4	8	7	2.73	0.10	2a	15.8
10	9	2.77	0.19	2a	11.2	10	5	2.90	0.14	2a	15.6
12	10	2.88	0.18	2a	11.1	12	11	3.09	0.19	2a	15.5
18	4	3.17	0.28	2a'	11.2	18	11	4.15	0.24	2a'	16.3
20	18	3.64	0.24	2a'	11.5	20	23	4.50	0.33	2b	15.5
26	38	4.40	0.50	2b	11.6	26	31	5.39	0.61	3a',4a'	16.0
29	5	4.54	0.40	2b	11.9	29	5	5.40	0.71	3b,4b	17.6
40	68	5.32	0.64	3b,4b	12.5	40	60	7.66	0.55	5	17.1
44	5	5.99	0.37	3b,4b	12.7	44	7	8.16	0.49	5	17.5
63	24	8.22	0.83	5	13.3	53	18	10.02	0.84	M	
65	24	8.83	1.61	5-M	13.8	61	50	12.15	0.81	M	17.5
						76	18	14.02	1.25	M	17.2
						83	3	14.15	1.63	M	
						88	19	16.74	3.02	M	17.7

M = metamorphosed

[a] = see text for explanation

[b] = Days 0-4 at ambient seawater temperature

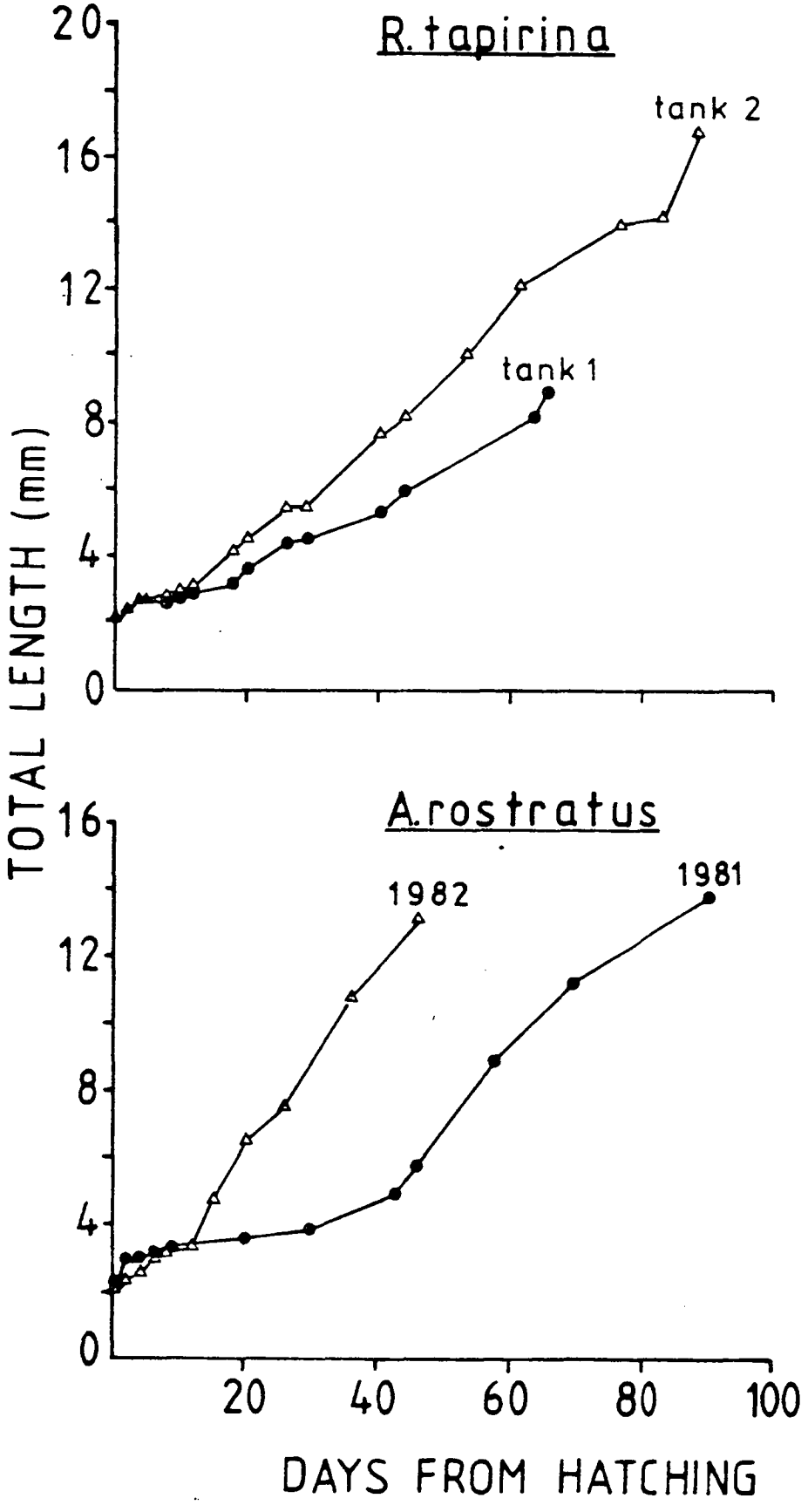


FIGURE 6.1 Growth in length of *R. tapirina* and *A. rostratus* larvae from hatching to post-metamorphosis at different temperature regimes

The greatest mortality (4%) occurred from day 36 to 39 amongst the smallest larvae in tank 1; they probably had not accepted the diet of *Artemia* nauplii. About 10% of the larvae were unpigmented except for the eyes, but their growth and survival rates seem unaffected.

The variation in length of larvae was high at metamorphosis in tank 1 which contained the greatest density of larvae and least food per larvae. A wide range in lengths occurred only after metamorphosis in tank 2 when the juveniles were weaned on to a diet of dried food, as described below. Similarly, the time taken for all larvae to undergo metamorphosis in tank 1 was 28 days compared with 7 days in tank 2.

Attempts were made to wean newly-metamorphosed juveniles from tank 2 onto a dried food diet of 0.5 mm trout starter crumbles. Three weaning trials were conducted:

(1) abrupt change to dried food,

(2) frozen and freeze-dried *Artemia* were fed for 10 days, followed by trout crumbles and freeze-dried *Artemia* for 10 days, and then trout crumbles only. During this transition period, small quantities of live *Artemia* were added every second day, and

(3) live *Artemia* were slowly decreased and dried food of trout crumbles and freeze-dried *Artemia* increased in the diet over four weeks.

All juveniles of trial 1 died and approximately 30% of the juveniles in trials 2 and 3 died after 7-10 days on dried food only. The remaining juveniles and all of tank 1 were then transferred to a private fish-farming hatchery for ongrowing.

(iii) Ammotretis rostratus

In 1981 eggs and larvae were reared in static and flowing seawater tanks; approximately 75% of fertilized eggs hatched in both tanks. Larvae were fed *c.* 0.8 rotifers.mℓ^{-1} from days 5-7 after hatching when most larvae, including unfed controls, died. The mortality was lower in the static water tank, presumably due to the rotifer food supply

being conserved. The few remaining larvae were reared in this tank and 65 animals reached metamorphosis.

In 1982, eggs and larvae were cultured in static water at room temperature. Eggs and milt were obtained from adults in poor condition; about 50% of the eggs were fertilized and 50% of these hatched. Although larval survival rates were high (*c.* 65%) and growth rates appeared normal, many larvae developed abnormally.

Hatching occurred after 93-105 h at ambient seawater temperature (12.5 - 12.7°C) in 1981 and after 61-69 h in 1982 at room temperature (16.2 - 17.1°C). Larvae metamorphosed after *c.* 69 days at a mean length of 11.2 mm in 1981 at temperatures of 12.7 - 16.5°C, and after *c.* 36 days, mean length 10.7 mm in 1982 at 16.0 - 17.4°C (Table 6.4, Figure 6.1).

6.3.2 Egg and Larval Development of *R. tapirina* and *A. rostratus*

The developmental stages at ambient seawater temperature are detailed for *R. tapirina* and summarized for *A. rostratus;* the lengths of larvae at most developmental stages are listed in Tables 6.3 and 6.4 respectively.

(i) Rhombosolea tapirina

Embryonic Development

Mature, unfertilized eggs were almost transparent with a brown tint and smooth chorion. They ranged in diameter 0.75 - 0.99 mm and contained 1-7 oil droplets (Table 6.5, Figure 6.2.1). Most fertilized eggs floated at the water surface and dead or dying eggs sank to the tank bottom.

After fertilization, the brown tint vanished, a small perivitelline space developed and a small depression, probably the micropyle, quickly disappeared (Figure 6.2.2). After *c.* 1.5 h the blastodisc formed (Figure 6.2.3). The first cleavage was completed after 2 - 2.5 h (Figures 6.2.4, 6.2.5), second by 2.5 - 3 h (Figure 6.2.6), third by

TABLE 6.4 Growth in length and stages of development of *A. rostratus* larvae at different temperatures

1981 Ambient Seawater Temperature						1982 Ambient Room Temperature					
Days from hatching	Sample size	Mean length (mm)	S.D.	Developmental stage[a]	Temperature (°C)	Days from hatching	Sample size	Mean length (mm)	S.D.	Developmental stage[a]	Temperature (°C)
0	26	2.32	0.29	1a	12.8	0	8	2.01	0.30	1a-1b	17.2
1	12	2.38	0.37	1b	12.8	1	4	2.08	0.26	1b	17.3
2	9	2.89	0.21	1b'	12.7	2	4	2.39	0.17	1c	17.0
3	20	2.83	0.18	1c	12.7	4	8	2.43	0.17	1d	16.9
4	6	2.89	0.17	1c'	12.7	6	5	2.98	0.22	2a	16.4
5	25	2.96	0.23	1d	12.7	8	7	3.16	0.19	2a	16.0
6	6	3.09	0.20	1d	12.7	12	4	3.24	0.17	2a'	17.4
9	7	3.28	0.31	2a	12.9	15	3	4.68	0.61	2b	17.2
20	4	3.57	0.36	2a	13.1	20	4	6.45	0.96	3a	17.4
30	5	3.82	0.43	2a	13.9	26	36	7.72	1.39	3c	17.0
43	4	4.86	0.44	3a	13.8	36	55	10.72	1.74	5-M	16.1
46	6	5.75	0.89	3a'	14.3	46	50	12.95	1.65	M	16.7
57	8	8.76	1.34	3b'	14.4						
69	16	11.21	2.43	5-M	15.3						
90	7	13.86	2.83	M	16.5						

M = metamorphosed

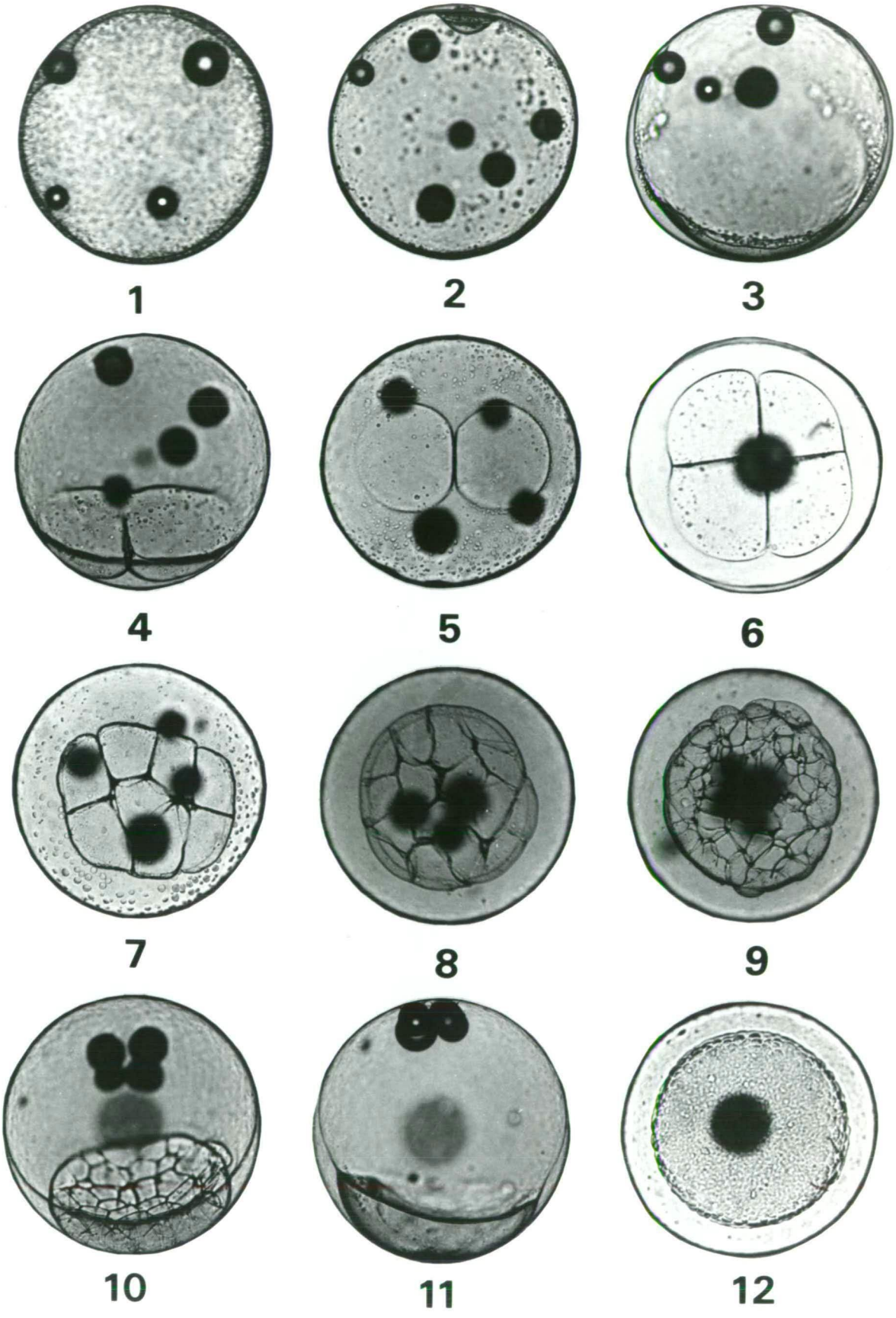
[a] = see text for explanation

TABLE 6.5 Egg diameters and number of oil droplets in eggs of *R. tapirina* and *A. rostratus*

	Adults		Eggs					Oil Droplets			
	Date of capture	Total length (cm)	F/U[a]	Sample size	Mean diameter (mm)	Range	S.D.	Mean number	Range	Mean diam. largest oil droplet (mm)	Range
Rhombosolea tapirina	7-8-80	28.6	U	68	0.80	0.75-0.99	0.06	1.0	1	0.18	0.17-0.19
	23-8-80	37.4	F	79	0.77	0.74-0.87	0.02	1.1	1-2	0.17	0.15-0.19
	2-9-81	29.2	F	22	0.75	0.73-0.75	0.07	1.3	1-2	-	-
	24-8-82	31.5	U	48	0.76	0.70-0.83	0.03	1.4	1-5	0.15	0.13-0.17
	24-8-82	31.5	F	67	0.75	0.73-0.81	0.02	2.9	1-7	0.12	0.10-0.16
Ammotretis rostratus	24-7-81	28.2	U	72	1.03	0.90-1.18	0.05	2.4	1-13	-	-
	28-9-81	28.4	F	52	0.99	0.93-1.05	0.03	17.3	6-53	0.14	0.63-0.35
	1-11-82	29.3	U	45	0.99	0.94-1.02	0.02	5.8	1-17	-	-
	16-10-80	32.9	U	20	1.02	0.80-1.12	0.08	55.0	50-67	-	-

[a] F = fertilized, U = unfertilized

FIGURE 6.2 Embryonic development of *Rhombosolea tapirina*. The stages of development in photographs 1-24 are described in the text.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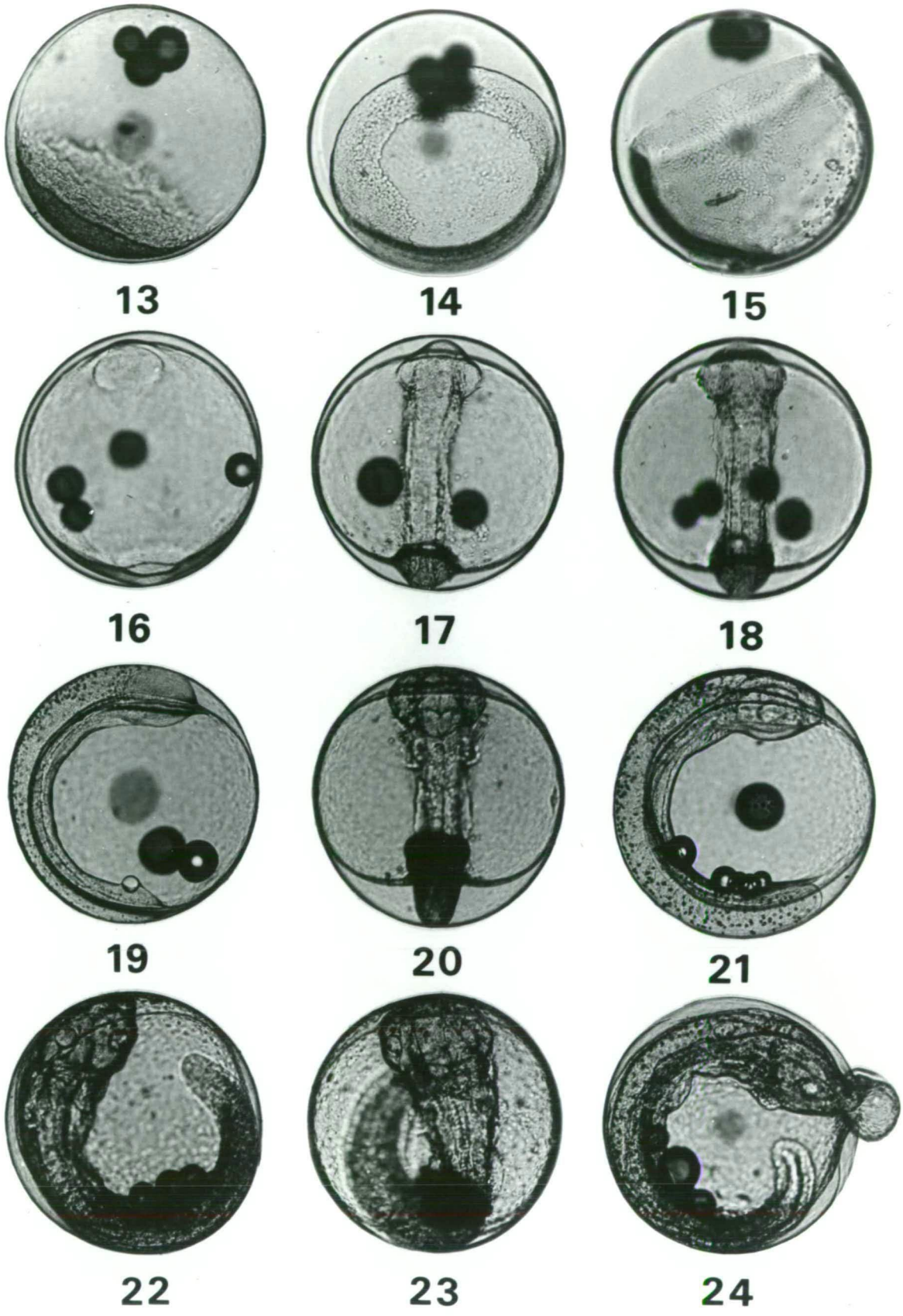
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3 - 3.5 h (Figure 6.2.7) and fourth by 4 - 4.5 h (Figure 6.2.8). After 5.5 h and fifth cleavage, the blastoderm was 2 cell layers in thickness (Figure 6.2.9). Further cleavage was difficult to follow as the cell boundaries became indistinct (Figure 6.2.10).

At 6 - 9 h the blastodermal cap formed (Figure 6.2.11); the peripheral periblast was visible after 11 - 15 h (Figure 6.2.12) and the blastodermal cap flattened out over the yolk during 15 - 20 h (Figure 6.2.13). After 21 - 24 h the germ ring and start of the embryonic shield were visible (Figure 6.2.14). The blastoderm covered over one-half of the yolk, the embryonic shield was larger and neural keel was obvious after 25 - 30 h (Figure 6.2.15). The blastopore closed at 30 - 36 h (Figure 6.2.16); the cephalic region and embryonic axis were also developing (Figure 6.2.17).

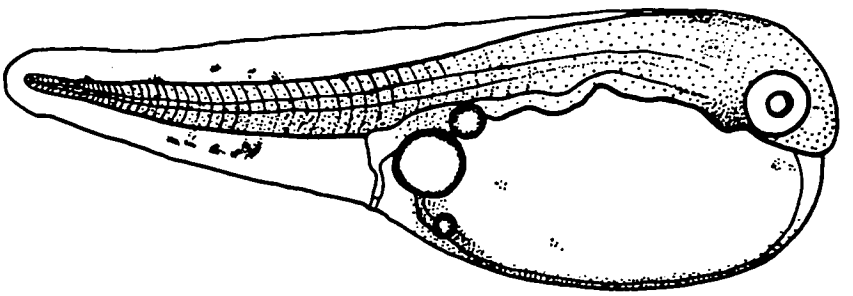
At 40 - 45 h the first somites and Kupffer's vesicle were visible (Figure 6.2.18). Pigmentation had developed on the embryo but not on the yolk and 10 - 15 somites were apparent after *c.* 50 h (Figure 6.2.19). After 55 - 65 h three primary divisions of the brain, optic lenses and auditory capsules were visible and the pericardial cavity was developing (Figures 6.2.20, 6.2.21). The heart was seen beating after 72 - 78 h and twitching movements of the embryo, which had developed many more chromatophores, were observed. The pectoral fins and the finfold at the tip of the curled tail were developing and otoliths were visible (Figure 6.2.22). Just before hatching, the embryo almost surrounded the yolk and movements were more vigorous (Figure 6.2.23). Hatching occurred 82 - 93 h after fertilization (Figure 6.2.24).

Larval Development

Stage 1 - yolk sac present

Newly hatched larvae (mean length 1.92 mm, smallest 1.45 mm) floated passively at the surface with yolksac uppermost and wriggled occasionally. The yolksac measured 0.85 x 0.51 mm, mouth and jaws were not visible and about 35 somites were developed (Stage 1a, Figure 6.3.1). One day posthatching the yolksac measured 0.69 x 0.39 mm. Two bands of dentritic chromatophores on the dorsal finfold above the midgut region and half-way along the finfold and one band on the ventral finfold just

FIGURE 6.3 Larval development of *Rhombosolea tapirina*. The stages of development in drawings 1-9 are described in the text.



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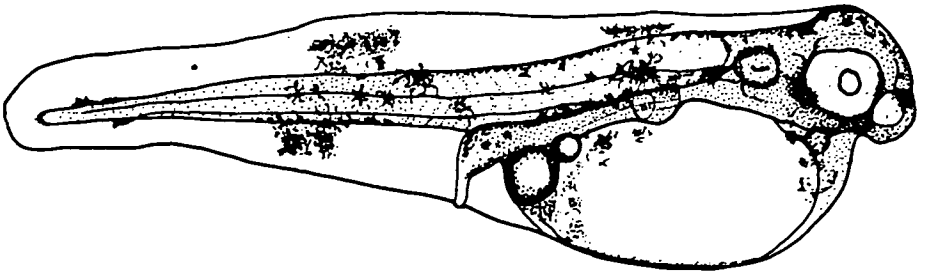

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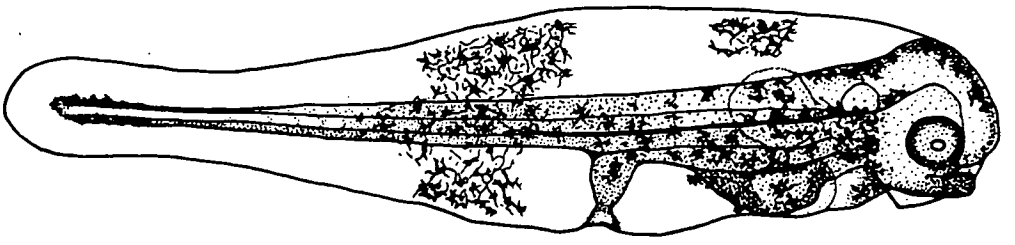

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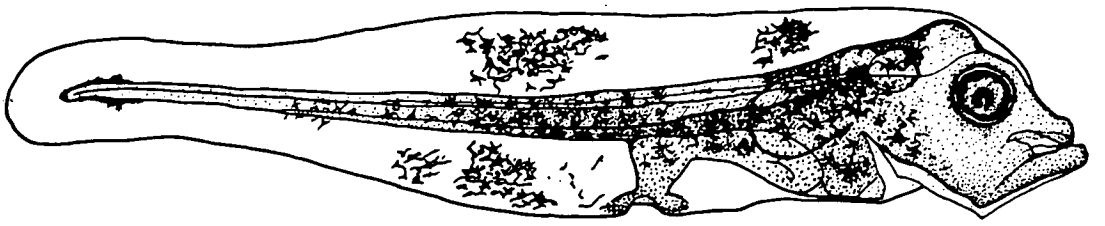

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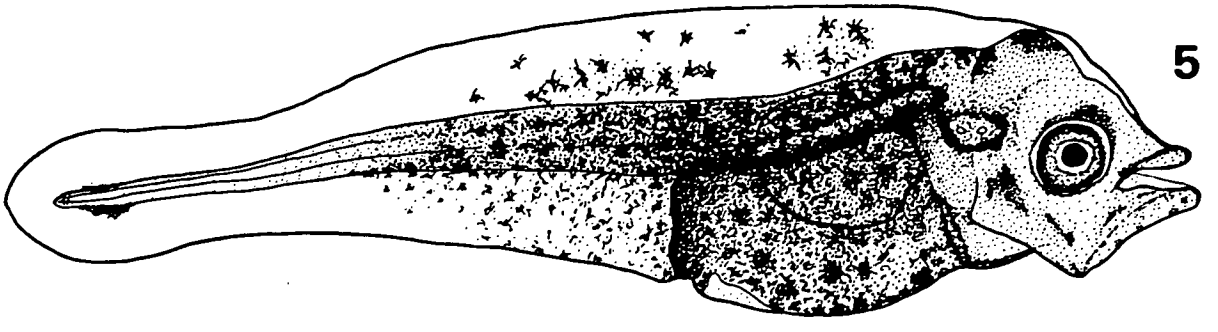

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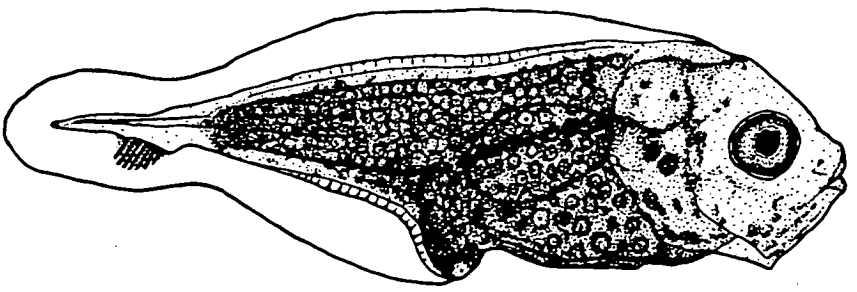

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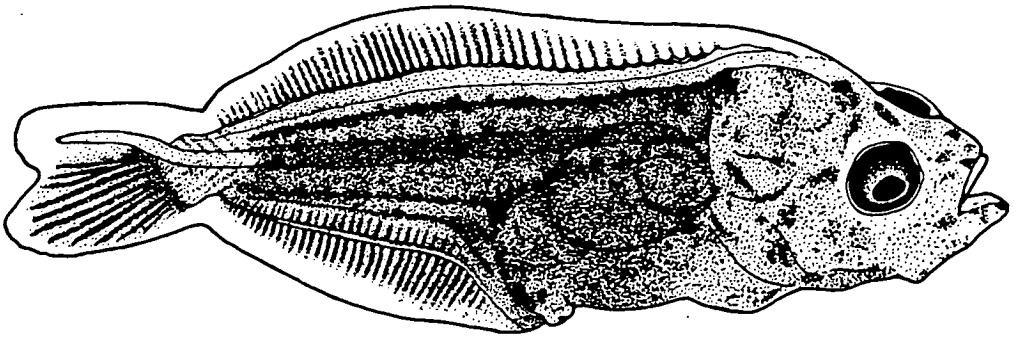

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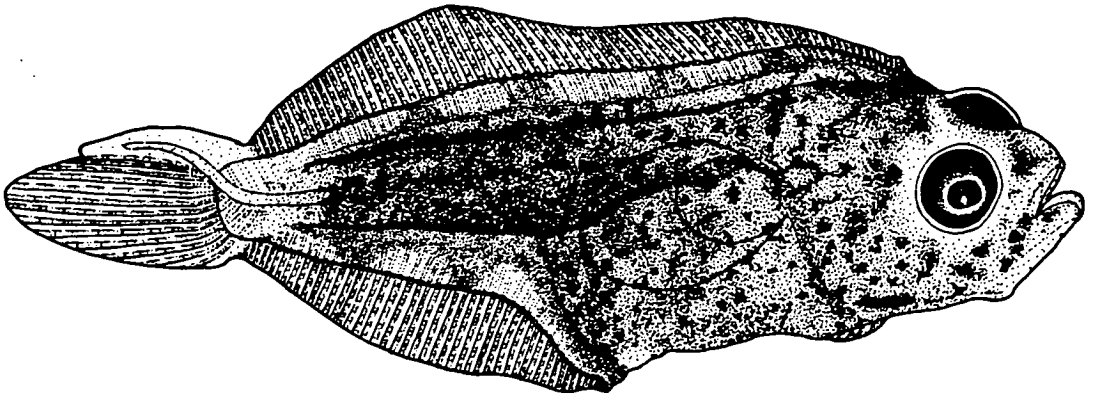

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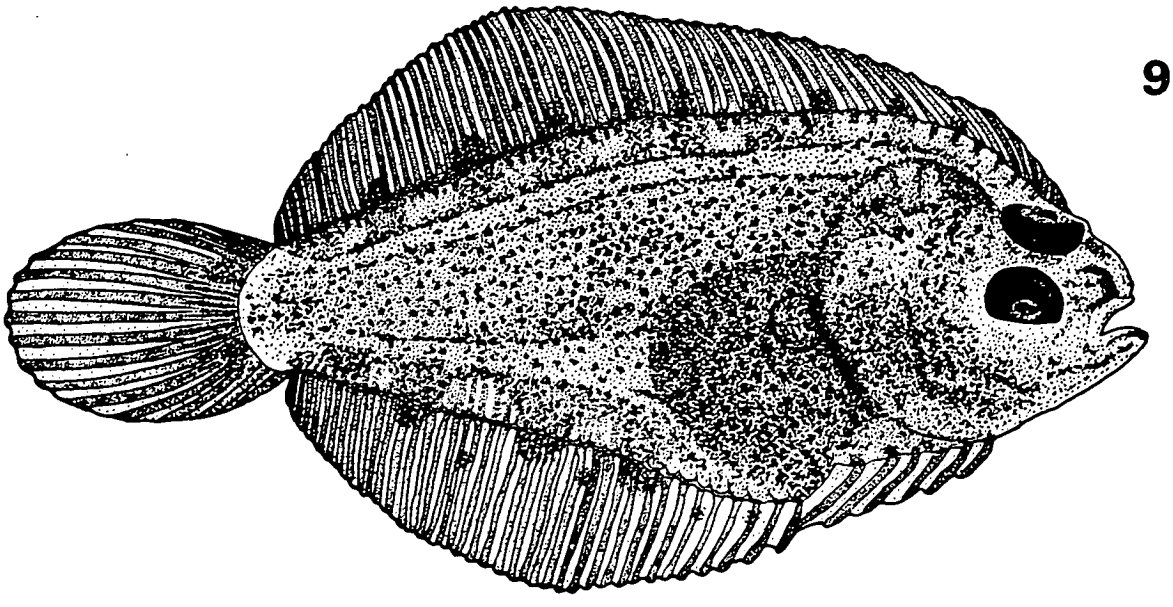

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posterior to the second dorsal band were distinct in preflexion larvae (Stage 1b, Figure 6.3.2). After three days most of the yolksac had been resorbed and measured 0.47 x 0.26 mm. Eyes were light brown, pectoral fins were functional and a pair of spines were present in the otic region (Stage 1c, Figure 6.3.3). By day 5 the mouth, jaws, gut and anus were functional and feeding had commenced. A large loop reaching to the body wall had developed in the originally straight-tubed gut. Pigmentation was heavier and the eyes were black. The larvae swam more vigorously (Stage 1d, Figure 6.3.4).

Stage 2 - yolk resorbed, notochord straight

Larvae developed in size and the body became relatively deeper; the otic spines were more prominent (Stage 2a, Figure 6.3.5). After 26 - 29 days, approximately 5 caudal rays were developing (Stage 2b, Figure 6.3.6).

Stages 3 and 4 - flexion of notochord and migration of left eye

The notochord was upturned near the posterior extremity at an angle of *c.* 20° after 34 days. The left eye had started migrating and was just visible from the right side; the caudal fin margin was extended and caudal rays were further developed (Stage 3a, 4a, Figure 6.3.7). The left eye was clearly visible from the right side and the notochord was upturned at 45° or more after 40 days. About 12 caudal rays were distinct. By day 46 the notochord was further upturned, anal and dorsal fin rays had formed completely and pelvic fin rays were visible (Stage 3b, 4b, Figure 6.3.8). Caudal fins were fully developed and the extremity of the notochord was just visible after 50 days. Larvae were strongly pigmented on both sides and settling on the bottom.

Stage 5 - eye on or over edge of head

About 80% of the larvae had settled on the bottom by day 63, pigment on the blind side was reduced, the otic spines had regressed, the pelvic fin was completely formed and larval pectoral fins were replaced by adult pectoral buds. Bands of pigment on the ocular side, 5 - 10 dorsally and 3 - 5 ventrally, extended from the pterygiophore region onto the fins.

Metamorphosis

By day 72 *c.* 50% of larvae had metamorphosed and all by day 85. Pigment on the blind side was reduced to isolated punctate or stellate chromatophores. Fin ray counts were 57 - 65 dorsal, 40 - 46 anal and 6 pelvic rays (Figure 6.3.9).

(ii) Ammotretis rostratus

Embryonic Development

Mature, unfertilized eggs were transparent with smooth chorion, ranged in diameter 0.9 - 1.18 mm, and contained from one large to 67 small oil droplets (Table 6.5, Figure 6.4.1). Fertilized eggs developed a small perivitelline space and floated at the surface. Two blastomeres were visible after 2.5 - 3 h, 4 after 3 - 3.5 h, 8 after 3.5 - 4 h and 16 after *c.* 5 h. The blasto-dermal cap had developed by 8 - 10 h. After 25 - 28 h the germ ring and start of the embryonic shield were visible (Figure 6.4.2). The blastoderm covered three-quarters of the yolk after *c.* 34 h (Figure 6.4.3). After 40 - 44 h the blastopore was closed (Figure 6.4.4) and the embryo had developed to the stage of Figures 6.4.5 and 6.4.6 after 49 - 53 h. The pericardial cavity, auditory visicles, somites and divisions of the brain were visible after 72 h (Figures 6.4.7, 6.4.8). The heart was beating and the embryo moved periodically after 80 - 85 h (Figure 6.4.9). Hatching occurred after 93 - 105 h.

Larval Development

Stage 1 - yolksac present

After hatching, larvae floated passively at the surface, moving occasionally. Chromatophores were scattered over the body, yolksac and finfolds. After one day, a triangular extension of the finfold anterior to the head, which contained a protruberance at the position of the developing mouth, was distinct (Figure 6.5.1). The anus was open by day 3 and mouth, jaws, gut and pectoral fins were functional by day 5 when the yolksac had been resorbed, retinal pigment was dark brown and the anterior extension of the finfold had disappeared.

FIGURE 6.4 Embryonic development of *Ammotretis rostratus*. The stages of development in photographs 1-9 are described in the text.

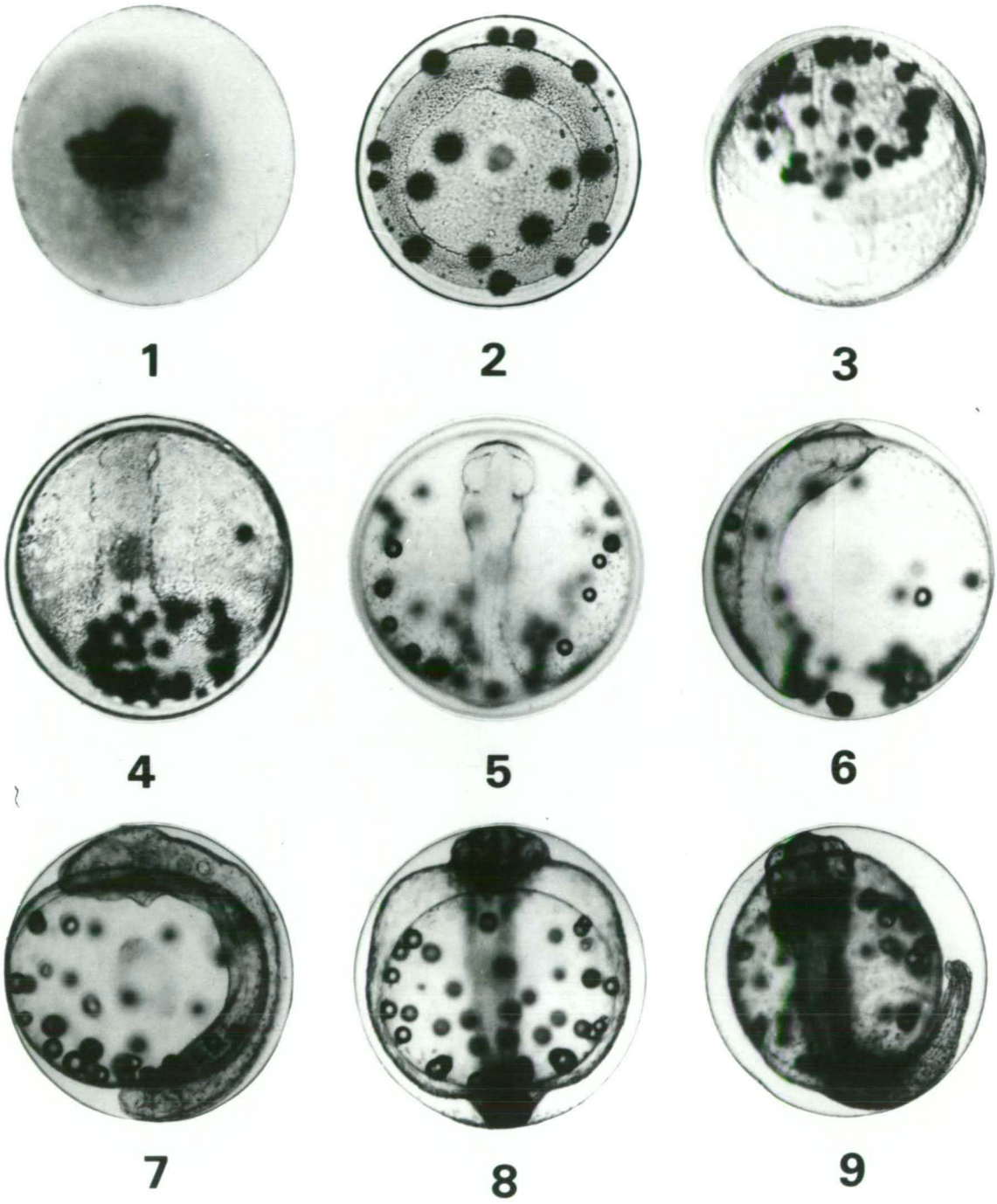
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FIGURE 6.5 Larval development of *Ammotretis rostratus*. The stages of development in drawings 1-6 are described in the text.

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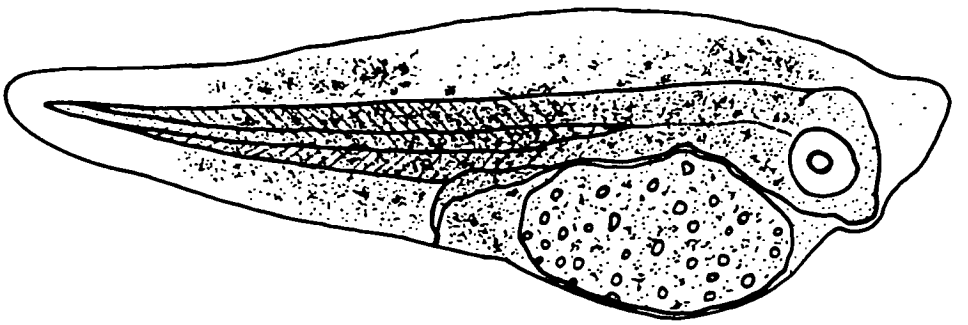

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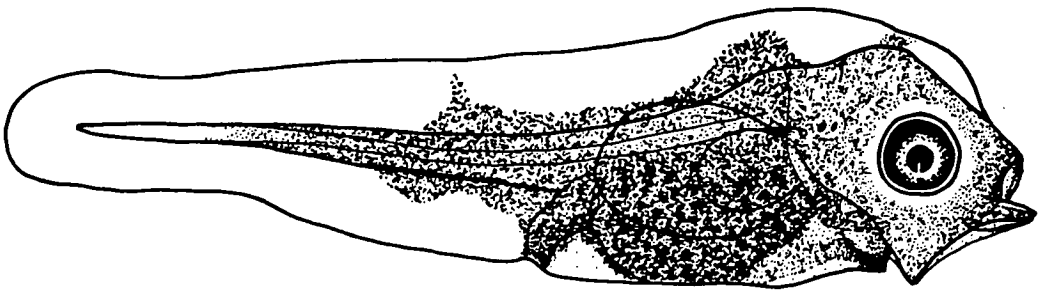

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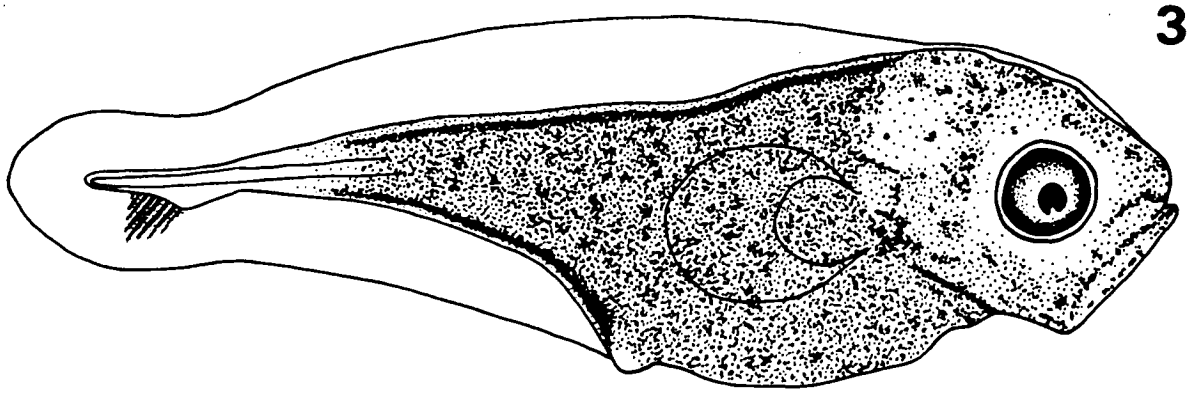

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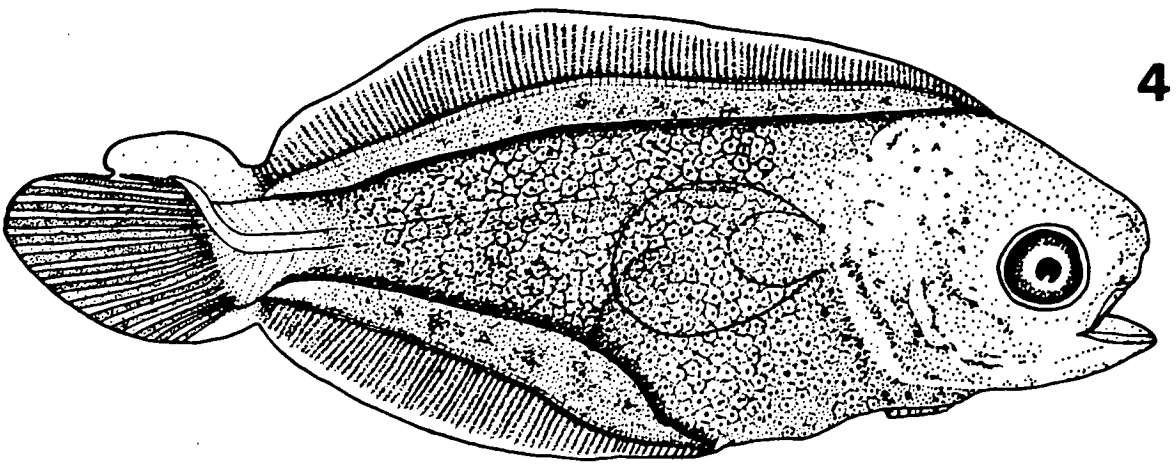

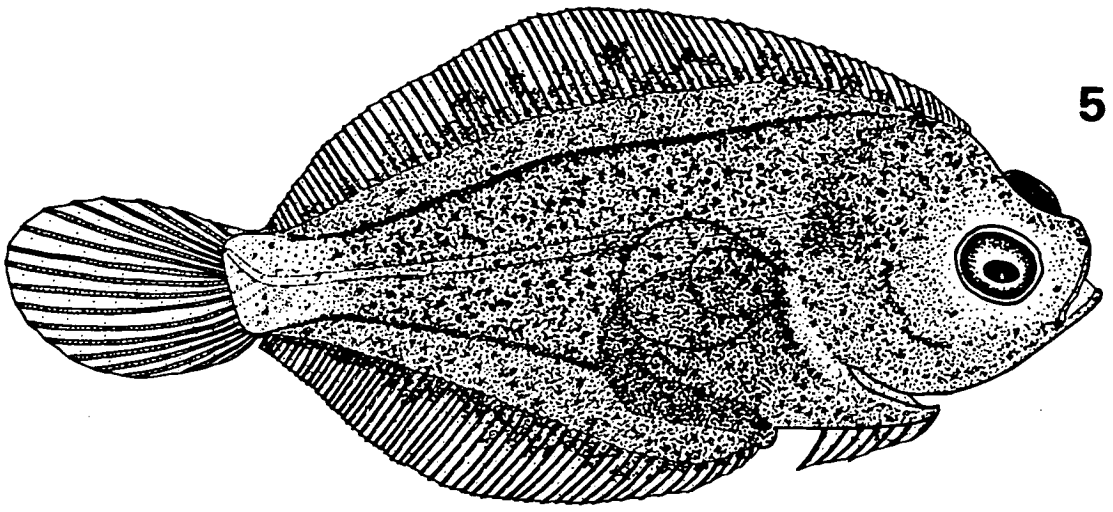

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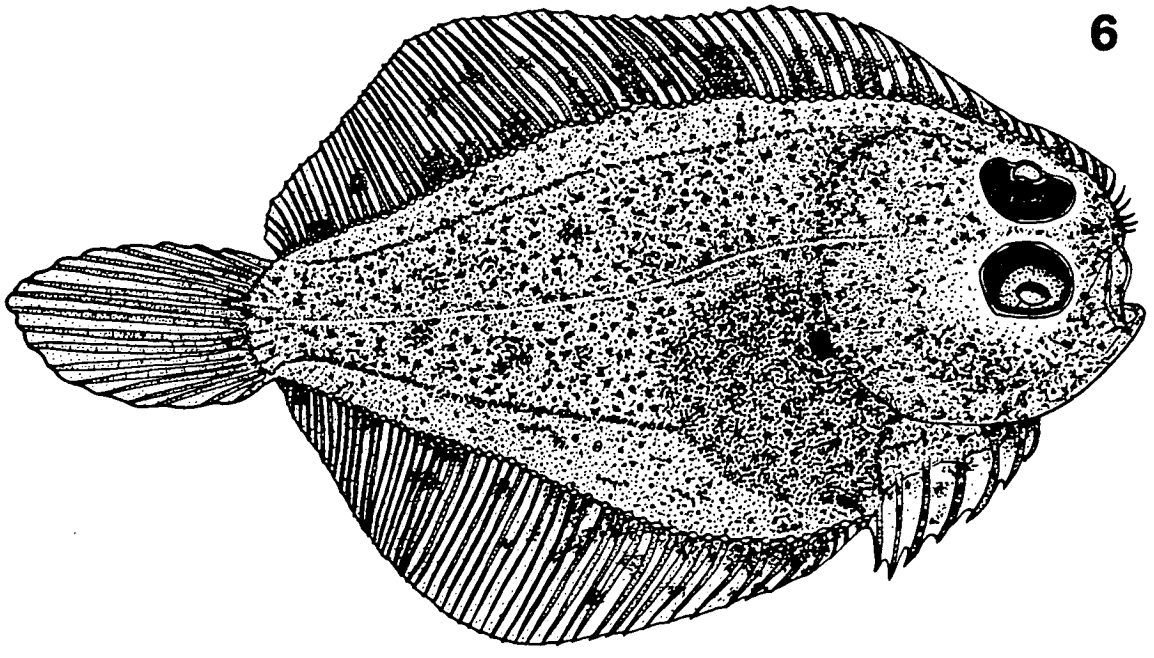

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Stage 2 - yolk resorbed, notochord straight

The pattern of pigmentation shown in Figure 6.5.2 occurred on all preflexion larvae until the pattern of Figure 6.5.3 developed. At this time, *c.* day 32, caudal fin rays and the extension of the caudal finfold margin were obvious.

Stage 3 - flexion of notochord

The notochord was upturned at 20-30° angle by day 42 and greater than 45° by day 52. Caudal rays and the fin margin were further developed (Figure 6.5.4).

Stage 4 - migration of the left eye

The left eye was migrating by day 56. It was visible from the right side after *c.* day 62 when larvae were settled on the bottom with caudal, dorsal and anal fins completely formed (Figure 6.5.5).

Stage 5 - eye on or over edge of head

By *c.* day 68, the right pelvic fin was joined to the anal, the left pelvic fin was almost complete, larval pectoral buds were lost and adult pectoral fin buds were formed.

Metamorphosis

Most larvae had metamorphosed by day 75 and all had by day 88. The pigment pattern on the ocular side is shown in Figure 6.5.6; it was reduced to isolated punctate melanophores on the blind side. Fin ray counts were 73 - 82 dorsal, 46 - 54 anal and 7 right pelvic rays.

6.4 DISCUSSION

The larvae of both *R. tapirina* and *A. rostratus* are small at hatching, first-feeding and metamorphosis when compared with other species of flatfish (Table 6.6) but it is difficult to compare the times taken to reach these stages of development because of the various temperature regimes used. When both species were cultured at ambient

TABLE 6.6 Length at, and time to, hatching, yolk sac absorption and metamorphosis for several species of flatfish

Species	Days to hatching and length (mm)	Days to yolk sac absorption from hatching and length (mm)	Days to metamorphosis from hatching and length (mm)	Rearing Temperature (°C)	Reference
Greenback flounder *Rhombosolea tapirina*	3.4-3.9 (2.0)	5 (2.71)	57-85 (8.8-9.5)	11.1-13.8	Crawford (this study)
Long-snouted flounder *Ammotretis rostratus*	3.9-4.4 (2.32)	5 (2.96)	60-88 (10.6-11.5)	12.5-16.5	Crawford (this study)
Sand flounder *Rhombosolea plebia*	4.75-5 (1.78)	5-5.3 (3.0-3.2)		10.1	Robertson & Raj (1977)
Lemon sole *Microstomus kitt*	7	10-11 (5.8)	*c.* 110 (19.5)	10.0±1.0 eggs 9.3-13.5 larvae	Howell (1972)
Dover sole *Solea solea*	2.75-6.5 (3.35)	1.75-4.5 (4.45)	21.5-63 (9-10)	19-10[a]	Fonds (1979)
Turbot *Scophthalmus maximus*	5.8-9.8 (2.7-3.0)	3.75-8 (3.6-3.8)	50-80 (23-30)	14.4-10 eggs[a] 15.0-10 y.s. larvae[a] 13.5-18 larvae	Jones (1972, 1973b)
Plaice *Pleuronectes platessa*	14 (6)	7	42-56 (10)	9-10 eggs 10-12 larvae	Blaxter (1968)
Starry flounder *Platichthys stellatus*	2.8 (1.93-2.08)	4-5 (*c.*3.5)	(>10.5)	12.5	Orcutt (1950)
Roundnose flounder *Eopsetta grigorjewi*	3.13 (2.9-3.2)	7 (4.8-5.3)	24 (>7)	approx. 14	Imaoka & Misu (1974)
Yellowtail flounder *Limanda ferruginea*	6-7 (2.75)	4-5	54-69 (17 SL)	10	Smigielski (1979)

[a] Eggs and larvae were cultured at different temperatures in several experiments

seawater temperature, which was 1-2° warmer for *A. rostratus*, the maximum growth rate was highest for *A. rostratus* but the size at, and time taken to, metamorphosis was less for *R. tapirina*.

The growth rates of both species were suppressed for several days after first-feeding. The larvae possibly took several days to establish successful feeding or there were insufficient rotifers of the required size range. Rotifers eaten at first-feeding by Black Sea turbot (*Scophthalmus maeoticus*) were much smaller than the average size of the rotifer population (Spectorova *et al.* 1974; Spectorova and Doroshev, 1976). Laurence (1977) also found that the food concentration required by first-feeding winter flounder larvae (*Pseudopleuronectes americanus*) was higher than at later larval stages due to the inefficient manner of prey capture.

The 94 and 98% survival rates for *R. tapirina* larvae from first-feeding to metamorphosis in 1982 are high compared with the survival rates of other species of flatfish, especially those which are too small to feed initially on *Artemia* nauplii. For example, survival rates of turbot larvae from first-feeding to metamorphosis have been about 50% (Kuhlmann *et al.*, 1981) and 10% (Howell, 1979) on an experimental scale and an average of 15.1% (Kingswell *et al.*, 1977) and 3-6% (Jones *et al.*, 1981) on a pilot commercial scale. An average survival rate of 97.5% was obtained by Kingswell *et al.* (1977) on a large scale for Dover sole larvae from first-feeding on *Artemia* nauplii to metamorphosis but weaning on to artificial diets and ongrowing have presented difficulties (Jones *et al.*, 1981). Although many attempts have been made to culture other species of flatfish, few have been reared to metamorphosis in large numbers. High mortalities have generally occurred at the time of first-feeding because the larvae were unable to establish feeding on an exogenous food source (e.g. Robertson and Raj, 1970; Imaoka and Misu, 1974; Spectorova *et al.*, 1974; Policansky and Sieswerda, 1979). The culture techniques developed and feeding strategies employed in this study contributed to the high survival rates of the flounder larvae.

It has been well documented that flatfish larvae benefit from the daily addition of unicellular algae to larval rearing tanks during the rotifer feeding stage (Spectorova and Doroshev, 1976; Howell, 1979; Scott and Baynes, 1979). However, no flatfish larvae can survive on

algae alone; any benefits of algae to larval nutrition must come from rotifer digested algae (Scott and Baynes, 1979; Scott and Middleton, 1979). In these experiments, the addition of algae to the larval rearing tanks was found to be not necessary. Only adding rotifers daily has the advantage that the rearing system is kept as simple as possible and accumulation of algal deposits, which enhance bacterial growth, do not occur.

The eggs and larvae of *R. tapirina* and *A. rostratus* can be readily separated using the following characteristics: egg diameter, oil droplet numbers and diameter of largest oil droplet, oil droplet numbers in yolksac larvae and the triangular extension of the finfold anterior to the head in *A. rostratus*, formation of a pair of spines in the otic region of *R. tapirina* larvae, pattern of pigmentation on the finfolds of preflexion larvae and on the body and fins from flexion to metamorphosis, development of the caudal fin relative to the migration of the left eye, dorsal, anal and right pelvic fin ray counts after settling, length of larvae at metamorphosis and the formation of a left pelvic fin and hooked snout in *A. rostratus*.

These descriptions will aid in the identification of flounder eggs and larvae caught in plankton samples. The descriptions of pigment patterns of *R. tapirina* larvae are similar to those of Roper (1979) except that he observed less variation.

Although these results are preliminary, the high survival rates from hatching to metamorphosis indicate that both species could be readily cultured commercially using the techniques developed in this study. However, survival and growth rates during weaning on to dried foods and ongrowing need to be examined further before large-scale culturing of these flounder species could be attempted.

CHAPTER 7

GENERAL DISCUSSION

This study has shown that estuaries, and to a lesser extent marine inlets, are important nursery grounds for *Rhombosolea tapirina* and *Ammotretis rostratus*. *A. lituratus* juveniles, however, occur only on semi-exposed and exposed beaches after they have attained a length of about 3.5 cm. It has been well documented that estuaries are favoured as nursery grounds for many species of fish because they provide an abundant and suitable food supply, sheltered conditions and protection from larger piscivorous predators (e.g. Pollard, 1976; Lenanton, 1977; Kilner and Akroyd, 1978; Blaber and Blaber, 1980). Moreover, Toole (1980) and Rosenburg (1982) suggest that juveniles which occur predominantly in very shallow water are generally segregated from larger fish, and any intraspecific and interspecific competition is consequently reduced.

There have been numerous studies on the factors affecting the distributions of larval and juvenile fish. As concluded by Shrode *et al.* (1982), fish distribution is a complex phenomenon subject to control and modification by a multitude of variables. Habitat selection by fishes has, however, been little studied and factors selected for have generally been suggested from observed field distributions (e.g. Day, 1951; Lenanton, 1977; Weinstein, 1979; Blaber and Blaber, 1980; Larson, 1980; Riley *et al.*, 1981).

A correlation between the distribution of an animal and the variation in an environmental parameter, however, only indicates that this parameter may be used as a cue for habitat selection. Experimental studies determine whether the animal is capable of behaviourally responding to the factor (Sale, 1969a). Nevertheless, the selection for a particular factor in the laboratory does not prove that the factor is most important in habitat selection in the natural environment because responses to other factors, especially biotic ones (e.g. food availability, predators, competition), may override the expression of a particular orientating mechanism (Reynolds and Thompson, 1974a). The investigations by Sale (1968, 1969a,b) and Reynolds and Thompson, 1974a,b) are amongst

the few to examine habitat selection in fishes from both field and experimental studies. Juvenile manini, *Acanthurus triostegus sandvicensis*, were observed by Sale (1969b) to select mainly for presence of substrate, cover, algal food and a suitable depth of water. Reynolds and Thompson (1974a) found that juvenile grunion, *Leuresthes sardina*, responded to gradients of light intensity, temperature, turbulence and dissolved oxygen in the laboratory in a manner which was in accordance with their field distributions. The grunion also displayed an ontogenetic change in preferred salinity during the larval to the juvenile stage (Reynolds and Thompson, 1974b).

The present study has shown, from a combination of field and laboratory studies, that *R. tapirina* and *A. rostratus* larvae are probably dependent on water movements for transport towards nursery grounds, and that preferences for shallow water, low salinities and sandy substrates are probably important in drawing flounder larvae into estuaries. The occurrence of smaller numbers of newly-metamorphosed juveniles in the marine inlet at Cremorne suggests, nevertheless, that low salinities are a preferred, but not essential, requirement of nursery grounds. The depth of water and the substrate type are probably more important.

Once on the nursery grounds, the two species show minor differences in distribution which appear to be related to their preferred depths and substrate types, and also possibly to the levels of turbulence and the swimming abilities of newly-metamorphosed juveniles. The observed differences in natural distributions of the two species by depth and by degree of penetration of the estuary were in accordance with experimentally-determined substrate preferences and current tolerances. Temperature and salinity preferences, of the two species were, however, apparently not important. Although they both preferred very low saline conditions in the laboratory, they were most abundant, particularly *A. rostratus*, at much higher salinities in the field. This suggests that salinity preferences were overridden by a preference for other factors. Nevertheless, at site D2b where the salinity fluctuated markedly during the tidal cycle, both species were caught in the highest densities. Similarly, temperature preferences of the two species appeared to be subordinated by other factors. Unfortunately, it is very difficult in practise to simultaneously examine preferences for several environmental variables, particularly when salinity gradients are involved.

The results indicate that *R. tapirina* and *A. rostratus* juveniles co-exist by partially partitioning the spatial and trophic resources of the habitat. The two species were not obviously segregated temporally either by seasonal recruitment or diurnal movements and feeding patterns. Similarly, other studies of sympatric juvenile flatfish have shown that they partition the habitat spatially and trophically (Macer, 1967; Edwards and Steele, 1968; Pearcy, 1978; Pearcy and Hancock, 1978; Roper, 1979; Burchmore, 1982), although temporal differences may occur also (Macer, 1967; Edwards and Steele, 1968).

Moyle and Cech (1982), in a general discussion on flatfishes consider that co-existing species segregate ecologically by having different depth distributions and feeding habits, the latter being reflected in the structure of the mouth and the pharyngeal teeth. Adults of many sympatric species of flatfish have been observed to partition the spatial resources (e.g. Hartley, 1940; Pearcy, 1978; Livingston, 1981; Rainer and Munro, 1982) and the trophic resources (e.g. Hartley, 1940; Tyler, 1972; Hatanaka *et al.*, 1974; Stickney *et al.*, 1974; Kravitz *et al.*, 1976; Pearcy and Hancock, 1978; Livingston, 1981). Schoener (1974) reviewed resource partitioning in ecological communities, mainly terrestrial, and concluded that spatial dimensions were more important than food-related dimensions, which in turn were more important than temporal dimensions for segregating species. Co-existing species of fish have been observed to mainly partition the spatial resources (e.g. Werner *et al.*, 1977). However, spatial partitioning is often correlated with trophic partitioning in fish communities (e.g. Keast, 1970; Chao and Musick, 1977; George and Hadley, 1979; Anderson *et al.*, 1981; Prince *et al.*, 1982).

Although juvenile *R. tapirina* and *A. rostratus* have very similar patterns of habitat utilization, the existence of competition between them for space or food cannot be assumed. Competition between species generally occurs when a shared resource becomes limited in supply (Pianka, 1976). However, competition is difficult to demonstrate in natural communities because it is always advantageous for an organism to reduce or avoid competition (Pianka, 1976), and because competition may only occur sporadically (Wiens, 1977). Also, co-existence and competition in natural communities are commonly complex and involve strong factor interactions (Colwell and Fuentes, 1975).

Measures of overlap have been commonly used as an index of interspecific competition for resources (e.g. Levins, 1968; May and MacArthur, 1972). However, overlap does not necessarily imply that competition is occurring (Colwell and Futuyma, 1976; Rathcke, 1976; Hulbert, 1978; Slobodchikoff and Schulz, 1980). In particular, the shared resource may be so abundant that it is not limiting to either species. In fact, it has been proposed by Pianka (1974) that the maximal tolerable overlap should be lower in intensively competitive situations than in environments with lower demand/supply ratios, i.e. extensive overlap may be correlated with reduced competition. Dietary overlaps in several co-existing fish species have been found to be highest when food resources were abundant and least when food resources were scarce (e.g. Keast, 1970; Zaret and Rand, 1971; Cadwallader, 1975). Hartley (1948) and Cadwallader (1975) suggested that a major method by which co-existing species of fish avoid severe competition for food is to change the relative proportions of dietary components. This also possibly occurs in *R. tapirina* and *A. rostratus* juveniles.

The availability of food resources, however, was not determined in the present study. Several measures have been widely used to compare the diets of fishes with the potential availability of food resources in the habitat (e.g. Ivlev's (1961) index of electivity and the forage ratio (Hess and Rainwater, 1939)). In recent years, however, such measures have been criticized. Measurements of food availability, or food selection by predators are often fraught with difficulties (Strauss, 1979; Wallace, 1981). For example, it is often difficult to obtain an unbiased sample from the habitat which accurately represents the relative abundances of potential prey species to the predator due to the patchy distributions of prey species and to the differential availability of prey to predator in various microhabitats. Moreover, Moore and Moore (1976) found that food selection in the flounder, *Platichthys flesus*, was affected by factors other than availability of food, including hunting efficiency of fish, their conditioning for certain foods, the degree of concealment of prey and the turbidity and temperature of the water. Similarly, Petraitis (1979) and Johnson (1980) considered that the more general measures of resource utilization are often not appropriate as the comparison of usage to availability of a resource is dependent on the subjective evaluation by the investigator of the relative availability of the resource to the consumer.

Interspecific competition in natural populations has often been investigated by comparing the ecology of a species when it occurs allopatrically to when it occurs sympatrically. Morphological changes (character displacement) and ecological changes (niche shifts) in co-existing species have been considered to be indicative of interspecific competition (e.g. MacArthur, 1972; Schoener, 1974). Many species typically converge when they occur in allopatry and diverge when they occur together. However, such comparative studies often lack a suitable control and it may be impossible to separate the effects of competition from other factors, e.g. the relative availability of habitat types or food (Colwell and Fuentes, 1975; Werner and Hall, 1976; Connell, 1980; Dunham, 1980). Thus, although the diets of *R. tapirina* and *A. rostratus* juveniles have been examined in areas where they occur together, and where they occur separately, it is considered inappropriate to make comparisons, especially as both species are apparently opportunistic species and their diets vary seasonally and between geographical areas.

Other biotic factors which may be important in determining the distributions of the two species and could influence habitat partitioning and competition, include the effects of predation and the interactions between the two species and with other fish species of the inshore environment. These factors generally have been examined in natural populations of sympatric species by controlled manipulations of densities or distributions of the species (see reviews by Colwell and Fuentes, 1975; Connell, 1975). Such experiments, however, would be extremely difficult to conduct with the two flounder species studied because of their movements up and down the shore with the tide and because of the effects of wave action on sandy substrates in the intertidal zone.

Experimental manipulations of densities of other animals in the field have indicated that predators can maintain the populations of potential competitors below levels required for competition (e.g. Paine, 1966, 1974; Connell, 1975; Menge and Sutherland, 1976). As discussed in Chapter 2, the decrease in abundance of *R. tapirina* and *A. rostratus* after the peak recruitment period may be due to predation by larger fish. It is possible, therefore, that predation may similarly serve to reduce competition between the two species.

Controlled manipulations under field conditions also have provided evidence of interference competition between co-existing species. The abundance or distribution of one species has typically changed when the density or distribution of its purported competitor was altered. This has been observed in sympatric fish species by Low (1971), Sale (1974, 1975), Robertson *et al.* (1976) and Larson (1980). Fish with strongly marked territories typically exhibit competitive interactions. However, as *R. tapirina* and *A. rostratus* juveniles apparently are not territorial and were not observed to interact when kept together in aquaria, interference competition between them is probably not as likely as exploitative competition, i.e. competition for shared resources.

The maximum densities of juvenile flounder observed on the nursery grounds in the present study were high. Combined mortality and emigration rates of *R. tapirina* and *A. rostratus* juveniles after peak recruitment were also similar to those of other species of flatfish. This raises the question of why the adult flounder populations in Tasmania are so low in comparison with the size of flatfish stocks overseas, for example plaice *Pleuronectes platessa*. Densities of juvenile plaice on the nursery grounds are of the same order as those of *R. tapirina*. However, the plaice fishery is one of the largest in Europe. There are several factors which may be important in determining these differences.

Newly-metamorphosed plaice are spread over a much wider depth range, extending from the intertidal zone to 4-6 m below low water mark (Steele *et al.*, 1970), than *R. tapirina* or *A. rostratus* juveniles which occur in 0-1 m depth. Moreover, the topography of the intertidal zone of plaice nurseries is quite different. Around the British Isles the average tidal height is 4.6 m and 3.5 m at spring and neap tides, respectively, although it may be much higher in some areas. Also, in many regions the beaches have shallow gradients and the slope of the shore in estuaries may be as low as 1:600 (Perkins, 1974). By contrast, in south-eastern Tasmania the tidal height reaches a maximum of 1.6 m and 1.0 m at spring and neap tides, respectively, and the gradient of the intertidal zone is relatively steep. Thus, although the maximum densities of 0-group *R. tapirina* and plaice are similar, plaice occupy a much larger area than *R. tapirina* because of their wider depth

distributions and because the intertidal zone of plaice nurseries is more extensive. This large intertidal zone provides an enormous area of favourable feeding conditions for juvenile plaice. Moreover, there are no major estuarine systems with extensive sandflats in Tasmania which are suitable nursery grounds for flounder in comparison with, for example, the Wadden Sea for 0-group plaice. This sea has an intertidal zone of about 6000 km^2 and juvenile plaice are thought to be abundant throughout (Kuipers, 1977). Also, as juvenile flounder are concentrated in a smaller, shallower region, predominantly in estuaries, they would be more vulnerable to the deleterious activities of man than juvenile plaice.

Similarly, the range of depths occupied by adult plaice, and the area of bottom available at those depths, compared with that of flounder may influence the size of adult populations. Plaice are generally caught in depths of up to 70 m although they may be found to 180 m depth (Bagenal, 1966). Furthermore, the continental shelf (i.e. above 200 m depth) is extensive around the British Isles and almost the entire North Sea is within the shelf region (Tait, 1968). By contrast, *R. tapirina* are found mostly in depths of less than 45 m although they may occur to 55 m depth (unpublished data, Tasmanian Fisheries Development Authority). The continental shelf around Tasmania is comparatively narrow and much of the bottom topography is unsuitable for demersal fish species (Olsen, 1965). Moreover, Australian marine waters are some of the most nutrient poor regions of the world (Rochford, 1980). They are, therefore, probably not capable of supporting large stocks of fish.

The flatfish fishery in New Zealand is also much larger than that in Australia; 8803 tonnes of flatfish were landed there in 1980 (Anon, 1982). This is probably related to the larger area of continental shelf and higher productivity of the waters around New Zealand than in Australia. Productivity and zooplankton biomass values for much of New Zealand are similar to, and in certain areas higher than, those from upwelling areas in other parts of the world (Bradford and Roberts, 1978). However, the densities of juvenile flounder found in New Zealand by Roper and Jillett (1981) could not be directly compared with those observed in the present study because nets of different mesh size were used.

Another factor which may be important in reducing flounder stocks is amateur spearfishing. Although the numbers of adults, as well as juveniles, taken by this method are not known, they are thought to be considerable because all sizes of *R. tapirina* frequent the intertidal zone in all months of the year. By contrast, adults of most other species of flatfish do not move in so close to shore and therefore are inaccessible to spearfishing. For example, plaice move into deeper water and further from the coast as they increase in size (Wimpenny, 1953).

The effects of predation and interspecific competition, in particular for food, on the population size of *R. tapirina* and *A. rostratus* juveniles were not examined. These factors may, however, be important. Edwards and Steele (1970) found that food supplies and mortality rates, rather than initial numbers of fish, were the controlling factors of O-group plaice population size at the end of the summer growth period. There was apparently an upper limit to the rate of energy intake by the population from available food supply in any given year, so that numbers of fish and their average length were determined by variations in mortality. They suggested that yearly variations in mortality rate were probably due to varying population densities of predators. Density-dependent mortality of juvenile plaice due to predation has also been suggested by Lockwood (1981). Nevertheless, Kuipers (1977) observed that the population numbers of O-group plaice remained much more constant in the Wadden Sea, probably due to a lower rate of predation and because of an abundant food supply. Plaice at this stage mainly consume regenerating parts of benthic invertebrates. Their food source is therefore renewable. *R. tapirina* and *A. rostratus* juveniles, however, only feed on whole animals. Their food supplies may possibly become limiting during the peak recruitment period when they occur in high densities over a narrow depth range.

The reproductive strategy of both *R. tapirina* and *A. rostratus* of an apparently long spawning season, serial spawning and comparatively high fecundity contrasts with that of many other species of flatfish, including plaice, which have a shorter spawning season, spawn only once each season, and have lower fecundities. The strategy of the two flounder species studied tends to maximize reproductive potential and appears to be most suited to the survival of newly-metamorphosed juveniles

which have a narrow distributional range in the harsh environment of the intertidal zone. The chances of at least a proportion of eggs, larvae or juveniles finding favourable environmental conditions are enhanced and the juveniles are less crowded on the nursery grounds as recruitment is spread over several months.

It has been suggested by several people, including Leggett and Carscadden (1978), Moyle and Cech (1982) and Roff (1981) that, although each fish species has a reproductive strategy which is most suited to the set of fluctuating environmental conditions in which it occurs, iteoparity (i.e. repeat spawning) is usually favoured because environments, and therefore reproductive success, are rarely predictable. Thus, most fish adopt a 'bet-hedging' strategy so that all their energy reserves are not allocated to one spawning only. For example, Leggett and Carscadden (1978) observed that in populations of the American shad (*Alosa sapidissima*), the proportion of repeat spawners increased with higher environmental variability, particularly temperature which influenced the survival of eggs and larvae. Conversely, the relative fecundity decreased as a greater amount of energy was allocated to migration than to the gonads. Serial spawning in combination with high fecundity may be a further adaptation to highly unpredictable environments. It would be interesting to examine the reproductive strategies of *R. tapirina* and *A. rostratus* in areas of lower latitude which are generally considered to be more stable, particularly as Burchmore (1982) observed distinct year classes of *A. rostratus* in the warmer waters of Botany Bay, New South Wales.

Another aspect of this study, the cultivation of *R. tapirina* and *A. rostratus*, has provided descriptions of the eggs and larvae of the two species which can be used to identify those caught in the plankton. However, until the planktonic eggs of other fish species are described, the positive identification of *R. tapirina* eggs remains difficult. The success of the rearing experiments indicates that both species could be cultivated commercially. Nevertheless, further research is required on ongrowing and conversion onto an artificial diet of dried food.

Research on the environmental conditions promoting maximum growth in other fish species has shown that growth rates are generally highest at the preferred temperature and salinity regimes. Jobling (1981)

reviewed the temperature requirements of several species of fish and concluded that the preferred temperature is a good estimate of that at which maximum growth occurs. Similarly, Deubler and White (1962) and Stickney and White (1973) found that the growth rates of larval *Paralichthys dentalus* and *P. lethostigma*, respectively, were highest at the salinities commonly recorded in the areas where they were most abundant. Thus, ongrowing of *R. tapirina* and *A. rostratus* juveniles may be best conducted at their experimentally-determined preferred temperatures of 12-15°C for *R. tapirina* and 17-19°C for *A. rostratus*, and salinities of 0-10‰ for both species.

The rearing experiments therefore suggest that it may be possible in the future to supplement the flounder fishery by artificial cultivation of the two species. However, in the meantime the results of this study have emphasised the importance of estuaries, particularly the intertidal zone, in the life histories of *R. tapirina* and *A. rostratus*. These areas must be maintained in an ecologically viable condition and protected from despoliation if flounder populations are to remain at the present level.

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APPENDIX 1: CHANGES IN WEIGHT AND LENGTH OF JUVENILE FLOUNDER DUE TO PRESERVATION IN FORMALIN

The lengths and weights of 40 *R. tapirina* and 38 *A. rostratus* were measured when the fish were alive and at 2, 5, 10 and 20 days after preservation in 5% V/V formalin. The lengths of live *R. tapirina* ranged from 1.8 to 6.9 cm and weights from 0.056-3.81 g; live *A. rostratus* were 1.7 to 6.9 cm in length and from 0.033-3.59 g weight.

Lengths (ℓ) and weights (w) of fish after preservation in formalin for a given time period were expressed as a percentage of the fresh lengths (L) and weights (W), respectively, and means were calculated. Linear regression equations of preserved length against live length, and preserved weight against live weight were computed for each species for each period of preservation. The results are shown in the table below.

Days in Preservative	Mean $\frac{\ell}{L}.100$	S.D.	Mean $\frac{w}{W}.100$	S.D.	Regression Equation
			R. tapirina		
0	100		100		
2	99.52	± 1.34	92.56	± 4.13	L=1.00ℓ+0.01, W=1.080w+0.006
5	98.95	± 1.81	90.89	± 5.27	L=1.00ℓ+0.04, W=1.072w+0.012
10	98.33	± 2.62	90.83	± 3.54	L=0.99ℓ+0.06, W=1.075w+0.008
20	97.60	± 3.76	88.25	± 5.08	L=0.98ℓ+0.11, W=1.118w+0.012
			A. rostratus		
0	100		100		
2	99.22	± 1.56	95.32	± 4.01	L=1.00ℓ+0.07, W=1.051w+0.007
5	97.91	± 1.87	92.11	± 4.55	L=0.99ℓ+0.12, W=1.083w+0.007
10	97.45	± 2.61	91.89	± 4.85	L=0.98ℓ+0.18, W=1.074w+0.011
20	96.56	± 3.87	90.90	± 5.01	L=0.97ℓ+0.21, W=1.092w+0.013

APPENDIX 2: Total volume (V) of water filtered in m^3 per plankton tow, percentage (P) of plankton sample sorted and number *R. tapirina* larvae (N) in that portion of the sample, at six sites in different months. The number of *A. rostratus* larvae are shown in brackets.

Date	Site DP1			Site DP2			Site FP3			Site FP4			Site FP5			Site FP6		
	V	P	N	V	P	N	V	P	N	V	P	N	V	P	N	V	P	N
6 Aug. 1980	88.53	50	0	102.04	70	1	63.47	40	0	67.58	60	2	103.15	60	1	118.82	20	1
3 Sept. 1980	67.88	100	0	80.36	100	0	73.19	100	1	79.21	100	0	55.43	80	2	83.34	60	2
1 Oct. 1980	69.85	80	0	67.69	80	2	71.38	100	3	71.83	100	8	76.45	100	1	77.01	30	0
12 Nov. 1980	63.01	100	1	68.33	100	3	84.75	100	5(1)	70.86	100	0	72.83	100	0	75.67	100	0
9 Jan. 1981	102.67	100	0	97.11	100	0	70.67	70	0	84.59	70	0	81.75	80	0	91.77	50	0
5 Feb. 1981	75.84	100	(1)	56.35	100	2	48.04	100	0	51.72	100	0	54.06	100	0	51.17	100	0
11 Mar. 1981	32.95	100	0	37.05	100	0	38.05	100	0	43.31	100	0	44.78	100	0	37.42	100	0
8 Apr. 1981	46.25	90	0	39.32	100	0	41.06	100	0	33.81	100	0	38.35	100	0	32.39	100	0
5 May 1981	67.5	100	0	64.3	100	0	37.98	100	0	31.10	100	1	38.86	100	1	46.3	100	1
25 June 1981	13.86	100	0	22.91	100	0	16.64	100	0	13.52	100	0	17.36	100	1	20.56	100	0
20 July 1981	36.78	100	1	39.75	100	1	35.02	100	3	35.91	100	1	34.18	100	1	28.34	100	0
31 Aug. 1981	39.11	100	0	36.15	100	1	37.64	100	2	43.88	100	3	43.31	100	3	48.73	100	2
12 Oct. 1981	73.03	100	2	92.79	100	6	90.38	100	3(2)	111.00	50	1	123.07	100	0	112.84	50	1
25 Nov. 1981	107.44	100	0	87.40	100	0	99.51	100	0	132.29	100	0	90.55	100	0	117.48	100	0
14 Dec. 1981	99.63	100	0	102.39	100	0	92.17	100	0	88.65	100	0	89.37	100	0	99.76	100	0

APPENDIX 3a: The area (A) swept by the push-net in m^2 and the number of *R. tapirina* (Rt) and *A. rostratus* (Ar) juveniles caught at each of three depths per month at site D1 (Nutgrove) (no sample taken is indicated by -).

Depth	10-30 cm			50-70 cm			90-110 cm		
Date	A	Rt	Ar	A	Rt	Ar	A	Rt	Ar
3 Nov. 1980	345	3	2	345	1	0	345	1	17
12 Dec. 1980	345	4	0	345	60	39	-	-	-
14 Jan. 1981	345	4	1	500	0	3	500	3	3
12 Feb. 1981	345	7	3	500	2	12	-	-	-
24 Mar. 1981	255	1	1	255	13	3	500	0	0
23 Apr. 1981	225	0	0	500	21	41	500	24	38
26 May 1981	240	0	1	500	4	0	500	0	1
19 June 1981	390	2	0	500	15	9	500	9	9
"							500	12	4
7 July 1981	210	1	1	500	24	29	500	22	15
12 Aug. 1981	202.5	7	0	202.5	30	1	500	111	3
"							500	97	2
23 Sept. 1981	-	-	-	500	268	6	500	143	8
21 Oct. 1981	279	5	0	500	51	7	500	59	10
19 Nov. 1981	270	48	2	500	809	22	500	13	7
7 Dec. 1981	187.5	6	0	187.5	63	0	500	196	20
14 Jan. 1982	262.5	70	3	500	29	32	500	1	8

APPENDIX 3b: The area (A) swept by the push-net in m^2 and the number of *R. tapirina* (Rt) and *A. rostratus* (Ar) juveniles caught in each of 2-4 samples per month at site D2b (Browns Rivulet)

Date	A	Rt	Ar	Date	A	Rt	Ar
3 Nov. 1981	180	7	1	26 May 1981	105	1	0
	225	6	3		150	1	1
	450	4	0		150	5	3
	225	11	3				
				19 Jun.1981	127.5	2	0
10 Dec. 1981	240	53	65		150	0	1
	450	78	8		214.5	2	0
	300	31	95				
	225	58	8	7 Jul.1981	172.5	0	4
					133.5	0	4
14 Jan. 1981	180	23	22		105	0	0
	165	35	31				
	105	3	10	12 Aug.1981	168	68	13
					115.5	65	17
14 Feb. 1981	150	3	0				
	108	1	1	23 Sep.1981	135	119	1
	125	4	1		165	26	12
					135	29	7
14 Mar. 1981	217.5	7	7				
	157.5	3	2	21 Oct.1981	117	383	18
					105	50	8
23 Apr. 1981	187.5	9	9		147	56	9
	363	13	9				
				19 Nov.1981	150	308	9
					150	68	10
				7 Dec.1981	150	66	2
					127.5	37	15
				14 Jan.1982	129	15	0
					129	14	11

APPENDIX 3c: The area (A) swept by the push-net in m^2 and the number of *R. tapirina* (Rt) and *A. rostratus* (Ar) juveniles caught at each station a,b,c,d at 50-70 cm depth, and at station c at 10-30 cm and 90-110 cm depth, each month at site F3 (Pittwater) (Depth: 1 = 10-30 cm, 2 = 50-70 cm, 3 = 90-110 cm; no sample taken is indicated by -).

Date	Depth	Station a			Station b			Station c			Station d			Date	Depth	Station a			Station b			Station c			Station d		
		A	Rt	Ar	A	Rt	Ar	A	Rt	Ar	A	Rt	Ar			A	Rt	Ar	A	Rt	Ar	A	Rt	Ar	A	Rt	Ar
5 Nov. 1980	1							135	62	0				4 Aug. 1981	1							180	40	0			
	2							500	37	4					2	500	7	12	500	26	1	500	30	0	500	12	0
	3							-	-	-					3							500	35	11			
3 Dec. 1980	1							300	206	1				4 Sept. 1981	1							138	22	0			
	2							500	102	18					2	500	291	15	500	239	3	500	171	1	500	33	1
	3							-	-	-					3							500	420	5			
6 Jan. 1981	1							225	18	0				8 Oct. 1981	1							195	11	2			
	2	500	5	17	500	20	3	500	67	10	500	20	2		2	500	31	2	500	141	3	500	164	5	500	164	5
	3							-	-	-					3							500	61	7			
9 Feb. 1981	1							450	30	1				3 Nov. 1981	1							172.5	27	0			
	2	500	3	2	500	20	5	500	19	2	500	3	5		2	500	14	7	500	14	1	500	23	0	500	82	0
	3							-	-	-					3							500	48	4			
10 Mar. 1981	1							150	2	0				2 Dec. 1981	1							234	15	0			
	2	500	0	2	500	8	1	500	4	0	500	34	1		2	500	0	1	500	67	2	500	88	0	500	114	0
	3							500	33	1					3							500	239	9			
7 Apr. 1981	1							114	1	0				15 Jan. 1982	1							234	0	0			
	2	500	0	0	500	9	1	500	34	0	500	32	0		2							500	146	22			
	3							500	4	5					3							500	25	8			
7 May, 1981	1							150	7	0																	
	2	500	1	5	500	4	0	500	8	1	500	6	0														
	3							500	7	3																	
5 Jun. 1981	1							147	24	1																	
	2	500	0	2	500	121	0	500	64	0	500	15	3														
	3							500	15	4																	
3 Jul. 1981	1							150	7	0																	
	2	500	0	0	500	220	0	500	43	0	500	15	0														
	3							500	99	0																	

APPENDIX 3d: The area (A) swept by the push-net in m^2 and the number of *R. tapirina* (Rt), *A. rostratus* (Ar) and *A. lituratus* (Al) juveniles caught in each sample at stations a,b,c in different months at site F4 (Cremorne)

Date	Station a				Station b				Station c			
	A	Rt	Ar	Al	A	Rt	Ar	Al	A	Rt	Ar	Al
26 Nov. 1980					500	47	0	0				
					500	52	0	0				
					500	14	0	0				
31 Dec. 1980	500	0	0	4	500	49	14	0	495	19	0	0
					120	14	0	0				
27 Jan. 1981	500	0	0	0	500	13	4	0	500	17	0	0
					500	26	0	0	150	6	0	0
					500	12	2	0				
24 Feb. 1981	500	0	0	2	150	1	1	0	200	7	0	0
					500	18	2	0				
					500	10	1	0				
22 Apr. 1981	500	0	0	1	500	8	1	0	150	0	0	0
					500	44	1	0	500	1	0	0
18 Jun. 1981					500	13	11	0	150	0	0	0
					500	12	5	0				
					500	3	2	0				
					247.5	2	0	0				
20 Jul. 1981	500	0	0	0	500	30	0	0	321	10	0	0
					500	5	2	0				
					330	4	0	0				
19 Aug. 1981	500	1	1	1	307.5	2	0	0	258	3	0	0
					500	17	1	0				
					500	121	0	0				
21 Sept. 1981					225	22	0	0	375	114	0	0
					500	33	0	0				
					500	8	2	0				
20 Oct. 1981					345	17	0	0	210	125	0	0
					500	31	2	0				
					500	9	4	0				
17 Nov. 1981	500	0	0	1	337.5	10	0	0	450	104	0	0
					500	32	5	0				
					500	56	10	0				
16 Dec. 1981					240	10	0	0	180	9	0	0
					500	25	10	0				
					500	17	13	0				
28 Jan. 1982					225	17	0	0	165	8	0	0
					500	8	9	0				
					500	2	6	0				

APPENDIX 4a: The area (A) swept by the beam trawl in m^2 and the number of *R. tapirina* (Rt) and *A. rostratus* (Ar) juveniles caught in each sample per month at site D1 (Nutgrove)

Date	Site D1		
	A	Rt	Ar
20 Aug. 1980	1000	2	2
18 Sept. 1980	1000	4	8
14 Oct. 1980	1000	8	18
"	1068	0	0
2 Feb. 1981	1600	0	0
24 Mar. 1981	1600	0	0
23 Apr. 1981	1068	1	0
26 May 1981	1600	0	0
19 Jun. 1981	1600	1	1
7 Jul. 1981	1600	0	1
12 Aug. 1981	1600	0	0
"	1068	0	0
21 Oct. 1981	1600	0	0
19 Nov. 1981	1600	0	0
"	1068	0	0
7 Dec. 1981	1600	1	0
14 Jan. 1982	1600	0	0

APPENDIX 4b: The area (A) swept by the beam trawl in m^2 and the number of *R. tapirina* (Rt), *A. rostratus* (Ar) and *A. lituratus* (Aℓ) juveniles caught in each sample per month at site D2a (Kingston Beach)

Date	Site D2a			
	A	Rt	Ar	Aℓ
18 Sept. 1980	1600	2	0	0
14 Oct. 1980	1600	0	2	0
3 Nov. 1980	1600	0	1	0
10 Dec. 1980	1600	0	4	0
14 Jan. 1981	1600	0	1	0
12 Feb. 1981	1600	0	0	0
24 Mar. 1981	1068	1	3	0
23 Apr. 1981	1600	0	1	0
"	1600	42	45	19
26 May 1981	1600	6	20	6
19 Jun. 1981	1600	0	2	1
"	1068	0	0	7
7 Jul. 1981	1600	0	0	0
12 Aug. 1981	1600	0	0	0
23 Sept. 1981	1600	0	7	0
"	1600	2	5	5
21 Oct. 1981	1600	0	1	9
"	1600	0	3	4
19 Nov. 1981	1600	1	5	9
"	1600	0	6	0
7 Dec. 1981	1600	0	6	7
14 Jan. 1982	1600	0	0	0
	1068	0	0	0

APPENDIX 4c: The area (A) swept by the beam trawl in m^2 and the number of *R. tapirina* (Rt) and *A. rostratus* (Ar) juveniles caught in each sample at stations b and c in different months at site F3 (Pittwater)

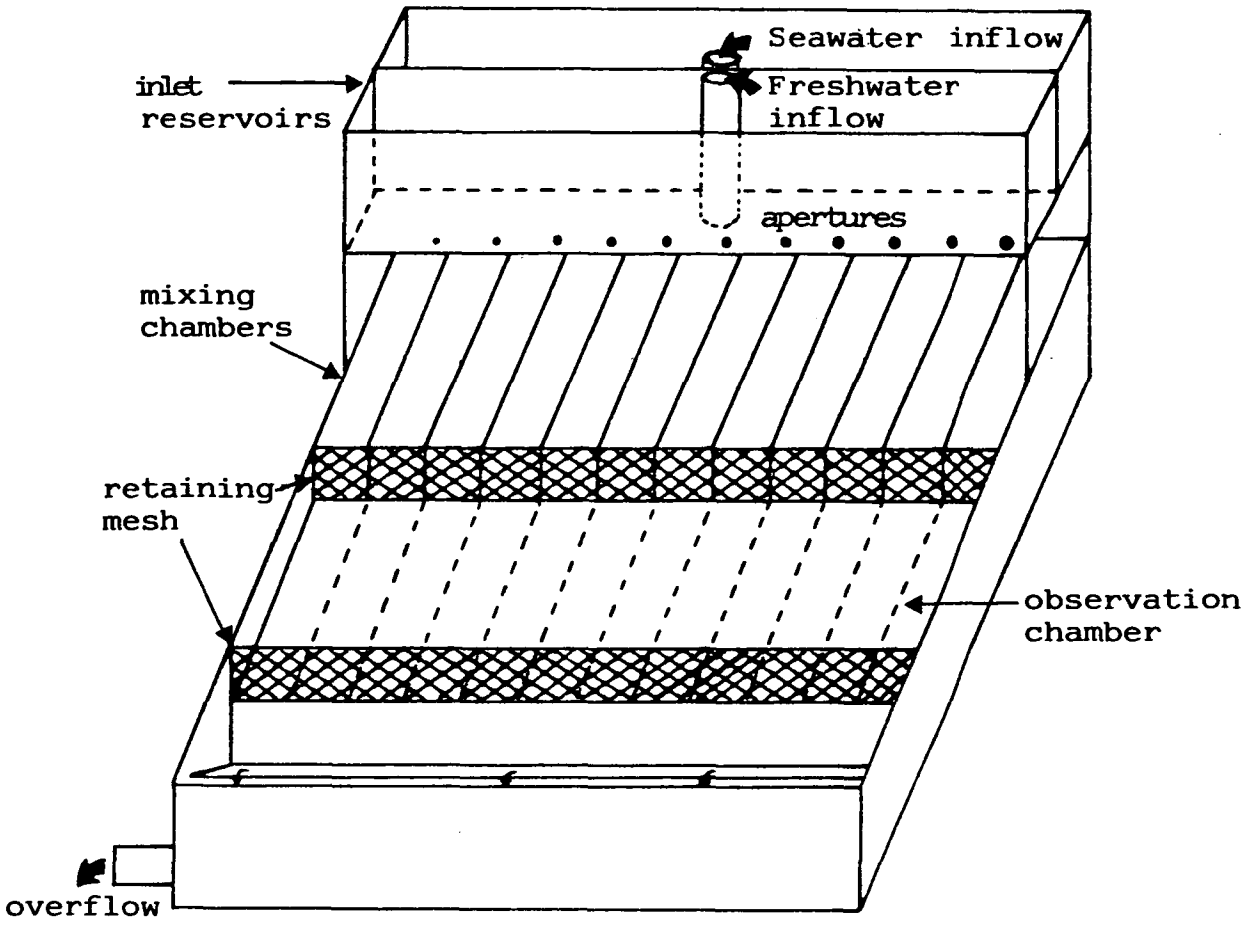
Date	Stations b and c		
	A	Rt	Ar
16 Aug. 1980	1600	7	4
1 Oct. 1980	1600	21	1
"	1600	34	10
5 Nov. 1980	1068	10	5
"	1068	2	0
3 Dec. 1980	1600	49	2
"	1068	62	7
6 Jan. 1981	1600	6	4
11 Feb. 1981	1068	2	1
"	1068	2	0
10 Mar. 1981	1068	47	12
"	1600	0	0
7 Apr. 1981	1068	0	2
"	1600	18	0
7 May 1981	1600	2	0
"	1068	2	9
5 Jun. 1981	1600	2	2
"	1068	5	1
3 Jul. 1981	1600	6	0
"	1600	1	0
4 Aug. 1981	1600	0	0
8 Oct. 1981	1600	0	0
"	1600	0	0
3 Nov. 1981	1600	12	2
2 Dec. 1981	1600	3	1
"	1600	0	0
15 Jan. 1982	1600	2	3
"	1600	0	0

APPENDIX 4d: The area (A) swept by the beam trawl in m^2 and the number of *R. tapirina* (Rt), *A. rostratus* (Ar) and *A. lituratus* (Aℓ) juveniles caught in each sample at stations a and b+c in different months at site F4 (Cremorne)

Date	Station a				Station b+c			
	A	Rt	Ar	Aℓ	A	Rt	Ar	Aℓ
28 Aug. 1980	1600	0	0	0	2669	22	16	0
"	1600	0	0	0	1600	4	1	0
23 Sept. 1980	1600	0	1	2	1600	19	0	0
"	1600	2	1	1	1600	11	0	0
29 Oct. 1980	1600	4	2	5	1600	50	1	0
"	1600	11	3	5	1600	8	1	0
26 Nov. 1980	1600	4	0	6	1600	48	2	0
"	1600	4	6	5				
31 Dec. 1980	1600	13	8	2	1600	13	0	0
"	1600	0	3	1	1600	32	4	0
27 Jan. 1981	1600	3	0	0	1600	15	0	0
"	1600	0	1	0	1600	24	0	0
24 Feb. 1981	1600	5	2	0	1600	4	0	0
"					1600	17	7	0
4 Mar. 1981	1600	12	8	1	1600	24	8	0
22 Apr. 1981	1600	8	7	1	1068	9	6	0
"					1600	3	31	0
22 May 1981	1600	12	5	1	1600	8	3	0
"	1600	2	3	2	1600	1	0	0
18 Jun. 1981	-	-	-	-	640	3	2	0
20 Jul. 1981	1068	4	4	0	1600	3	1	0
"	1068	3	7	1	1600	0	0	0
19 Aug. 1981	1600	3	4	4	1600	1	0	0
"					1600	9	3	0
21 Sept. 1981	1600	2	1	0	1600	11	1	0
"	1600	5	6	1	1600	0	0	0
20 Oct. 1981	1600	6	2	0	1068	6	0	0
"	1068	9	3	2	1068	3	1	0
17 Nov. 1981	1600	38	8	4	1600	6	1	0
"					1600	19	5	0
16 Dec. 1981	1600	1	0	0	1600	5	3	0
"					1600	11	3	0
28 Jan. 1982	1600	7	2	0	1600	1	0	0
"	1600	12	0	0	1600	0	0	0

APPENDIX 5: SALINITY GRADIENT APPARATUS

The salinity gradient apparatus which regularly provided a gradient of 0-33‰ across the observational chamber is shown diagramatically in Figure 1. Seawater and freshwater flowed into separate reservoirs and drained through apertures of varying sizes into 12 mixing compartments below. Each reservoir contained 11 apertures of decreasing size from 8 mm to 3 mm and no aperture at the end, and the two reservoirs were arranged so that the apertures decreased in size towards opposite ends. Thus, one end of the mixing compartment received only freshwater and the volume of freshwater decreased by a consistent amount towards the other end whilst the volume of seawater increased. To enhance mixing, freshwater flowed down tubing from the apertures to the bottom of the mixing compartments. The mixing chambers therefore contained water of varying salinities which then flowed over the observational chamber and out through an overflow pipe. Mesh of 2 mm knot to knot was placed at either end of the observational chamber to retain larvae.



Appendix 5 : Fig.1. Salinity gradient machine.
Scale 1cm = 10cm.

APPENDIX 6: CULTIVATION OF MICROALGAE, ROTIFERS (*BRACHINONUS PLICATILIS*) AND *ARTEMIA SALINA* NAUPLII.

Microalgae, *Chlorella* sp., *Monochrysis lutheri* and *Phaeodactylum tricornutum* were cultured as food for rotifers using the methods of Chanley (1978, unpublished) and Guillard (1973). Only *Phaeodactylum* could be cultured on a semi-continuous basis, the batch method being used for the other algae. When possible, rotifers were fed a mixture of algal species.

Attempts to rear rotifers on torulose yeast at various concentrations, as described by Fontaine and Revera (1980), were unsuccessful. Subsequently, yeast was only added as a supplementary food when algal concentrations were low.

Approximately 4 x 10^6 rotifers were produced daily for up to 6 weeks using the methods of Theilacker and McMaster (1971) and Howell (1973). Initially, rotifers were cultured by progressive inoculation of dense algal cultures of increasing volume up to 200 ℓ. When the density of the 200 ℓ rotifer cultures reached approximately 50 organisms $m\ell^{-1}$, one quarter of the volume was siphoned off on to a 63 μm mesh sieve which retained the rotifers. The seawater was replaced with an equal volume of dense algae (1-2 x 10^6 cells $m\ell^{-1}$). As the algae was consumed faster than it was replaced, it was periodically necessary to replace larger volumes of the culture medium with dense algae.

The rotifers were cultured at 20-25°C in ultra-violet treated seawater of 33-34‰ salinity. They were continuously aerated from the bottom and illuminated in the centre of the tank.

Artemia cysts (up to 5 g) were incubated in seawater in 20 ℓ glass carboys, aerated from the bottom and illuminated for at least the first few hours (Sorgeloos and Persoone, 1975). The temperature was maintained at 25-28°C and the hatched nauplii were siphoned off 28-32 h later. Only newly hatched nauplii were fed to flounder larvae.

In 1981 the outer chorion of *Artemia* cysts were first removed using sodium hypochlorite (Sorgeloos *et al.*, 1977). However, few

nauplii hatched in 1982 when a different stock of cysts were treated in a similar manner. Large numbers of nauplii were obtained only from untreated eggs.

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