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The Life History and Breeding Biology of *Nicon aestuariensis* Knox (Annelida, Polychaeta)

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Abstract

Nicon aestuariensis Knox has a two-year life cycle. The worms metamorphose into heteronereids and swarm at breeding. This is exceptional in estuarine species. Swarming occurred at fortnightly intervals, after new and full moon. The mating worms swam zig-zag courses, not the usual circles. The development of artificially fertilised eggs was followed up to the four setiger larva. Larvae were not found in the field and their habitat is uncertain. Present information suggests that the reproductive biology is different from all previously described Nereid species.

INTRODUCTION

A great deal of work (recently reviewed by Clark, 1961) has been done on the reproductive biology of polychaetes belonging to the family Nereidae, but there are so many variations that study of further species is valuable. Estuarine species are particularly interesting as they often show reproductive adaptations to this demanding environment.

Most of the strictly marine species metamorphose at maturity and reproduce in a specially adapted free-swimming form, the heteronereid, but estuarine species in general do not form a heteronereid. Heteronereids of *Nicon aestuariensis* were recorded by Knox (1951: 225) in his description of the species, so a detailed study was made (as part of an M.Sc. degree thesis) when it was found to be one of the most abundant animals in the upper reaches of the Heathcote Estuary, near Christchurch.

HABITAT

The section of the Estuary from which the worms were obtained extends for about threequarters of a mile upstream from the Heathcote Bridge on the road from Christchurch to Sumner. It consists of wide, nearly level, rush-covered flats at high water neap tide level, with bare muddy slopes down to the meandering channel which the river occupies at low tide. The worms were found in burrows in the stiff grey mud of the slopes, with the maximum population density at mid-tide level.

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A series of samples of the surface water was taken and the salinities determined by direct titration of 10ml aliquots of the sample against a 27.25 grams per litre solution of silver nitrate, using potassium dichromate as the indicator (Barnes, 1959: 95). The extreme salinity range found was from 0.2 parts per thousand (‰) at low tide to 29.1‰ at high water spring tide, with a minimum of 3.1‰ at mid-tide level.

Breeding Cycle

Methods

The procedure adopted for study of the breeding cycle was based on that used by Dales (1951) for work on *Nereis diversicolor* O. F. Muller. Specimens of *Nicon aestuariensis* were collected from the area in which they were most numerous, initially once a fortnight, then once a month. A length of steel tubing was used to extract a vertical core of mud with a cross-sectional area of 0.01 m^2 and a length of about 30cm. The worms were sorted from the mud by hand. At first six of these cores were taken on each occasion, but after the April collection eight were taken to maintain an adequate total sample number. The cores were taken at intervals of a few metres along the bank all at the mid-tide level where the population density was greatest. Over-sampling was easy to avoid as the places from which the cores had been taken could be seen for many weeks afterwards.

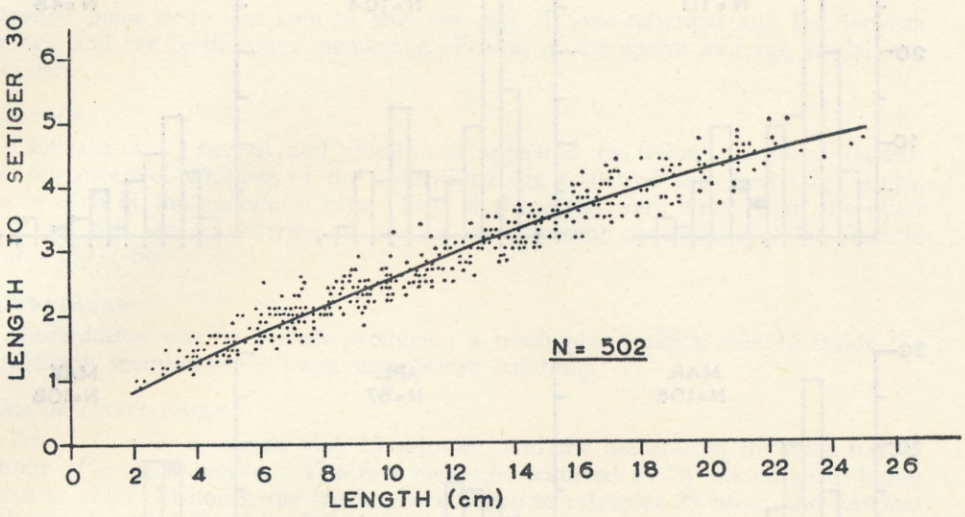
The worms were taken back to the laboratory and kept in seawater for twenty-four hours. In this time most of the mud was passed out of the gut and the worms were then anaesthetised in a solution of 60 grams of magnesium chloride ($MgCl_2$. $6H_2O$) per litre of tapwater. This concentration gave better results than a solution isotonic with seawater (80 grams per litre). Anaesthetisation to the point where the worms did not react to being extended for measuring took about 18 hours.

The worms were measured while extended along a wetted millimetre scale, and the measurements were made to the nearest millimetre. A low power magnifying glass above the scale gave greater accuracy and ease of reading. Two lengths were measured on complete specimens (a) the total length from the tips of the palps to the posterior end of the pygidium, excluding the anal cirri, and (b) the length from the tips of the palps to the posterior edge of the thirtieth setiger. On damaged specimens the length from the tips of the palps to the posterior edge of the thirtieth setiger was measured. Shorter fragments were counted for calculations of the population density but were not measured. Magnesium chloride has a relaxing effect, so the measured lengths are greater than in live specimens.

A graph (Text-fig. 1) of the relationship between the total length and the length to the thirtieth setiger was constructed from the measurements of complete specimens. Anterior fragments were assigned a total length from this curve. Worms which had been injured in the field and were regenerating a posterior end were treated in the same way. The total lengths were then grouped into classes with limits two centimetres apart and plotted as a length-frequency diagram for each month (Text-fig. 2). Specimens of a length such that they might have been placed in either of two classes were always put into the larger one.

The sex of the worms was determined by inspection under a stereoscopic microscope. This is practicable as the body wall of *N. aestuariensis* is not pigmented, but when necessary the body wall was slit and the coelomic contents teased out for closer observation.

After being counted, measured and sexed the worms were fixed in Bouin. Gametogenesis and the anatomical changes during metamorphosis were not studied.



TEXT-FIG. 1.—Relationship of the length of the anterior 30 setigers to the total length of intact specimens.

DISCUSSION OF METHODS

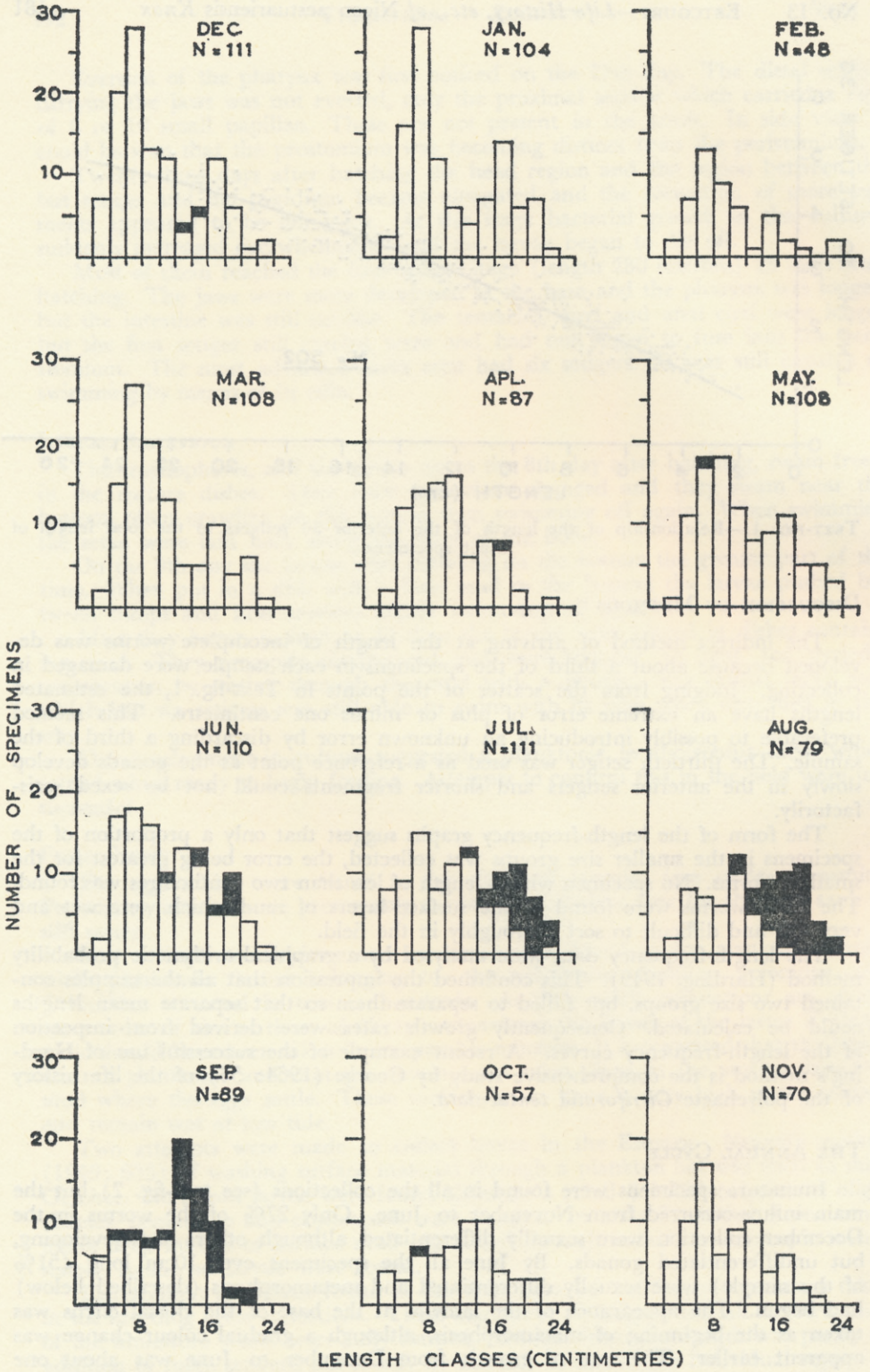
The indirect method of arriving at the length of incomplete worms was developed because about a third of the specimens in each sample were damaged in collecting. Judging from the scatter of the points in Text-fig. 1, the estimated lengths have an extreme error of plus or minus one centimetre. This seemed preferable to possibly introducing an unknown error by discarding a third of the sample. The thirtieth setiger was used as a reference point as the gonads develop slowly in the anterior setigers and shorter fragments could not be sexed satisfactorily.

The form of the length-frequency graphs suggest that only a proportion of the specimens in the smaller size groups was collected, the error being greatest for the smallest worms. No specimen with a length of less than two centimetres was found. The small worms were found in the surface layers of mud which were wet and very soft and difficult to sort thoroughly in the field.

The length-frequency data were analysed by a graphical arithmetic probability method (Harding, 1949). This confirmed the impression that all the samples contained two size groups, but failed to separate them so that separate mean lengths could be calculated. Consequently growth rates were derived from inspection of the length-frequency curves. A recent example of the successful use of Harding's method is the comprehensive study by George (1964: 53) of the life history of the polychaete *Cirriformia tentaculata.*

THE ANNUAL CYCLE

Immature specimens were found in all the collections (see text-fig. 2) but the main influx occurred from November to June. Only 27% of the worms in the December collection were sexually differentiated although others had developing, but undifferentiated gonads. By June all the specimens over 10cm long (51% of the sample) were sexually differentiated and metamorphosis (described below) had begun. The appearance of the lamella at the base of the dorsal cirrus was taken as the beginning of metamorphosis, although a gradual colour change was apparent earlier. The rate of growth from December to June was about one centimetre per month.



TEXT-FIG. 2.—Length-frequency diagrams. The blocked-in areas represent metamorphosing specimens.

Almost all of the sexually differentiated worms were metamorphosing by the first week in August, but some were much further advanced than others. The worms shrink as they metamorphose and so appear in the smaller size groups on the graphs. Some of the heteronereids may have spawned in the last week of August, but the main spawning periods were in September and October. Metamorphosis therefore took three to four months.

The heteronereids die after spawning and the population density decreased from 1180/m² in September to 760/m² in October (Table 1), then increased again in November and December as the immature worms appeared in the collections. The slow fall in the population density during the study period was perhaps due to increased pollution from the Heathcote River.

The specimens which were not large enough to metamorphose in time for the main spawnings grew rapidly through the spring (September and October) and formed the larger size group in the summer collections (December and January). Field observations of spawning suggested that these specimens metamorphosed and spawned in small numbers at least until May, and possibly right through to the next main breeding season. The presence of some small immature worms in all the collections supports this interpretation. The late spawning worms tended to grow to a larger size than the early spawners (14–16 centimetres against 10–12 centimetres) before beginning to metamorphose.

TABLE 1

Month	Number of Worms in each 0.01 sq. m. sample.								Mean	Standard Deviation.	Mean No. per sq. m.
1960 Dec.	16	13	22	25	20	25			19.8	4.5	1980 ± 450
1961 Jan.	16	19	19	19	21	12			17.7	3.2	1770 ± 320
Feb.	25	16	11						17.3	7.1	1730 ± 710
Mch.	20	21	23	15	18	15			18.6	3.3	1860 ± 330
Apl.	14	30	6	29	13				18.4		*1840
May	10	19	11	8	17	11	17	15	13.5	4.0	1350 ± 400
June	22	9	16	15	17	13	16	10	14.4	3.8	1440 ± 380
July	15	10	17	10	18	21	11	9	16.4	5.2	1640 ± 520
Aug.	14	12	9	10	14	12	8	6	10.6	2.9	1060 ± 290
Sep.	20	16	7	14	8	9	10	10	11.8	4.3	1180 ± 430
Oct.	11	6	8	5	10	6	7	8	7.6	2.6	760 ± 260
Nov.	9	8	16	10	6	10	7	9	9.4	3.1	940 ± 310
Dec.	14	6	10	9	15	13	16	11	11.8	3.4	1180 ± 340
1962 Jan.	12	11	14	10	7	10	8	11	10.4	2.2	1040 ± 220

*Two of the samples were merged in counting and the standard deviation was not meaningful.

There was a marked cyclical change in the sex ratio during the year. In the March collection there were 20 males to 14 females, in June 20 males to 37 females, in September 36 males to 10 females, and in November 12 males to 10 females. This suggests that the males differentiate later than the females but mature more quickly.

MORPHOLOGY OF THE HETERONEREID

Three stages in the metamorphosis of *N. aestuariensis* are illustrated in Plate 1. The process of metamorphosis appeared to be the same as has been described in other Nereids (see reviews by Fauval (1959: 135) and Clark (1961: 201)) and the heteronereid is similar to other three part heteronereids. The anterior section is little modified, the middle section has foliaceous parapodia bearing flattened swimming setae, while the posterior section remains unmodified.

Genital products first appeared in worms from 10 to 12 centimetres long as a small white mass in the ventral part of parapodia in the middle of the body. The eggs or sperms spread into anterior and posterior segments and completely filled the coelom before external morphological changes began. An 18 centimetres long worm at this stage is illustrated in Plate 1, fig. 1.

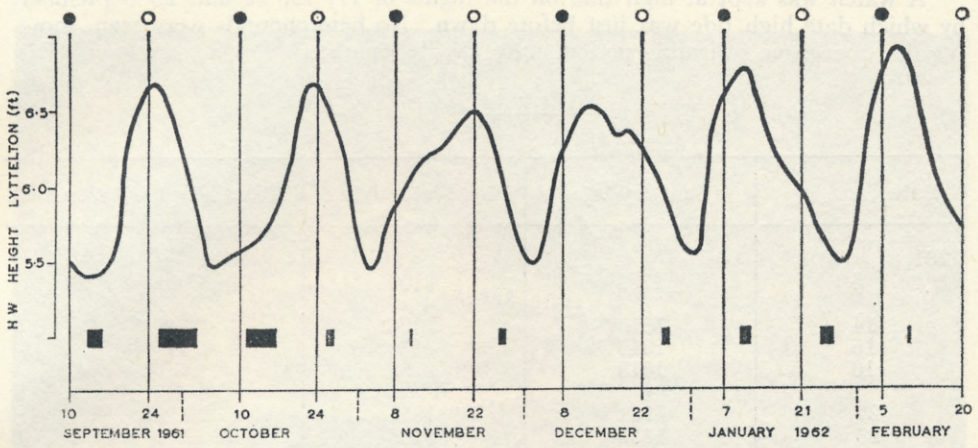
Plate 1, fig. 2 shows an intermediate stage in which the parapodia have begun to elongate, and the setal lamellae and the lamellae at the bases of the dorsal and ventral cirri are developing. The setae are unchanged. The eyes have begun to enlarge and the colour of the animal has changed with the development of a network of blood-vessels in the bodywall.

The modification of the parapodia spreads anteriorly and posteriorly but in the mature heteronereid (Plate 1, fig. 3) there are from 20 to 26 segments left unchanged anteriorly and from 20 to 60 posteriorly. Mature heteronereids were only 2 to 4.5 centimetres long, while the immature worms had a length of 20 to 25 centimetres. This reduction was due to longitudinal compression, as no segments were lost. The normal setae have been replaced by flattened, paddle-shaped natatory setae. The palps and antennae are now directed ventrally, bringing the much enlarged eyes into an anterodorsal position. The dorsal and ventral cirri of the first seven setigers are enlarged in the usual way, but the males do not have the crenulate dorsal cirri found in many species. This has a bearing on the swarming behaviour, which is discussed below. Male heteronereids are distinguished by the presence on the pygidium of a rosette of hollow papillae through which the sperm is emitted, and by their brighter colour.

The head and the first four or five setigers of male heteronereids are greenish, as in the atokous worms, while the rest of the unmodified segments are pink (red blood-vessels over white sperm) with a conspicuous dorsal blood-vessel. The parapodia of the modified segments are red-orange in colour, due to their extreme vascularisation, but there are few capillaries in the body wall between the bases of the parapodia and the dorsal blood-vessel. This forms a striking pattern with a glistening white stripe between the dorsal blood vessel and the parapodia on each side. The females have the same pattern but the eggs are pale green in colour, so the anterior and posterior regions tend to be pale green and the middle region is not so red as in the male. The difference is sufficient to allow the sexes to be distinguished in the field.

SWARMING PERIODICITY

At maturity the heteronereids swim to the surface where the gametes are released into the water. This aggregation (swarming) increases the concentration of gametes and therefore the efficiency of fertilisation. The swarming of *N. aestuariensis* was found to show a fortnightly or semilunar periodicity.



TEXT-FIG. 3.—The relationship of swarming to lunar and tidal cycles. The open circles represent full moon and the solid circles new moon. The dates on which swarming occurred are blocked in.

METHODS

Observations were made at night, using a 200 candle-power pressure lantern to attract the swimming heteronereids. Specimens were collected with a dipnet. Occasionally observations were made from a rowboat, but wading out to knee depth was just as effective. A surface water sample for salinity determination was taken at high tide, and water and air temperatures and weather conditions were noted. On most occasions a plankton sample was obtained by trailing a townet for ten minutes in the ebb current at a jetty immediately on the seaward side of the Heathcote Bridge. In this position the net sampled water draining from the area where the worms were most numerous.

Field work was begun in December 1960, but the first heteronereid was not caught and identified until April 1961. Regular observations were started in September 1961 and carried on until the end of February 1962. The dates on which heteronereids were seen, or swarming was indicated by the presence of developing eggs in the plankton, are given in Table II, and the relationship of swarming to the lunar and tidal cycles is illustrated in Text-fig. 3. The tidal curve in this figure was constructed by plotting the three-day running mean of the predicted heights of high water at Port Lyttelton (9 miles away by sea) then drawing a smooth curve through the points. The tides observed in the Estuary fitted these predictions very closely, although high water at the collecting area was about one hour later than at Lyttelton. Tidal heights, phases of the moon, and sunset times were taken from the N.Z. Nautical Almanac.

FIELD OBSERVATIONS

The results of these are set out in Table II. The times of high water are those observed in the Estuary.

The greatest numbers of heteronereids were seen in September and October. On 15 September, 82 specimens (70 males, 12 females) were netted in 55 minutes. On 27 September up to 40 heteronereids were visible at once in the swarm. Successively smaller numbers were seen on following nights, and from 12 November on only two or three heteronereids were seen in an evening.

A watch was kept at high tide on the nights of 17, 19, 22 and 25 September, by which date high tide was just before dawn. No heteronereids were seen, showing that successive swarming periods were clearly separated.

TABLE 2

Date	Sunset Time	High Water Time	Time First Heteronereid Seen
1961			
May 5	1728	2100	2100
Sep. 14	1816	2025	2035
15	1817	2120	2120
16	1818	2155	2215
27	1831	1951	1954
28	1832	2105	2028
29	1833	2150	2130
30	1834	2250	2230
Oct. 1	1836	2330	2320
3	1838	0015	0020
11	1847		eggs in plankton
12	1849	1935	2015
13	1850	2015	2030
14	1851	2110	2105
15	1852	2150	eggs in plankton
16	1854	2235	2242
27	1908	2030	2030
28	1909	2117	2104
Nov. 12	1929	2045	2116
27	1948	2140	eggs in plankton
28	1950	2215	2300
Dec. 26	2013	2120	2145
27	2013	2200	2230
1962			
Jan. 10	2011	2050	2153
11	2011	2200	eggs in plankton
25	2004	2112	2145
26	2003	2200	2200
27	2002	2235	2235
Feb. 10	1946	2230	2233

Discussion

After the observations between the periods beginning on 14 September and 27 September had shown that successive periods were clearly separated, observations were begun on a date predicted from tide tables and sunset times and continued until no signs of swarming were seen.

Swarming occurred once a fortnight on a maximum of six consecutive evenings, beginning from one to four days after the new and full moon. The first heteronereids appeared about high water when the tidal currents ceased, but once swarming started heteronereids could continue to appear for the next hour, by which time the ebb current had a speed of one to two knots.

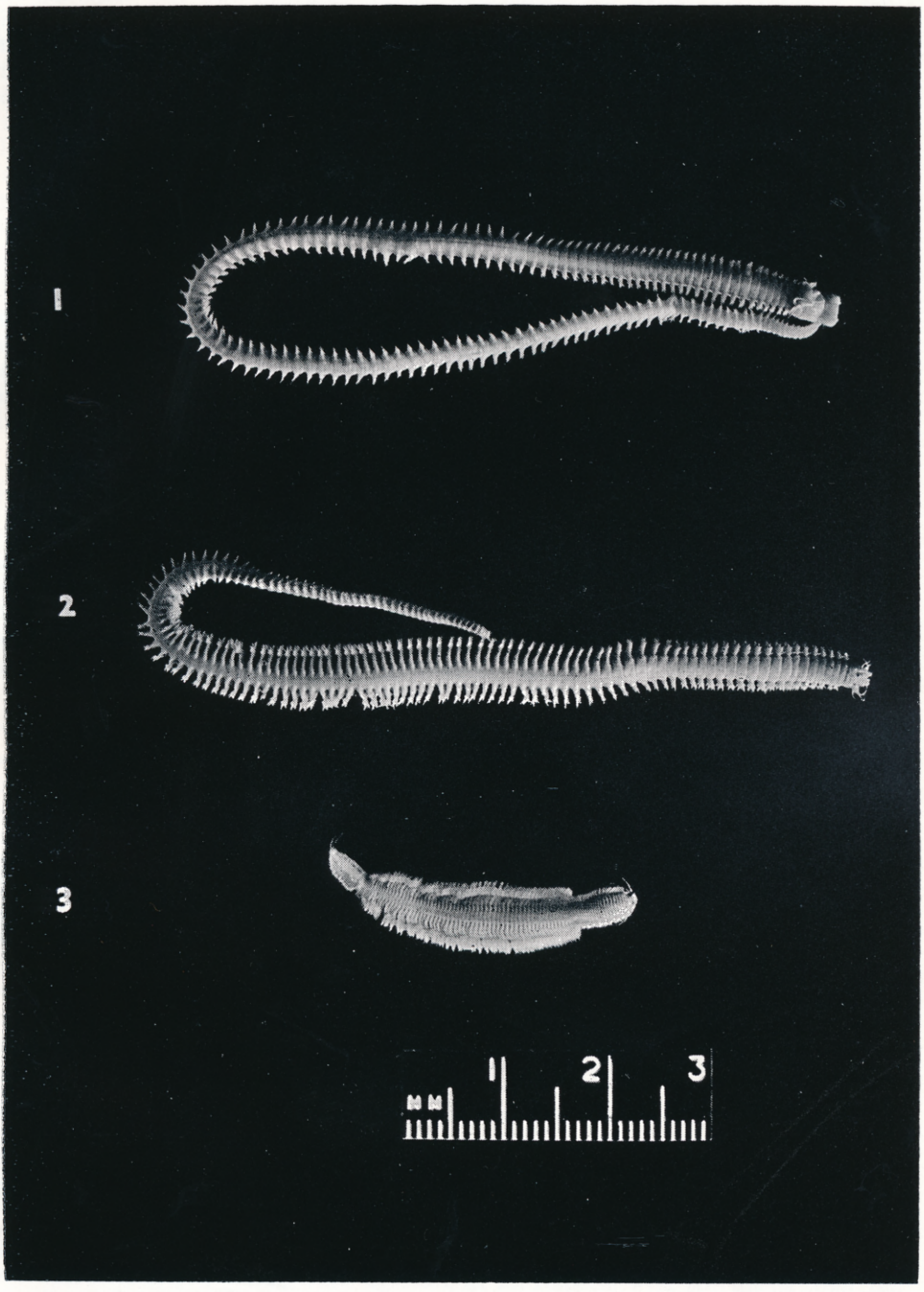


Photo: J. J. Whalan.

Stages in the metamorphosis of *Nicon aestuariensis* Knox.

Darkness was also necessary before swarming took place, the limiting light intensity being somewhere between dusk light and bright starlight. Consequently the appearance of the heteronereids grew later as the day length increased. The first heteronereids was seen at 8.35 p.m. on 14 September, but not until 9.53 p.m. on 10 January. The effect of full moon light is not known as a hill to the east shaded the area at high tide. The response to tide was modified slightly by the light intensity as on one occasion heteronereids did not appear until 30 minutes after high tide, when the last trace of daylight had faded. On the other hand, the first heteronereid never appeared more than 30 minutes before high tide even on nights when darkness fell two or three hours before.

Variations in the other environmental factors studied did not affect swarming. The salinity at high tide varied from 20.0‰ at neap tides to 29.1‰ at springs, while the highest and lowest water temperatures in which swarming occurred were 21.5° C. in January and 9° C. in September. Heavy rain inhibits swarming (Korringa, 1957: 927) but during this study heavy rain did not fall on any of the nights when swarming was expected. Consequently the effect of rain on the swarming of *N. aestuariensis* was not observed.

Korringa (1957: 921) in a review of lunar periodicity distinguished three components in breeding systems of this sort: " an *annual rhythm,* a breeding season of longer or shorter duration, which may be controlled by water temperature, a *lunar periodicity,* perhaps better called a tidal periodicity, which brings about maxima in spawning twice a month during the entire breeding season, and next a *daily rhythm* which limits spawning activities to certain hours of the day or tidal cycle ". *N. aestuariensis* fits this pattern with its breeding season in spring extending at a lower intensity into autumn, swarming restricted to a few nights in each fortnight, and to an hour just after high tide on those nights.

Generalising on fortnightly periodicities, Korringa further stated (1957: 922), " In all cases it is a question of rhythmical ripening of eggs and sperm synchronised with the cycle of neaps and springs. The maxima are therefore not necessarily observed at new or full moon ". But the swarming of *N. aestuariensis* maintained a close relationship to the new and full moon rather than following the shifting cycle of neaps and springs (Text-fig. 3). However, another characteristic of the tides, their timing, does have a constant relationship to the phases of the moon. The afternoon tide at Lyttelton on the day of new and full moon is always high between 4 p.m. and 6 p.m. and at the collecting area in the Heathcote Estuary it is about 50 minutes later. Consequently a few days after new and full moon high tide in the Estuary occurs just after dark, and this conjunction of the two factors which were necessary for swarming recurs once each fortnight. Furthermore swarming did not occur on all high tides at night but only on the first few in each fortnight, so that successive periods of swarming were completely separated.

The observed periodicity of swarming can be explained by assuming that swarming is initiated by the combination " high tide shortly after nightfall ". The correctness of this conclusion, and the exact stimuli to which the worms respond, could be established only by experiments with metamorphosing worms kept under controlled conditions of light and tide.

A collection of heteronereids from Bluff Harbour in the teaching collection of the Zoology Department, University of Canterbury, was examined. The heteronereids were *Nicon aestuariensis* agreeing in all respects with specimens from the Heathcote Estuary. They were netted from a wharf on 13 January 1951, at 11.15 p.m. This was five days after full moon and two and three-quarter hours after sunset, but four and three-quarter hours after high tide. There appears to be the same relation as at Heathcote between swarming and the moon and sunset, but a less marked relation to the tide.

The swarming behaviour of a single species of nereid may be different in different localities (e.g., *Platynereis dumerilii*, Fauvel 1959: 139) and the striking periodicity found in *N. aestuariensis* in a single season and a single locality requires further study in the field as well as in the laboratory.

SPAWNING BEHAVIOUR

Once a swarm has formed in response to an environmental stimulus the release of gametes into the water (spawning) is triggered by reactions of the heteronereids within the swarm. In most nereids (Clark, 1961: 214) there is a reciprocal stimulation of spawning; a substance diffusing from the ripe female or its eggs causes the males to release sperm and the presence of sperm in the water causes the female to emit the eggs. However, natural spawning was not observed in *Nicon aestuariensis* and the trigger mechanism is unknown.

FIELD OBSERVATIONS

Swarming always began with the appearance of one or two male heteronereids. There was often a slight pause before the first females appeared, but after this the numbers rapidly increased to a peak which was maintained for about half an hour then slowly decreased. The rapid erratic swimming of the heteronereids made it difficult to estimate the numbers in a swarm, but the greatest number in view at one time was about 40. The total number seen while a swarm lasted was several times greater as heteronereids joined the swarm, replacing those which drifted away or were eaten by yellow-eyed mullet, *Aldrichetta forsteri* (Cuv. and Val.).

Heteronereids were attracted to the light from a fairly wide area, for when the boat was moved quickly 50 metres away from a swarm, no heteronereids were seen until the same swarm reformed under the light. Under the light both sexes swam rapidly with abrupt changes of direction and depth. The observations were made in water less than a metre deep and here the worms swam from surface to bottom.

A large sample of heteronereids from one swarm contained six males to each female, but the swarms did not break up into groups each containing a female and several males.

The release of the eggs and sperms was not seen although both gravid and spent heteronereids were netted from the swarms.

LABORATORY OBSERVATIONS

Ripe males and females netted from swarms were brought back to the laboratory in separate jars then placed together in dishes of seawater. Different numbers of males and females were tried but spawning did not occur.

The worms could be stimulated to spawn by pinching the skin with forceps. Sperm was emitted through the anal rosette, and the eggs through rents in the lateral body wall between the parapodia of the last three or four modified segments. The septa have broken down so that the coelom is continuous along the length of the heteronereid. Under these conditions all the eggs or sperms were released at once, forced out by contraction of the longitudinal body musculature, and the heteronereids seemed to shrink in the process. Spawned heteronereids were quite flaccid although still able to swim, and in the laboratory they died in one or two days.

Ripe male and female heteronereids kept in separate dishes in the laboratory released their sperms or eggs at night and then died.

The heteronereids appeared to have a high oxygen requirement. Specimens kept in seawater without aeration soon stopped swimming and sank to the bottom of the dish, but they began swimming again when air was bubbled through the water.

DISCUSSION

Many, but not all, species which swarm have a preponderance of males (Clark, 1961: 213) and the six to one ratio found in *N. aestuariensis* is not unusual. Death after spawning is also normal (Clark, 1961: 200).

However the zig-zag swimming of the heteronereids and the lack of association between the sexes is unusual. Most nereids which swarm at the surface show the same behaviour, with several males swimming in tight circles round each female. This nuptial dance depends on special sense organs developed on the under surface of the dorsal cirri of the males (Clark, 1961: 214). Cirri carrying these sense organs have a distinctive appearance termed " crenulate ". The males of *N. aestuariensis* lack these structures. *Platynereis megalops,* which also lacks the cirral sense organs, undergoes a form of copulation instead of a nuptial dance. This, together with the fact that normal spawning was not seen, suggests that the complete behaviour pattern of *N. aestuariensis* was not observed, but it appears to be different from those described in other species.

LARVAL DEVELOPMENT

The embryology of all Nereid species studied is very similar, but there are differences in the stage at which hatching occurs and in the ecology of the larvae. Attempts were made to rear the larvae of *Nicon aestuariensis* in the laboratory and to find them in the field, so that the complete life cycle could be followed and especially so that the ecology of the larvae in the estuarine habitat could be studied.

REARING EXPERIMENTS

Since the worms could not be induced to spawn naturally the technique recommended by Costello *et al* (1957: 84) for *Nereis limbata* was used to obtain and fertilise gametes. Standard culture techniques were used and all cultures were kept at room temperatures. The development was followed by observation of live larvae and study of a series of stained whole mounts.

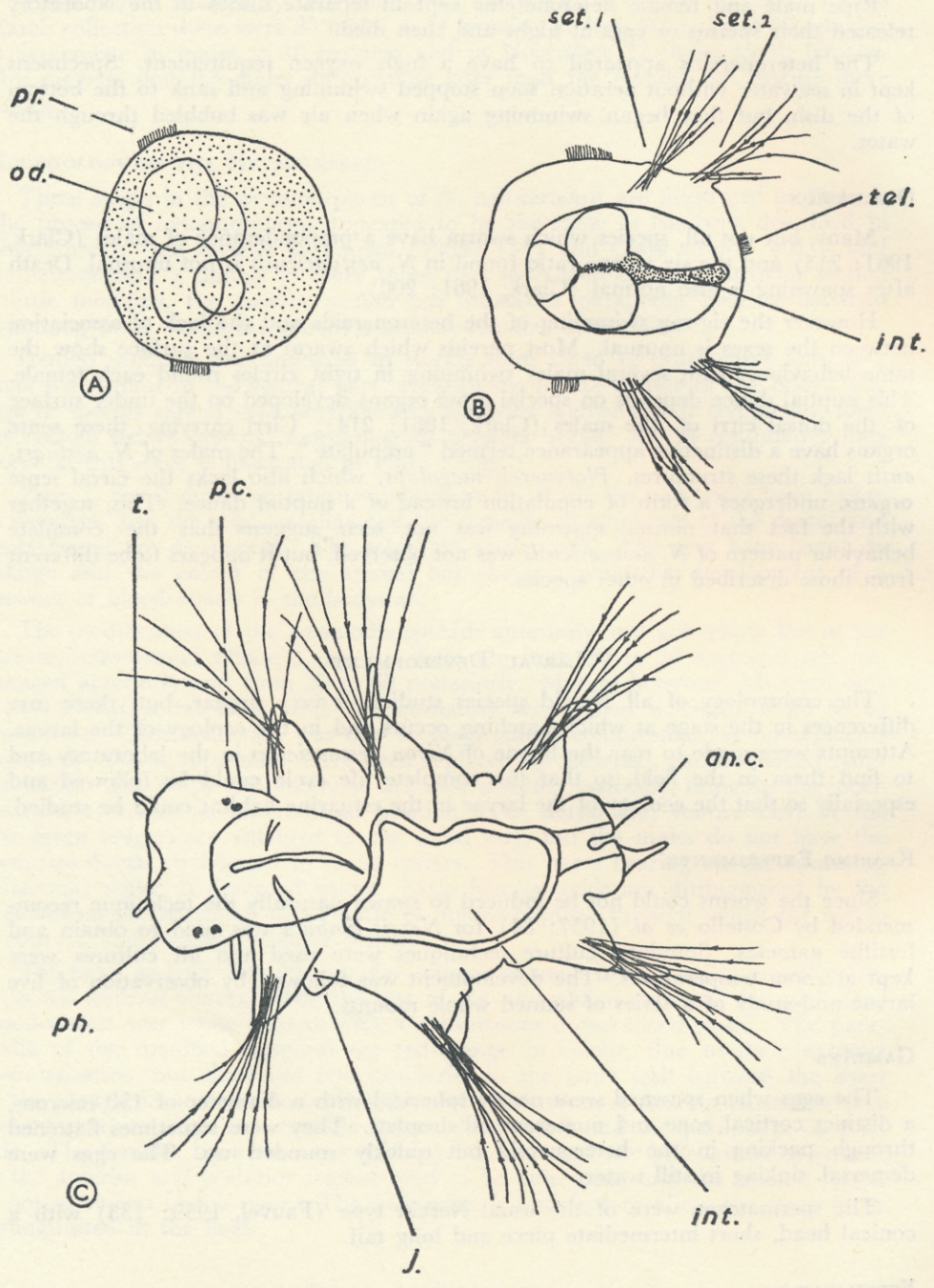
GAMETES

The eggs when spawned were nearly spherical with a diameter of 150 microns, a distinct cortical zone and numerous oil droplets. They were sometimes flattened through packing in the heteronereid but quickly rounded up. The eggs were demersal, sinking in still water.

The spermatozoa were of the usual Nereid type (Fauvel, 1959: 133) with a conical head, short intermediate piece and long tail.

FERTILISATION

Once activated by a sperm a layer of jelly with a thickness equal to the diameter of the egg appeared round the egg. This was produced by the cortical zone, which disappeared at the same time, leaving a space inside the now distinct fertilisation membrane. The maturation diversions and polar body formation followed. An



TEXT-FIG. 4.—A, Elongating Trochophore. B, Two setiger larva, length 105 microns. C, Three setiger larva, length 210 microns.

Abbreviations: *an.c.*, anal cirri; *int.*, intestine; *j*, jaw; *od.*, oil droplet; *p.c.*, peristomial cirri; *ph.*, pharynx; *pr.*, prototroch; *set. 1*, setiger 1; *set. 2*, setiger 2; *t*, tentacle; *tel.*, telotroch.

extruded polar body was seen in only one egg. It was spherical and lay between the egg and the fertilisation membrane. Fusion of the sperm and egg nuclei was not seen.

CLEAVAGE

Cleavage was unequal and spiral and appeared to follow the usual pattern The numerous oil droplets of the unfertilised egg coalesced into four large drops, one in each of the endoderm cells. Two of the drops were larger than the other two, and this was a criterion of normal development as mentioned by Costello *et al.* (1957: 86).

GASTRULATION

Gastrulation was by epiboly producing a trochophore which rotated inside the fertilisation membrane for some time before hatching.

RATE OF DEVELOPMENT

At 7° C. jelly extrusion took 15 minutes, and the maturation divisions started 1 hour after insemination. The first cleavage occurred at 2½ hours, gastrulation began at about 18 hours, the trochophore began to rotate at 25 hours and hatched 30 hours after insemination. Higher temperature increased the rate of development especially during cleavage and gastrulation. At 15° C. cleavage began 2¼ hours after insemination and the trochophores hatched after only 18 hours.

TROCHOPHORES

The trochophores (Text-fig. 4A) were spherical when hatched and did not begin to elongate until 3 days later. The only cilia seen were those of the prototroch. This did not form a complete ring, but the extent of the gap was not established. There was no sign of an apical tuft. The stomodaeum showed as a small indented thickened area just posterior to the prototroch. Pigment was limited to a discontinuous ring of red spots immediately anterior to the prototroch and parallel with it. The trochophores swam randomly in dishes without showing any reaction to light or preference for a particular level in the water.

LARVAE

The trochophores passed rapidly into the two setiger stage (Text-fig. 4B) 4½ days after hatching. No single setiger larvae were seen. The intestine was visible running from the oil droplets to the pygidial plate, which was distinct from the second setiger. The pigment spots of the trochophore had disappeared, and small eyespots were present. A small telotroch had developed. Muscular movements had begun and the compound setae could be spread laterally or folded back against the body. Length of the larvae was 105 microns.

Next day the third setiger had formed and the intestine was developing. On the 7th day the anal cirri were noticed.

The pharynx was forming on the 8th day after hatching and the oil droplets had moved posteriorly into the intestine. An incomplete ring of metatrochal cilia had developed on each setiger just posterior to the setae.

Nine days after hatching the tentacles and the first pair of peristomial cirri were present (see Text-fig. 4C). There were now clearly two pairs of eyespots.

Some larvae began to feed on bacteria growing on the sides of the dishes at 11 days and all were feeding by the 15th day. The larvae (Text-fig. 4C) now had a length of 210 microns. By this time the oil droplets in the intestine were very small and quickly disappeared once feeding began. Peristaltic waves were seen passing along the pharynx and feeding appeared to be by suction. The jaws were first noticed on the 10th day and were quite distinct by the 15th. The setae were arranged in dorsal and ventral rami with a lobe of the parapodium projecting between the two rami.

Eversion of the pharynx was first noticed on the 17th day. The distal section carrying the jaws was not everted, only the proximal section which carried a ring of 8 or 10 small papillae. These are not present in the adult. In side view it could be seen that the prostomium was becoming distinct from the peristomium.

Twenty-three days after hatching the head region and the region between the last setiger and the pygidium became elongated and the formation of more segments appeared to be imminent. At this stage bacterial growth in the cultures suddenly increased overwhelmingly and the larvae began to die off.

Most of them reached the four setiger stage (length 280 microns) 29 days after hatching. The jaws were more developed at the base and the pharynx was longer, but the intestine was still sac-like. The tentacles, cirri and anal cirri were longer but the first setiger still carried setae and had not begun to fuse into the peristomium. The most advanced larva seen had six setigers. It was still capable of swimming by means of its cilia.

LARVAL BEHAVIOUR

The trochophores, and the larvae up to the 8th day after hatching, swam freely in the culture dishes. Then their behaviour changed and they swam near the bottom, often crawling on the bottom then swimming off again. When swimming the setae were laid back along the sides of the body.

On the 9th day the larvae were crawling on the bottom the greater part of the time. When put in a dish with a little mud in the bottom the larvae pushed between lumps and into crevices before swimming off again.

After they began to feed at 11 days the larvae were placed in dishes containing mud. They promptly disappeared and were brought to light for periodic examination by sorting through the mud with a needle. The largest larva seen which had six setigers was still able to swim with its cilia when disturbed in this way.

The development in culture suggested a planktonic lecithotrophic larva which settled when ready to begin feeding. Attempts to confirm this in the field were unsuccessful.

FIELD STUDY

The eggs which were found in the plankton samples (see under Swarming Periodicity, above) were presumably carried by turbulent flow for the eggs sank in still water.

Only a single trochophore and no larvae were found in the plankton samples while planktonic larvae belonging to the polychaete families Spionidae and Orbiniidae occurred in tens or hundreds. As their adult populations are of the same order of size as those of *Nicon aestuariensis* the larvae of the latter if planktonic should have been taken in comparable numbers. It is suggested that the larvae are not planktonic and that the development takes place in the surface layers of mud where the eggs settle. These surface layers have a very high water content and remain wet at low tide.

Two attempts were made to collect larvae in the Estuary. Newell's method (1949: 635) of washing surface material through a plankton net was tried in mid-October. No *Nicon* larvae were found although a small Sabellid polychaete, oligochaetes, nematodes and crustaceans were present. In early December a one centimetre deep sample of surface mud was sorted through under a stereoscopic microscope. Again no larvae were found.

The smallest specimens found in the field were 2.5 centimetre specimens taken in the breeding cycle collections. These had about 70 setigers and all the structures of the immature worm were fully formed. Growth from this length was partly by addition of more setigers, but mostly by increase in size of the individual segments. The number of segments for a given length varied from 64 to 99 in the two to

four centimetre class and from 118 to 147 in the twenty to twenty-two centimetre class. These figures are for undamaged worms, but ones with a regenerating posterior end were common. The shortest ones seen had the head, pharynx and a few segments of the intestine intact.

LARVAL GROWTH RATE

Development to the four setiger stage took one month, but because of the failure to find later larvae the time taken to reach a length of 2.5 centimetres is not known.

DISCUSSION

The life cycle appears to take two years. Development to the four setiger stage took one month, and growth from a length of 2.5 centimetres to maturity and spawning one year. An estimate of 10 or 11 months for the unknown later larval development seems consistent with these rates.

The probable cycle is, worms hatched in spring reach a length of three to four centimetres in late spring of the following year, grow rapidly through the summer and autumn, then metamorphose during winter and spawn in the spring.

The breeding biology of brackish and freshwater Nereid species is highly modified because the adults are capable of osmoregulating but the developing eggs and larvae are not (Clark, 1961: 217). He states that of more than fifty brackish and freshwater species only four (including *Nicon aestuariensis*) undergo a complete metamorphosis, and only two, *Tylorrhynchus heterochaetus* and *Nereis japonica,* are known to swarm and spawn at the surface. Both these species migrate to the lower reaches of estuaries to spawn, and the larvae leave the bottom after they have acquired the ability to osmoregulate and are carried upstream by the flood tides.

Nicon aestuariensis is the third estuarine species known to swarm and spawn at the surface, but it appears to be unique as it does not migrate. This suggests that the larvae may be unusually tolerant of low salinities, but knowledge of the distribution of the larvae in the estuary and laboratory experiments are needed for confirmation.

The development as far as it was followed was of the normal Nereid type. There appears to be little correlation among Nereids between the type of larval development and the stage at which hatching occurs. *Nereis pelagica* (Wilson, 1932) has a planktotrophic larva which hatches with three setigers, *Nereis grubei* (Reish, 1954: 16) also hatches with three setigers but is lecithotrophic, while *Nereis diversicolor* (Dales, 1950) and *Nicon aestuariensis* (this report) have non-planktonic lecithotrophic larvae yet hatch as trochophores. The larvae of *N. diversicolor* and *Nicon aestuariensis* are anatomically very similar. There is insufficient evidence to decide whether the larvae of *Nicon aestuariensis* are planktonic or not, but if they are not the habitat of the larvae would also be similar. The adults appear to occupy similar ecological niches in estuaries, *N. diversicolor* in Europe (Smith, 1956) and *Nicon aestuariensis* in the South Island of New Zealand. However, there is one major difference between them; *N. diversicolor* does not metamorphose before spawning.

The fortnightly periodicity of swarming found in *Nicon aestuariensis* merits further study and experimental analysis, for no previous worker on lunar periodicity has suggested an environmental stimulus resembling the conjunction of high tide and nightfall postulated here.

The details of reproduction in Nereid species can vary in different localities and seasons, so the conclusions drawn from this study over one season in one locality may be modified by further work. However the spawning behaviour of the heteronereids and the ecology of the larvae are the only parts of the life history of *Nicon aestuariensis* which remain unknown.

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